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Associations between genetic and epigenetic variations in cytokine genes and mild persistent breast pain in women following breast cancer surgery

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ABSTRACT

Persistent pain following breast cancer surgery is a significant problem. Both inherited and acquired mechanisms of inflammation appear to play a role in the development and maintenance of persistent pain. In this longitudinal study, growth mixture modeling was used to identify persistent breast pain phenotypes based on pain assessments obtained prior to and monthly for 6 months following breast cancer surgery. Associations between the "no pain" and "mild pain" phenotypes and single nucleotide polymorphisms (SNPs) spanning 15 cytokine genes were evaluated. The methylation status of the CpG sites found in the promoters of genes associated with pain group membership was determined using bisulfite sequencing. In the multivariate analysis, three SNPs (i.e., interleukin 6 (*IL6*) rs2069840, C-X-C motif chemokine ligand 8 (*CXCL8*) rs4073, tumor necrosis factor (*TNF*) rs1800610) and two TNF CpG sites (i.e., c.−350C, c.−344C) were associated with pain group membership. These findings suggest that variations in *IL6*, *CXCL8*, and *TNF* are associated with the development and maintenance of mild persistent breast pain. CpG methylation within the *TNF* promoter may provide an additional mechanism through which TNF alters the risk for mild persistent breast pain after breast cancer surgery. These genetic and epigenetic variations may help to identify individuals who are predisposed to the development of mild levels of persistent breast pain following breast cancer surgery.

1. Introduction

Persistent pain in women following breast cancer surgery is common and associated with altered mood and sleep patterns, decreased quality of life, and disability [1,2]. Persistent postsurgical pain may result from ongoing nociceptor activation and/or nerve injury [3]. During the early postoperative period, inflammatory mediators produce sensitization at the affected area. These reversible changes in sensitivity discourage stimulation which serves as a protective mechanism that facilitates healing. Sustained activation of nociceptors may lead to maintenance of central sensitization and maladaptive phenotypic changes that alter the normal stimulus-response relationship and produce persistent pain [4].

Persistent alterations within nociceptors include changes in gene expression [5]. Evidence suggests that ongoing activation of inflammatory cells plays a role in the establishment of persistent pain [6]. In addition, peripheral nerve injury elicits an inflammatory reaction that prompts the aggregation of immune cells and increases the local concentration of pro-inflammatory cytokines [7]. These mediators participate in the initiation and maintenance of persistent pain after nerve injury by generating ectopic activity [8], altering neuronal connectivity [9], and reducing the number of inhibitory neurons [10].

Despite a clear connection between immune mechanisms and persistent pain [7], few studies were identified that evaluated for associations between polymorphisms in cytokine pathways and cancer-related pain [11–14]. Findings from these studies are difficult to interpret because pain was characterized using a dichotomized rating, the samples were small, and the number of polymorphisms evaluated was not comprehensive.

Emerging evidence suggests that acquired adaptations to genetic regulation (termed "epigenetics") are pervasive in biology [15]. Deoxyribonucleic acid (DNA) methylation is an epigenetic mechanism that regulates gene expression [15]. Acquisition of methylation at CpG dinucleotides provides an adaptive capacity for the organism to adjust to

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sustained changes in its environment. The methylation status of CpG sites within gene promoters has emerged as a promising biomarker for risk stratification and detection of human disease [16].

Recent work from our group used growth mixture modeling (GMM) to identify subgroups of women with distinct persistent breast pain trajectories prior to and for six months following breast cancer surgery [17]. Three distinct classes were identified using patients' ratings of worst pain in their breast. A fourth pain group was designated for those women who did not experience breast pain. The largest subgroup of women identified was the mild breast pain class (n = 173, 43.5%) who had a mean worst pain severity score of 2.5 (on a 0–10 numeric rating scale (NRS)). Of note, mild levels of persistent postsurgical pain are associated with diminished perceptions of overall health and reduced physical and social functioning [18]. Therefore, using data from women who were classified into the no breast pain and mild breast pain classes, the purposes of this study were to: (1) evaluate for associations between single nucleotide polymorphisms (SNPs) contained within cytokine genes and pain group membership and (2) determine the methylation status of CpG sites contained within the promoter of cytokine genes that harbored gene variations associated with pain group membership.

