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Associations between cytokine gene variations and severe persistent breast pain in women following breast cancer surgery

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Abstract

Persistent pain following breast cancer surgery is a significant clinical problem. While immune mechanisms may play a role in the development and maintenance of persistent pain, few studies have evaluated for associations between persistent breast pain following breast cancer surgery and variations in cytokine genes. In this study, associations between previously identified extreme persistent breast pain phenotypes (i.e., no pain versus severe pain) and single nucleotide polymorphisms (SNPs) spanning 15 cytokine genes were evaluated. In unadjusted analyses, the frequency of 13 SNPs and 3 haplotypes in 7 genes differed significantly between the no pain and severe pain classes. After adjustment for preoperative breast pain and the severity of average postoperative pain, one SNPs (i.e., interleukin (IL) 1 receptor 2 rs11674595) and one haplotype (i.e., IL10 haplotype A8) were associated with pain group membership. These findings suggest a

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role for cytokine gene polymorphisms in the development of persistent breast pain following breast cancer surgery.

Perspective—This study evaluated for associations between cytokine gene variations and the severity of persistent breast pain in women following breast cancer surgery. Variations in two cytokine genes were associated with severe breast pain. The results suggest that cytokines play a role in the development of persistent postsurgical pain.

Keywords

cytokines; polymorphism; breast cancer surgery; candidate genes; persistent pain

Introduction

Persistent pain in women following breast cancer surgery is common, with an estimated prevalence of between 21% and 55%.[5; 22; 39; 49; 50; 62; 64; 70; 71] Persistent pain is associated with depressed mood,[64] sleep disturbance,[14; 30] decreased quality of life,[5; 49; 62] and disability.[39; 70] Persistent postsurgical pain may result from ongoing nociceptor activation and/or nerve injury.[42] During the early postoperative period, release of numerous inflammatory mediators produce peripheral sensitization in and around the surgical site.[72] These reversible changes in sensitivity to innocuous and noxious stimuli discourage stimulation of the surgical incision and facilitate healing. However, sustained activation of nociceptors may lead to the maintenance of central sensitization and phenotypic changes that alter the normal stimulus-response relationship and produce persistent pain. Evidence suggests that ongoing activation of inflammatory and glial cells[45] and spinal inhibitory mechanisms[75] play a role in the establishment of persistent pain. In addition, peripheral nerve injury prompts the aggregation of immune cells that increases the local concentration of proinflammatory cytokines.[43] These mediators participate in the initiation and maintenance of persistent pain by generating ectopic activity,[23] altering neuronal connectivity,[29] and reducing the number of inhibitory neurons.[25]

While several studies have identified phenotypic characteristics that predispose patients to the development of persistent pain following breast cancer surgery,[2; 19; 33; 38; 69] less is known about the molecular mechanisms associated with this significant clinical problem. In fact, despite the strong evidence that persistent activation of immune mechanisms results in persistent pain,[43] only four studies evaluated for associations between polymorphisms in cytokine genes and cancer-related pain.[44; 54–56] Three of these studies[54–56] assessed pain intensity prior to the initiation of cancer treatment. Associations were found between severe pain (i.e., pain rated >6 on a 0 to 10 numeric rating scale (NRS)) and interleukin (IL) 1 beta (IL1B) rs1143627,[54] IL8 rs4073,[54; 55] and tumor necrosis factor alpha (TNFA) rs1800629.[56] However, findings from these studies are difficult to interpret because the pain phenotype was characterized using only a dichotomized pain severity rating, had modest sample sizes, and the number of polymorphisms evaluated was not optimal. Recent work from our group evaluated for associations between variations in cytokine genes and pain in the affected breast of women prior to breast cancer surgery.[44] Associations were found between the presence of preoperative pain and IL1 receptor 1 (IL1R1) rs2110726 and

IL13 rs1295686. Of note, no studies were found that evaluated for associations between cytokine gene polymorphisms and persistent postsurgical pain.

In this same sample of women assessed for pain prior to breast cancer surgery,[44] growth mixture modeling (GMM) was used to identify subgroups of women with distinct persistent breast pain trajectories prior to and for six months following breast cancer surgery.[46] In brief, three distinct classes were identified using patients' ratings of worst pain in their breast (i.e, mild, moderate, severe). A fourth pain class was identified of women who did not experience breast pain preoperatively or at any of the postoperative assessments. An evaluation of associations between extreme pain phenotypes may increase the effect size that can be detected in genetic association studies.[40] Therefore, using the extreme pain phenotypes identified in this GMM analysis, the purposes of this study were to evaluate for differences in demographic and clinical characteristics, as well as for variations in cytokine genes, between the no pain and severe pain classes.

Materials and Methods

Patients and Settings

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.[44; 46] 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 baseline assessment prior to surgery.

