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Evidence of an Association Between Pro- and Anti-Inflammatory Cytokine Genes and Pain in Women Prior to Breast Cancer Surgery

by

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Evidence of an Association Between Pro- and Anti-Inflammatory Cytokine Genes and Pain in Women Prior to Breast Cancer Surgery

Birha McCann

Abstract

The purposes of this study were to determine the occurrence rate for preoperative breast pain; describe the characteristics of this pain; evaluate for differences in demographic and clinical characteristics; and evaluate for variations in pro- and anti-inflammatory cytokine genes between women who did and did not report pain. Patients (n=398) were recruited prior to surgery and completed self-report questionnaires to obtain information on pain characteristics. Genotyping was done using the Golden Gate genotyping platform. Of note, 28.2% of the patients reported pain prior to surgery. Women who reported pain were significantly younger ($p < 0.001$), a higher percentage were non-white ($p= 0.018$), reported significantly lower Karnofsky Performance Status scores ($p = 0.008$), were less likely to have gone through menopause ($p = 0.012$), and had had significantly more biopsies ($p=0.006$). Carriers of the minor allele for a single nucleotide polymorphism (SNP) in IL1R1 (rs2110726; $p = 0.007$) were less likely to report breast pain prior to surgery. In contrast carriers of the minor allele for a SNP in IL13 (rs1295686; $p= 0.019$) were more likely to report breast pain prior to surgery. Findings from this study suggest that pain is a problem for over a quarter of women who are about to undergo breast cancer surgery. Based on the characteristics of the pain and the increased number of biopsies in women who reported pain, as well as the genetic associations found, this pain problem has an inflammatory component.

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INTRODUCTION

Acute pain following surgery for breast cancer and chronic pain associated with breast cancer and its treatment are common problems in women with breast cancer.[1,3,18,23,26,28,43,60-62] However, only five papers were found that described breast prior to surgery.[14,43,60-62] In a paper published in 1952,[14] Corry suggests that "the occurrence of pain in operable cases of carcinoma of the breast is well known to surgeons" and that the occurrence of pain ranged from 13.8% to 45%. In one of the early studies of chronic pain after breast cancer surgery,[62] 30% of the 93 patients surveyed reported preoperative pain in their affected breast. This pain had occurred for approximately 2 months prior to surgery. Pain intensity scores ranged from 0.6 to 6.9 on a 0 to 10 centimeter analog rating scale (ARS). Ten percent of these ninety-three women reported pain in both the affected breast and ipsilateral arm prior to surgery. Activities that aggravated the preoperative pain included reaching out, doing housework, driving a car, and sleeping on the affected side. In a second article that reported retrospective data from the same sample,[60] patients who reported pain in the ipsilateral arm prior to surgery recalled higher levels of postoperative pain compared to patients without preoperative pain. In the most recent study of risk factors for chronic pain following breast cancer surgery,[43] 28% of patients (n=93) reported preoperative breast pain. Preoperative pain status was not associated with the development of chronic pain following breast cancer surgery. No additional information was provided on pain characteristics. Taken together, these findings suggest that over a quarter of women who are about to undergo surgery for breast cancer experience breast pain. More detailed characterization of this pain is needed given its relatively high occurrence rate.

A potential cause for breast pain prior to surgery is the occurrence of inflammation around the site of the tumor or inflammation associated with tissue injury following breast biopsies. Both localized and systemic inflammation is associated with the growth and development of cancer.[24,25,40] Tissue injury following a biopsy can lead to inflammatory processes that result in swelling, redness, and pain. As noted in a number of recent reviews on pain mechanisms,[47,52,64] acute tissue injury causes the release of a number of inflammatory mediators including pro-inflammatory cytokines (e.g., interleukin 1 (IL1), and tumor necrosis factor alpha (TNF-α)).[4,58] The release of these cytokines, as well as a number of other neurotransmitters, results in inflammatory pain.