2. Methods

2.1. Patients and settings

This longitudinal study is part of a larger study of women who underwent breast cancer surgery [11,17,19]. Patients were recruited from a Comprehensive Cancer Center, two public hospitals, and four community practices. Patients were included if they: were female; ≥18 years of age; underwent unilateral breast cancer surgery; were able to read, write, and understand English; and gave written informed consent. Patients were excluded if they had bilateral breast cancer surgery and/or had distant metastasis. Of the 516 patients approached, 410 were enrolled in the study (response rate 79.4%). The major reasons for refusal were: being too busy, overwhelmed with the cancer diagnosis, or insufficient time available to do the baseline assessment prior to surgery.

2.2. Subjective measures

The Breast Symptoms Questionnaire (BSQ) and Post Surgical Pain Questionnaire, evaluated persistent and acute postoperative pain, respectively. Part 1 of the BSQ obtained information on the occurrence of pain and the occurrence of other symptoms in the breast scar area. Additional symptoms were based on the work of Tasmuth and colleagues [20,21]. Patients completed Part 2 of the BSQ if they had pain in the breast scar area. Patients rated the intensity of their average and worst pain using a 0 (no pain) to 10 (worst imaginable pain) NRS. The NRS is a valid and reliable measure of pain intensity [22].

Postsurgical Pain Questionnaire evaluated pain intensity, pain relief, and satisfaction with pain treatment in the first 24–48 h after surgery. Average and worst pain intensity were rated on a 0 (no pain) to 10 (worst imaginable pain) NRS. Pain relief was rated on a 0% (no relief) to 100% (complete relief) scale. Satisfaction with pain treatment was rated on a 0 (not satisfied at all) to 10 (extremely satisfied) NRS. Patients completed this questionnaire during the month 1 study visit.

2.3. Study procedures

The Committee on Human Research at the University of California, San Francisco and the Institutional Review Boards at our other sites approved this study. During the patient's preoperative visit, a clinician explained the study to the patient and determined her willingness to participate. For women who were willing to participate, clinicians introduced the patient to the research nurse. The research nurse determined the woman's eligibility, obtained written informed consent prior to surgery, had the patients complete the enrollment questionnaires (Assessment 0).

Patients were contacted two weeks after surgery to schedule the first post-surgical appointment. Patients were seen 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 visits, the women completed the study questionnaires and provided information on new and ongoing treatments. The blood sample was collected at the time of enrollment or during one of the monthly study visits. Patients' medical records were reviewed for disease and treatment information.

2.4. Characterization of the persistent breast pain phenotype

The characterization of the persistent breast pain phenotype used in the current study was described previously [17]. At each assessment, patients were asked, "Are you experiencing pain in your affected breast?" If the patient reported pain, she rated her "current pain at its worst" using a 0 (no pain) to 10 (worst pain) NRS. Prior to performing the GMM analysis, 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%). These patients were not included in the GMM analysis. The remaining 272 women's ratings of worst breast pain were used in the GMM analysis. GMM was used to assign each individual into a latent class based on similarities in worst pain ratings at enrollment and at 2, 3, 4, 5, and 6 months after surgery. Pain ratings obtained at the 1-month follow-up assessment were excluded from the model because it reduced the variability in pain ratings among the patients. Attempts to determine the latent classes failed when the month 1 ratings were included in the GMM analysis.

The GMM methods are described in detail elsewhere [23]. In brief, a single growth curve that represented the "average" change trajectory was estimated for the total sample. Then the number of latent growth classes that best fit the data was identified using established guidelines [24–26]. Descriptive statistics and frequency distributions for the no breast pain and mild 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, and Chi square and Fisher's Exact tests were used to evaluate for differences in demographic and clinical characteristics between the two breast pain classes. Adjustments were not made for missing data in comparisons between the GMM classes. Therefore, the cohort for each analysis was dependent on the largest set of available data across groups. A p-value of < 0.05 was considered statistically significant.

Logistic regression analysis was performed to evaluate for associations between phenotypic characteristics and pain group membership. Based on a review of the literature, all phenotypic characteristics that were identified in the bivariate analyses as being significantly different between the pain classes were evaluated for inclusion in the multivariate analysis. A backwards stepwise approach was used to create the most parsimonious model. Only predictors with a p-value of < 0.05 were retained in the final model. These same predictors were used in the models that evaluated the associations between genotype and pain group membership and CpG methylation level and pain group membership.

2.5. Genotype determination

Genomic 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/microliter (ng/μL) (diluted in 10 mM Tris/1 mM EDTA). A combination of tagging SNPs and literature driven SNPs (i.e., reported as being associated with altered function and/or symptoms) were chosen for this study. Tagging SNPs needed to be common (i.e.,