Subjective Measures

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.[35; 36] 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.[60] 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 using a "yes/no" format. If they indicated that they had a condition, they were asked if they received

treatment for it (yes/no; proxy for disease severity) and did it limit their activities (yes/no; indication of functional limitations). Patients were given the option to add three additional conditions not listed on the instrument. For each condition, a patient can receive a maximum of 3 points. Because there are 13 defined medical conditions and 2 optional conditions, the maximum score totals 45 points if the open-ended items are used and 39 points if only the closed-ended items are used. The SCQ has well-established validity and reliability and has been used in studies of patients with a variety of chronic conditions.[4; 8; 41; 60; 61]

Preoperative and persistent, as well as acute postoperative pain ratings were evaluated using the Breast Symptoms Questionnaire (BSQ) and Postsurgical Pain Questionnaire, respectively. The BSQ consists of two parts. Part 1 obtained information on the occurrence of pain and the occurrence of other symptoms in the breast scar area (i.e., swelling, numbness, strange sensations, hardness). The additional symptoms that were assessed were identified in studies by Tasmuth and colleagues.[65; 66] If the patient had pain in the breast scar area, they completed Part 2 of the BSQ. Patients were asked to rate the intensity of their average and worst pain using a 0 (no pain) to 10 (worst imaginable pain) NRS. A NRS is a valid and reliable measure of pain intensity.[31]

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 breast pain phenotype

Characterization of the persistent breast pain phenotype used in this study was described previously.[46] At each assessment, patients were asked, "Are you experiencing pain in your affected breast?" If the patient reported pain, she was asked to rate her "current pain at its worst" using a 0 (no pain) to 10 (worst pain) NRS. Prior to conducting GMM analyses, patients who reported no pain in their affected breast for all 6 assessments (i.e., enrollment and 2, 3, 4, 5, and 6 months) were identified (N=126; 31.7%) and were not included in the

GMM analysis. For the remaining 272 women, the six ratings of worst breast pain were used in the GMM analysis to assign each individual into a latent class. Acute pain ratings obtained at the 1-month follow-up assessment were excluded from the model. The high prevalence of pain at the month 1 assessment reduced the variability in pain ratings among the patients. This reduced variability prohibited the determination of latent classes when month 1 ratings were included in the GMM.

A single, unadjusted 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.[34; 48; 68] Model fit was assessed statistically by identifying the model with the lowest Bayesian Information Criterion (BIC). The parametric bootstrapped likelihood ratio test (BLRT) was used to evaluate whether a model with K classes fit the data better than a model with K-1 classes. In addition to using the BLRT to compare models, the Vuong-Lo-Mendell-Rubin Likelihood Ratio Test (VLMR) for the “K” versus “K-1” class models were examined. When the VLMR test is non-significant, it provides evidence that the K-class model is not better than the K-1 class model. The fourth index used to evaluate model fit was entropy, with >.80 being preferred.[7; 47] Finally, the best fitting model was visually inspected by plotting observed against model-predicted values to determine whether the predicted trajectories followed the empiric trajectories for the classes, and to evaluate whether the predicted plots “made sense” theoretically and clinically.[34; 48; 68] The GMM analyses were done using MPlus 6.1.[47]

Descriptive statistics and frequency distributions for the no breast pain and severe breast pain classes were generated for demographic and clinical characteristics using Stata version 12.1 (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 two breast pain classes. Logistic regression analysis was 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 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 the logistic regression analyses to evaluate the associations between genotype and pain group membership.

Genotype determination

Genomic deoxyribonucleic acid (DNA) was extracted from peripheral blood mononuclear cells using the PUREGene DNA Isolation System (Invitrogen, Carlsbad, CA). DNA was available from 310 of the 398 patients. 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). Genotyping was performed blinded to clinical status and positive and negative controls were included. Samples were genotyped using the Golden Gate genotyping platform (Illumina, San Diego, CA) and processed according to the standard protocol using GenomeStudio (Illumina, San

Diego, CA). Two blinded reviewers visually inspected signal intensity profiles and resulting genotype calls for each single nucleotide polymorphism (SNP). Disagreements were adjudicated by a third reviewer.

A combination of tagging SNPs and literature driven SNPs (i.e., reported as being associated with altered function and/or symptoms) 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 (e.g., HapMap, release 24). Tag SNP selection was performed across all four available HapMap samples (i.e., CEU, YRI, JPT, CHB). In order to ensure robust genetic association analyses, quality control filtering of SNPs was performed. SNPs with call rates <95% (n=0), sample call rates <95% (n=0), Hardy-Weinberg $p < .001$ (n=11), and/or a MAF of <5% (n=11) were excluded. A total of 82 SNPs from 15 inflammatory cytokine genes (i.e., interferon gamma (IFNG): 5 SNPs; IFNG receptor 1 (IFNGR1): 1 SNP; IL1B: 12 SNPs; IL1R1: 4 SNPs; IL1 receptor 2 (IL1R2): 3 SNPs; IL2: 3 SNPs; IL4: 2 SNPs; IL6: 9 SNPs; IL8: 3 SNPs; IL10: 7 SNPs; IL13: 4 SNPs; IL17A: 5 SNPs; nuclear factor kappa beta-1 (NFKB1): 11 SNPs; NFKB2: 4 SNPs; TNFA: 9 SNPs) passed all quality control filters and are included in subsequent analyses. Potential functional roles of SNPs associated with persistent breast pain were examined using PUPASuite 2.0,[11] a comprehensive search engine that examines for a series of putative functional effects (i.e., non-synonymous changes, altered transcription factor binding sites, exonic splicing enhancing or silencing, splice site alterations, microRNA target alterations).

Statistical analysis

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 from the patients' genotypes with Haploview 4.2. Linkage disequilibrium (LD)-based haplotype block definition was based on the D' confidence interval method.[21]

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.[63] 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. Haplotypes were evaluated assuming a dosage model (i.e., analogous to the additive model).