In addition, recent evidence suggests that variations in a number of genes in inflammatory pathways (e.g., cyclooxygenase 2,[51] TNFα,[48,50,51] nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, alpha (NFKBIA),[51] IL1,[4.49] IL8,[49-50] and IL16[22]) are associated with increases in acute[4,74] and cancer[48-51] pain. For example, in a study of patients with pancreatic cancer,[49] a polymorphism in the promoter region of IL8 (rs4073), which is known to correlate with increased IL8 production, was associated with higher pain intensity scores. A polymorphism in IL16 (rs4778889) was associated with pain in patients with endometriosis.[22] Additionally, inhibition of IL1, either directly through an antagonist, or through IL1R gene deletion, decreased algesia in mice.[63,74] Collectively, findings from these studies suggest that cytokine polymorphisms are associated with a variety of pain mechanisms in animals and humans.

Given the paucity of research on breast pain in women prior to breast cancer surgery and emerging evidence that cytokine gene polymorphisms may be associated with acute pain, the purposes of this study, in a sample of women who were to undergo surgery for

breast cancer were to: determine the occurrence rate for preoperative breast pain; describe the characteristics of this pain; evaluate for differences in demographic and clinical characteristics between women who did and did not report pain prior to surgery; and evaluate for variations in pro- and anti-inflammatory cytokine genes between the two pain groups.

MATERIALS AND METHODS

Patients and Settings

This descriptive cross-sectional study is part of a larger study that evaluated for neuropathic pain and lymphedema in women who underwent breast cancer surgery. 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 underwent 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, 410 were enrolled in the study (response rate 79.4%), and 398 completed the baseline assessment. The major reasons for refusal were: too busy, overwhelmed with the cancer diagnosis, or insufficient time available to do the baseline assessment prior to surgery.

Instruments

The demographic questionnaire obtained information on age, gender, marital status, education, ethnicity, employment status, living situation, and financial status.

Karnofsky Performance Status (KPS) scale is widely used to evaluate functional status in patients with cancer and has well established validity and reliability.[33-34] 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).

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.[55] 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 two 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.[5,11]

Breast Symptoms Questionnaire (BSQ), which consists of three parts, was used to obtain information on a number of pain characteristics. Part 1 obtained information on the prevalence, frequency, severity, and distress of symptoms in the breast (i.e., pain, swelling, numbness, strange sensations, hardness) prior to surgery. The symptoms that were assessed by Part 1 of the BSQ were identified in studies by

Tasmuth and colleagues.[61-62] The assessment of these symptoms is based on the format used in the Memorial Symptom Assessment Scale (MSAS).[44-45] Frequency of occurrence of the symptom, if present, was rated using a 1 to 4 scale (1= rarely to 4 = constantly). Severity was rated on a 1 to 4 scale (1=slight to 4=very severe). Distress was rated on a 0 to 4 scale (0=not at all to 4=very much). Prevalence rates for each symptom were determined using the responses in the "did not have" and frequency portions of the symptom assessment scale. Adaptations of the MSAS were used in previous studies.[35-36]

If the patient had pain in the breast, they completed Part 2 of the BSQ. Patients were asked to rate the intensity of their pain (i.e., pain right now and average and worst pain) using a NRS that ranged from 0 (no pain) to 10 (worst imaginable pain). Numeric rating scales are valid and reliable measures of pain intensity.[30]

Patients who completed Part 2 were asked to complete Part 3. With Part 3 of the BSQ, patients rated the level of interference caused by breast pain with sixteen activities using a 0 (does not interfere) to 10 (completely interferes) NRS. This interference scale is an adaptation of the interference scale from the Wisconsin Brief Pain Inventory (BPI).[15] This interference scale is a valid and reliable measure that has been used to evaluate the extent to which a person's pain interferes with their ability to function.[12,56] In addition to the original eight items on the interference scale of the BPI (i.e., general activity, mood, walking ability, normal work, relations with other people, sleep, enjoyment of life, sexual activity), the eight additional activities that were evaluated were those that were evaluated in the studies by Tasmuth and colleagues[61-62] (i.e., ability to sleep on the

operated side, touch, ability to reach out, ability to carry things, ability to get up from bed, ability to do handicrafts, ability to drive a car, ability to write).