Ancestry informative markers (AIMs) were used to minimize confounding due to population stratification.[24; 27; 67] Homogeneity in ancestry among patients was verified by principal component analysis[51] 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). This procedure was repeated until no discernible clustering of patients by their self-reported race/ethnicity was possible (data not shown). 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 12.1.

As was done in our previous studies,[28; 44] based on recommendations in the literature, [26; 58] the implementation of rigorous quality controls for genomic data, the nonindependence 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 (Table 1) 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 Supplemental Table 1 to allow for subsequent comparisons and meta-analyses.

Results

Differences in demographic and clinical characteristics between pain classes

Of the 398 women who completed the presurgical assessment, 126 (31.7%) were classified into the no breast pain class and 46 (11.6%) were classified into the severe breast pain class. Differences in demographic and clinical characteristics among the four breast pain classes at the time of enrollment are described in detail elsewhere.[46] Differences in demographic and clinical characteristics between the no breast pain and severe breast pain classes are provided in Table 2.

Women who were classified into the severe breast pain class were significantly younger, had fewer years of education, and were more likely to have an annual household income below $20,000 than women in the no breast pain class. In terms of ethnicity, post-hoc analyses revealed that the representation of Whites was greater in the no breast pain class (73%) than in the severe breast pain class (41%) (Bonferroni-corrected p-value = 0.006).

In terms of preoperative clinical characteristics, women in the severe breast pain class reported a higher number of comorbidities (i.e., SCQ score), lower functional status (i.e., KPS score), and were more likely to have a history of depression, back pain, and rheumatoid arthritis than women in the no breast pain class. Forty-three percent of women in the severe breast pain class, compared to 2.4% in the no breast pain class, reported pain in the affected

breast prior to surgery. Women in the severe breast pain class were more likely to report swelling, numbness, strange sensations, and hardness in their affected breast prior to surgery compared to women in the no breast pain class.

Differences between the no breast pain and severe breast pain classes were found in a number of surgical and postoperative characteristics. Compared to the no breast pain class, women in the severe pain class had a greater number of lymph modes removed, reported higher average and worst postoperative pain scores, were more likely to have undergone an axillary lymph node dissection, and were more likely to have re-excision or mastectomy within 6 months after surgery.

Regression analysis for phenotypic characteristics

As shown in Table 3, the only predictors that remained significant in the final regression model of clinical, demographic, and surgical characteristics were the occurrence of pain in the breast prior to surgery and the severity of average postoperative pain. For patients who reported pain in their affected breast prior to surgery, the odds of being in the severe pain class increased 8.71-fold (95% confidence interval (CI): 1.14, 66.48; p=0.037). For each one-unit increase in the severity of average postoperative pain, the odds of being in the severe pain class increased 2.02-fold (95% confidence interval (CI): 1.43, 2.84; p<0.001).

Regression analyses for candidate genes

As summarized in Table 1, no associations were found between pain group membership and SNPs in IFNGR1, IL1B, IL2, IL6, IL8, IL17A, NFKB2, and TNFA. However, the genotype frequency was significantly different between the no breast pain and severe breast pain classes for 13 SNPs and 3 haplotypes among 7 genes (IFNG: 2 SNPs, 1 haplotype; IL1R1: 1 SNP; IL1R2: 1 SNP; IL4: 1 SNP; IL10: 3 SNPs, 1 haplotype; IL13: 4 SNPs, 1 haplotype; NFKB1: 1 SNP).

In order to better estimate the magnitude (i.e., odds ratio, OR) and precision (i.e., CI) of genotype on pain group membership, multivariate logistic regression models were fit. In addition to genotype, the phenotypic variables included in the regression models were genomic estimates of and self-reported race/ethnicity (i.e., White, Black, Asian, Hispanic/Mixed ethnic background/other), occurrence of pain in the affected breast prior to surgery, and severity of average postoperative pain. As shown in Table 4, the genetic associations that remained significant were for IL1R2 rs11674595 and IL10 haplotype A8 (see Table 4 and Figures 1 and 2).

In the regression analysis for IL1R2 rs11674595 (Figure 1), individuals who were homozygous for the rare “C” allele (i.e., TT+TC versus CC) had a 36.1-fold increase in the odds of belonging to the severe breast pain class (95% CI: 2.02, 643.37, p=0.015).

In the regression analysis for IL10 haplotype A8, each dose of this haplotype decreased the odds of belonging to the severe breast pain class by 79% (95% CI: 9%, 95%, p=0.037). The IL10 haplotype A8 is composed of seven SNPs (i.e., rs3024505 “C“ allele, rs3024498 “G“ allele, rs3024496 “C“ allele, rs1878672 “G“ allele, rs1518111 “A“ allele, rs1518110 “T“ allele, rs3024491 “T“ allele) (Figure 2).

Discussion

This study is the first to evaluate for associations between variations in cytokine genes and the development of persistent breast pain in women following breast cancer surgery. Consistent with previous reports (for review see Anderson and Kehlet[2]), differences in a number of demographic and clinical characteristics were found between the no pain and severe breast pain classes in the bivariate analyses. However, as shown in Table 3, pain in the breast prior to surgery and the severity of average postoperative pain were the only phenotypic characteristics that remained significant in the multivariate analysis. Only one study was found that reported pain in the breast prior to surgery as a risk factor for persistent pain in the breast following surgery.[39] Of note, compared to the no pain class (2.8±2.1), the mean average postoperative pain intensity score reported by patients in the severe pain class (6.5±2.2) represented not only a statistically significant ($p<0.001$), but a clinically meaningful difference ($d=1.53$) in pain intensity scores. While the postoperative pain scores for patients in the no pain class are in the moderate range, based on work by Dihle and colleagues,[17] average pain scores for patients in the severe pain class are in the severe range.

Several review articles[33; 52; 69] have concluded that severe postoperative pain is a well-established risk factor for the development of phantom breast pain and other neuropathic pain syndromes following breast cancer surgery. Findings from this study suggest that inadequately treated preoperative and postoperative pain are significant risks factor for the development of severe persistent breast pain in women following breast cancer surgery. One can hypothesize that sensitized and injured peripheral nerves produce intense and prolonged afferent ectopic activity that is transmitted to dorsal horn neurons in the central nervous system.[12] This excessive ectopic activity may alter the morphological and biochemical properties of the pre- and post-synaptic membranes and change the excitability of the dorsal horn neurons. Prolonged central sensitization leads to permanent alterations in the structures responsible for processing nociceptive stimuli.[9] Prolonged stimulation of peripheral nociceptors both preoperatively and postoperatively by pain of moderate to high intensity maintains a hyperexcited state in dorsal horn neurons.[37]

Pro- and anti-inflammatory cytokines are known to modulate nociceptive signaling during acute and chronic inflammation and following tissue injury and nerve lesions.[74] However, significant inter-individual variability exists in the development and resolution of postsurgical pain. In this study, one SNP and one haplotype in two cytokine genes were associated with pain group membership after adjusting for the occurrence of breast pain preoperatively and the severity of average postoperative pain.

Findings from this study suggest that the rare “C” allele of IL1R2 rs11674595 increases the risk for the development of severe persistent breast pain. To date, no associations were reported between any SNP in IL1R2 and a pain phenotype. IL1R2 rs11674595 is located in a non-coding though evolutionarily conserved region and its impact on IL-1R2 production is unknown. However, IL1R2 encodes for the IL-1 type II receptor that inhibits inflammatory signaling by titrating IL-1β away from binding to IL-1R1.[10] Upon binding to IL-1R1, IL-1β initiates signaling cascades that promote the production and subsequent release of

nitric oxide, bradykinin, and prostaglandins.[13; 18; 59] These mediators alter the biophysical properties and kinetics of ion channels and receptors present in neuronal membranes to augment nociceptor excitability.[3]

IL-10 reduces the bioavailability of proinflammatory cytokines by downregulating expression of IL-1, IL-6, and TNF alpha (α) in activated macrophages.[15; 20] Activated macrophages are the major source of proinflammatory cytokines in the periphery following tissue injury and inflammation. In addition, IL-10 alters the production of antagonists of proinflammatory cytokines by decreasing the expression of IL-1 receptors,[16] increasing the production of soluble TNFα receptors,[32] and preventing the degradation of IL-1ra mRNA.[6] The increased availability of an antagonist for the proinflammatory cytokines further attenuates proinflammatory cytokine signaling and dampens their positive feedback loops. Findings from this study suggest that the IL10 haplotype A8 decreases the risk for the development of severe persistent pain. To date, no associations were reported between any of the SNPs contained in IL10 haplotype A8 and a pain phenotype.

Although the SNPs that comprise this haplotype are located in introns or in the 3' untranslated region of IL10, the functional significance of some of these SNPs were evaluated previously.[53; 57; 73] These studies provide conflicting evidence for a potential effect of the IL10 haplotype A8 on IL10 expression. IL10 rs3024498 is located in the 3' untranslated region of IL10 and falls in a putative transcription factor binding site region. The rare G allele of IL10 rs3024498 was associated with elevated serum IL-10 levels in patients with tuberculosis.[1] IL10 rs1518111 is located in a non-coding region of IL10. The rare A allele of IL10 rs1518111 is associated with decreased mRNA expression of IL-10.[53] Further investigation is necessary to determine how SNPs contained within this haplotype alter IL-10 gene expression and protein production.

Study limitations need to be acknowledged. First, no direct measurements of serum cytokines were done to provide additional data on the mechanisms that underlie the development of persistent breast pain. Second, future studies with a larger sample size may increase the power to detect differences in other cytokine genes. Third, although rigorous quality control analyses and adjustment for potential confounding due to population substructure were performed, some of the relationships identified may be due to type 1 errors. Fourth, an optimal examination of IL4 could not be done due to the large number of SNPs that failed quality controls. Finally, a number of clinical characteristics identified in bivariate analyses may be significant predictors of severe persistent breast pain in larger samples.

In conclusion, our findings suggest that polymorphisms in cytokine genes play a role in the development of severe persistent breast pain in women following breast cancer surgery. The genes and SNPs found in this study may help to identify individuals who are predisposed to the development of persistent, postsurgical breast pain. Future studies are warranted to confirm our findings and to determine if these associations are present in other persistent postsurgical pain syndromes and to determine the mechanism(s) underlying these associations.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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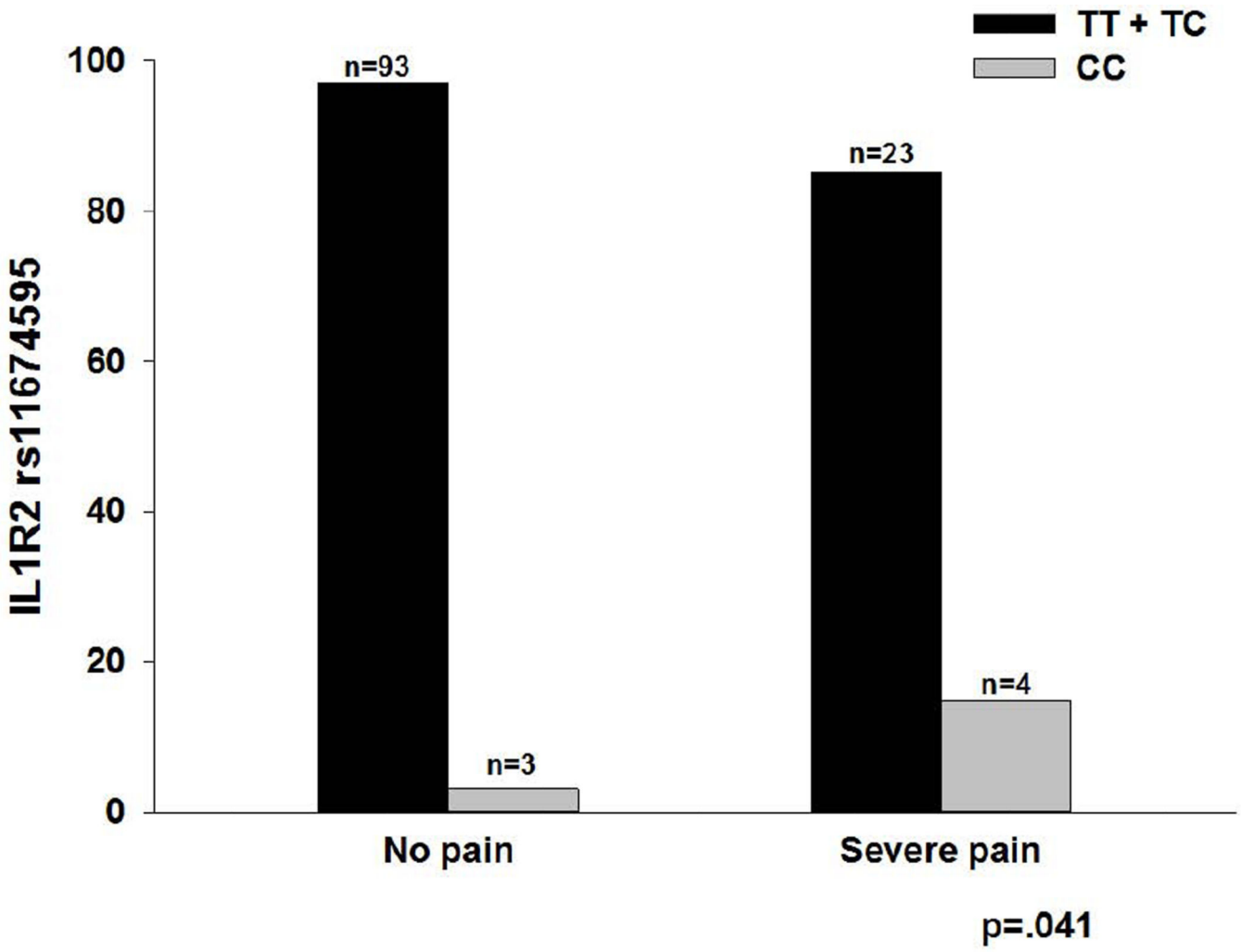
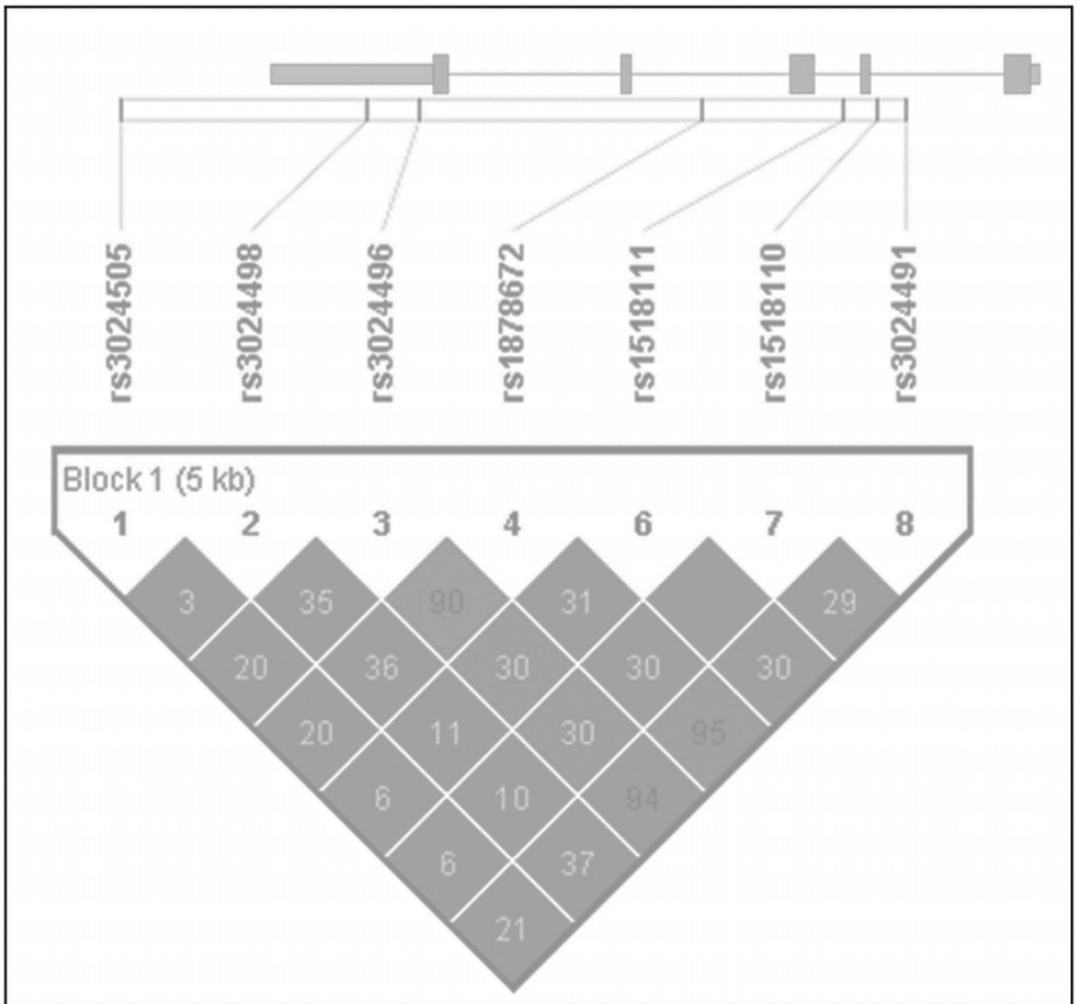


Figure 1.

Differences in the percentages of patients in the no breast pain and severe breast pain latent classes who were homozygous for the common allele or heterozygous (TT+TC) or homozygous for the rare allele (CC) for rs11674595 in IL1R2.



Haplotype	No Pain (n=142, s=284)	Severe Pain (n=32, s=64)
HapA1: CATCGGG	43 (30.3%)	12 (37.5%)
HapA2: CATCATG	32 (22.5%)	11 (34.4%)
HapA3: CATGATG	1 (<0.01%)	0 (0.0%)
HapA5: CACCATG	1 (<0.01%)	1 (0.01%)
HapA7: CACGATT	11 (8.7%)	2 (6.3%)
HapA8: CGCGATT #	33 (23.2%)	3 (9.4%)
HapA9: TACGATT	21 (14.8%)	3 (9.4%)

Figure 2.

IL10 linkage disequilibrium-based heatmap and haplotype analysis. In the figure embedded in the top row of the table, an ideogram of interleukin 10 (IL10) is presented above the white bar that represents the physical distance along human chromosome 1 (position 206,940,948 to 206,945,839; genome build 37.10, NG_012088.1). Exons are represented as boxes. Gray lines connecting the exons represent introns. The direction of transcription is from right to left. Reference sequence identifiers (rsID) for each single nucleotide polymorphism (SNP) are plotted both in terms of their physical distance (i.e., the white bar at the top of the figure)

and also equidistantly in order to render the pairwise linkage disequilibrium (LD) estimates that were calculated and visualized with Haploview 4.2. The gene structure for IL10 (i.e., hg18 NM_000572) was rendered with FancyGene 1.4. The correlation statistics (r^2 and D') are provided in the heatmap. LD-based haplotype block definition was based on D' confidence interval [11]. The haploblock is indicated in a bolded triangle and its component SNPs are rendered in bold font. Pairwise D' values (range: 0–1, inclusive) were rendered in shades of grey, with dark grey diamonds representing D' values approaching 1.0. When the r^2 values (range of 0–100, inclusive) are not equal to 0 or 100, they are provided in a given diamond. The haplotypes observed in the haploblock are listed in each row, starting with the nucleotide composition across the seven SNPs that compose the haplotype (i.e., rs3024505, rs3024498, rs3024496, rs1878672, rs1518111, rs1518110, rs3024491) and the count frequency (%) of each haplotype observed in the no breast pain and severe breast pain classes.

The haplotype (i.e. CGCGATT) identified in the bivariate analyses (Table 1) remained significant after controlling for relevant confounders.

n=number of individuals; s = number of alleles.

Table 1

Single nucleotide polymorphisms in cytokine genes with significant differences in the associations between the no breast pain and the severe breast pain classes

Gene	SNP	Position	Chr	MAF	Alleles	Chi square	p-value	Model
IFNG1	rs2069727	66834490	12	.384	A>G	FE	0.025	R
IFNG1	rs2069718	66836429	12	.494	C>T	10.09	0.006	A
IFNG1	HapA5					6.58	0.037	
IL1R1	rs3917332	96560387	2	.187	A>T	FE	0.037	D
IL1R2	rs11674595	96374804	2	.258	T>C	FE	0.041	R
IL4	rs2243248	127200946	5	.086	T>G	FE	0.033	D
IL10	rs3024498	177639855	1	.204	A>G	FE	0.015	D
IL10	rs1878672	177642039	1	.416	G>C	FE	0.029	D
IL10	rs3024491	177643372	1	.408	G>T	FE	0.035	D
IL10	HapA8					6.39	0.041	
IL13	rs1881457	127184713	5	.210	A>C	FE	0.043	D
IL13	rs1800925	127185113	5	.233	C>T	FE	0.007	D
IL13	rs1295686	127188147	5	.265	G>A	FE	0.014	D
IL13	rs20541	127188268	5	.212	C>T	7.81	0.017	A
IL13	HapA1					8.70	0.013	
NFKB1	rs4648141	103755947	4	.180	G>A	FE	0.041	R

Abbreviations: A = additive model; Chr = chromosome; D = dominant model; FE = Fisher's exact test; Hap = haplotype; IFNG = interferon gamma; IL = interleukin; MAF = minor allele frequency; NFKB = nuclear factor kappa beta; R = recessive model; SNP= single nucleotide polymorphism

Table 2

Differences in demographic and clinical characteristics between the breast pain classes prior to surgery

Demographic Characteristics	No Pain n=126 Mean (SD)	Severe Pain n=46 Mean (SD)	Statistics
Age (years)	58.6 (11.4)	52.4 (9.4)	t=3.30; p=0.001
Education (years)	15.8 (2.8)	14.3 (2.9)	t=2.99; p=0.003
	% (N)	% (N)	
Ethnicity			
White	73.0 (92)	41.3 (19)	
Black	7.1 (9)	21.7 (10)	X^2=16.03; p=0.001
Asian/Pacific Islander	10.3 (13)	21.7 (10)	
Hispanic/mixed ethnic background/other	9.5 (12)	15.2 (7)	
Lives alone	20.8 (26)	29.5 (13)	FE; p=0.298
Marital status			
Married/partnered	41.3 (52)	58.1 (25)	FE; p=0.076
Single/separated/widowed/divorced	58.7 (74)	41.9 (18)	
Currently working for pay	52.0 (65)	34.8 (16)	FE; p=0.057
Total annual household income			
<$10,000 to $19,999	8.5 (9)	39.5 (15)	
$20,000 to $99,000	48.1 (51)	44.7 (17)	Z=−4.26; p<0.001
≥$100,000	43.4 (46)	15.8 (6)	
Clinical Characteristics	Mean (SD)	Mean (SD)	
Body mass index (kg/m^2)	27.1 (7.0)	28.6 (6.3)	t=−1.29; p=0.197
Karnofsky Performance Status score	96.2 (8.7)	87.6 (14.9)	t=3.66; p=0.001
Self-administered Comorbidity Scale score	4.0 (2.3)	5.6 (3.2)	t=−3.01; p=0.004
Number of breast biopsies	1.4 (0.7)	1.6 (1.1)	Z=−1.80; p=0.072
	% (N)	% (N)	
Occurrence of comorbid conditions (% and number of women who reported each comorbid condition from the Self-Administered Comorbidity Questionnaire)			
Heart disease	4.0 (5)	2.2 (1)	FE; p=1.000
High blood pressure	34.9 (44)	45.7 (21)	FE; p=0.217
Lung disease	2.4 (3)	6.5 (3)	FE; p=0.344
Diabetes	7.1 (9)	17.4 (8)	FE; p=0.079
Ulcer	3.2 (4)	2.2 (1)	FE; p=1.000

Demographic Characteristics	No Pain n=126 Mean (SD)	Severe Pain n=46 Mean (SD)	Statistics
Kidney disease	1.6 (2)	0.0 (0)	FE; p=1.000
Liver disease	3.2 (4)	4.3 (2)	FE; p=0.659
Anemia	7.9 (10)	13.0 (6)	FE; p=0.374
Depression	16.7 (21)	34.8 (16)	FE; p=0.020
Osteoarthritis	17.5 (22)	19.6 (9)	FE; p=0.823
Back pain	22.2 (28)	41.3 (19)	FE; p=0.020
Rheumatoid arthritis	1.6 (2)	13.0 (6)	FE; p=0.005
Diagnosed with mastitis	11.2 (14)	7.0 (3)	FE; p=0.564
Diagnosed with fibrocystic disease	18.6 (22)	11.4 (5)	FE; p=0.347
Ever breast fed	48.0 (60)	41.3 (19)	FE; p=0.491
Surgery to affected breast unrelated to cancer	7.9 (10)	6.5 (3)	FE; p=1.000
Surgery to affected arm unrelated to the cancer			
Post-menopausal	71.0 (88)	62.8 (27)	FE; p=0.343
Received neoadjuvant chemotherapy	17.5 (22)	17.4 (8)	FE; p=1.000
On hormonal replacement therapy prior to surgery	19.0 (24)	6.7 (3)	FE; p=0.058
Stage of disease			
Stage 0	17.5 (22)	13.0 (6)	
Stage 1	41.3 (52)	34.8 (16)	Z=−1.50; p=0.1334
Stage IIA and IIB	35.7 (45)	39.1 (18)	
Stage IIIA, IIIB, IIIC, and IV	5.6 (7)	13.0 (6)	
Pain in breast prior to surgery	2.4 (3)	43.2 (19)	FE; p<0.001
Swelling in affected breast	3.2 (4)	23.9 (11)	FE; p<0.001
Numbness in affected breast	2.4 (3)	17.4 (8)	FE; p=0.001
Strange sensations in affected breast	12.7 (16)	28.3 (13)	FE; p=0.022
Hardness in affected breast	7.9 (10)	30.4 (14)	FE; p=0.001
Surgical Characteristics	Mean (SD)	Mean (SD)	
Number of lymph nodes removed	4.3 (4.7)	8.0 (9.0)	t=−2.61; p=0.012
Number of drains placed during surgery	0.4 (0.7)	0.5 (0.6)	t=−0.92; p=0.359
	% (N)	% (N)	
Type of surgery			

Demographic Characteristics	No Pain n=126 Mean (SD)	Severe Pain n=46 Mean (SD)	Statistics
Breast conservation	84.1 (106)	82.6 (38)	FE; p=0.818
Mastectomy	15.9 (20)	17.4 (8)	
Sentinel lymph node biopsy	84.1 (106)	71.7 (33)	FE; p=0.081
Axillary lymph node dissection	29.4 (37)	52.2 (24)	FE; p=0.007
Intercostobrachial nerve sacrificed	0.8 (1)	4.3 (2)	X^2=2.481; p=0.289
Placement of surgical drain			
No drain	69.0 (87)	56.5 (26)	X^2=6.941; p=0.074
Only in the breast	14.3 (18)	8.7 (4)	
Only in the axilla	12.7(16)	28.3 (13)	
Both in the breast and axilla	4.0 (5)	6.5 (3)	
Reconstruction at the time of surgery	15.9 (20)	13.0 (6)	FE; p=0.811
Postoperative Characteristics	Mean (SD)	Mean (SD)	
Number of postoperative complications	0.2 (0.5)	0.3 (0.6)	t=−1.17; p=0.246
Severity of average postoperative pain	2.8 (2.1)	6.5 (2.2)	t=−9.66; p<0.001
Severity of worst postoperative pain	4.2 (2.6)	7.9 (2.5)	t=−8.13; p<0.001
	% (N)	% (N)	
Received radiation therapy during the 6 months	56.3 (71)	41.3 (19)	FE; p=0.087
Received adjuvant chemotherapy during the 6 months	31.7 (40)	39.1 (18)	FE; p=0.369
Received hormonal therapy during the 6 months	45.2 (57)	30.4 (14)	FE; p=0.115
Received biological therapy during the 6 months	8.7 (11)	6.5 (3)	FE; p=0.762
Received complementary therapy during the 6 months	23.8 (30)	23.9 (11)	FE; p=1.000
Received physical therapy during the 6 months	9.5 (12)	19.6 (9)	FE; p=0.111
Had breast reconstruction during the 6 months	4.8 (6)	2.2 (1)	FE; p=0.676
Had re-excision or mastectomy during the 6 months	18.3 (23)	39.1 (18)	FE; p=0.008

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

Table 3

Final multiple logistic regression model with phenotypic characteristics that predicted pain class membership

GMM Class Comparison	Predictor	Odds Ratio	Standard Error	95% CI	Z	p-value
	Self-reported race/ethnicity					
	White versus Black	0.01	0.02	0.00, 0.95	−1.98	0.047
	White versus Asian/Pacific Islander	0.08	0.16	0.00, 3.40	−1.31	0.189
	White versus Hispanic/mixed/other	0.23	0.32	0.01, 3.68	−1.04	0.298
	Pain in the breast prior to surgery	8.71	9.03	1.14, 66.48	2.09	0.037
	Severity of average postoperative pain	2.02	0.35	1.43, 2.84	4.01	<0.0001
	Overall model fit: $\chi^2 = 47.38$; $p < 0.0001$; $R^2 = 0.4006$					

Abbreviations: CI = confidence interval; GMM = growth mixture model

Table 4

Multiple logistic regression analyses for IL1R2 and IL10 candidate gene markers

GMM Class Comparison	Predictor	Odds Ratio	Standard Error	95% CI	Z	p-value
No breast pain versus severe breast pain (n=116)	IL1R2 genotype	36.07	53.03	2.02, 643.37	2.44	0.015
	Pain in the breast prior to surgery	10.40	11.61	1.17, 92.75	2.10	0.036
	Severity of average postoperative pain	2.15	0.40	1.48, 3.10	4.05	<0.001
	Overall model fit: $\chi^2 = 54.64$, $p < 0.0001$; $R^2 = 0.4619$					
No breast pain versus severe breast pain (n=116)	IL10 Haplotype A8	0.21	0.16	0.05, 0.91	−2.08	0.037
	Pain in the breast prior to surgery	12.63	14.70	1.29, 123.61	2.18	0.029
	Severity of average postoperative pain	2.03	0.37	1.42, 2.91	3.88	<0.001
	Overall model fit: $\chi^2 = 52.82$, $p < 0.0001$; $R^2 = 0.4466$					

Notes: Multiple logistic regression analysis of the no pain and the severe pain GMM classes based on rating of worst breast pain. For each model, the first three principal components identified from the analysis of ancestry informative markers as well as self-report race/ethnicity were retained in all models to adjust for potential confounding due to race or ethnicity (data not shown). Predictors evaluated in each model included genotype (IL1R2 rs11674595: TT + TC versus CC; IL10 haplotype A8 composed of rs3024505- rs3024498-rs3024496-rs1878672-rs1518111-rs1518110-rs3024491: zero, one, or two doses of the C-G-C-G-A-T-T haplotype), occurrence of pain in the breast prior to surgery, and severity of average postoperative pain.

Abbreviations: CI = confidence interval; GMM = growth mixture model; IL1R2 = interleukin 1 receptor, type 2; IL10 = interleukin 10