Pain Qualities Assessment Scale (PQAS)[31,69] is an adaptation of the Neuropathic Pain Scale developed by Galer and Jensen[21] that consists of 20 items. The first 18 items are measured with NRSs that evaluate the magnitude of the different pain qualities (e.g., sharp, hot, aching, cold). The last two questions ask for an estimate of the intensity of deep pain and surface pain. Scores for individual pain qualities are reported and a mean score across the 20 items was calculated. In addition, three subscale scores were calculated (i.e., surface pain, paroxysmal pain, deep pain).[69] The PQAS has well-established validity and reliability.[31,69]

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 clinical staff member explained the study to the patient and determined her willingness to participate. For those women who were willing to participate, the staff member 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 consent, patients completed the baseline study questionnaires. Medical records were reviewed for disease and treatment information.

Genomic analyses

Gene selection - Cytokines and their receptors are classes of polypeptides that exercise a major influence on the inflammatory process.[68] Their dysregulation is associated with increased inflammatory responses in acute pain[68,70-73] and in a variety of

chronic medical conditions.[2,9,22,42,53,58,65] These polypeptides are divided into pro- and anti-inflammatory cytokines. Pro-inflammatory cytokines promote systemic inflammation and include: IL1R1, IL2, IL8, IL17A, and TNFα.[57,68] Anti-inflammatory cytokines suppress the activity of pro-inflammatory cytokines and include: IL1RII, IL4, IL10, and IL13.[57,68] Of note, IL1β and IL6 possess pro- and anti-inflammatory functions.[57]

Blood collection and genotyping - Genomic DNA was extracted from archived buffy coats maintained by the UCSF Genomic Markers of Symptoms Tissue Bank using the PUREGene DNA Isolation System (Invitrogen, Carlsbad, CA). Of the 398 who completed the baseline assessment, 302 provided a blood sample from which DNA could be recovered from the archived buffy coats. No differences were found in any demographic and clinical characteristics between patients who did and did not choose to participate in the study or in those participants who did and did not provide a blood sample for genomic analyses. Genotyping was performed blinded to clinical status and positive and negative controls were included. DNA samples were quantitated with a Nanodrop Spectrophotometer (ND-1000) 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 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 SNP. Disagreements were adjudicated by a third reviewer. If consensus could not be reached, the SNP was excluded.

SNP selection - 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 (defined as having a minor allele frequency ≥ 0.05) in public databases (e.g., HapMap). In order to ensure robust genetic association analyses, quality control filtering of SNPs was performed. SNPs with call rates <95%, or Hardy-Weinberg $p<0.001$ were excluded. As shown in Table 1, a total of 81 SNPs among the 11 candidate genes (IL1B: 12 SNPs; IL1R1: 5 SNPs; IL1R2: 3 SNPs; IL2: 5 SNPs; IL4: 9 SNPs; IL6: 14 SNPs; IL8: 3 SNPs; IL10: 8 SNPs; IL13: 5 SNPs; IL17A: 6 SNPs; TNFA: 11 SNPs) passed all quality control filters and were included in the genetic association analyses. Potential functional roles of SNPs associated with specific symptoms were examined using PUPASuite 2.0,[13] a comprehensive search engine that tests a series of functional effects (i.e., non-synonymous changes, altered transcription factor binding sites, exonic splicing enhancing or silencing, splice site alterations, microRNA target alterations).

Statistical Analyses for the Phenotypic Data

Data were analyzed using SPSS version 18 (SPSS, Chicago, IL) and STATA Version 9 (STATA Corp). Descriptive statistics and frequency distributions were generated for sample characteristics. Independent sample t-tests (for continuous variables), Mann-Whitney U test (for continuous variables not normally distributed), and Chi square analyses (for categorical variables) were used to evaluate for differences in demographic and clinical characteristics between patients who did and did not report breast pain prior to surgery. All calculations used actual values. Adjustments were not

made for missing data. Therefore, the cohort for each analysis was dependent on the largest set of available data between groups.

Statistical Analyses for the Genetic Data

Allele and genotype frequencies were determined by gene counting. Hardy-Weinberg equilibrium was assessed by the Chi-square exact test. Measures of linkage disequilibrium (i.e., D' and r^2) were computed from the participants' genotypes with Haploview 4.1 (http://www.broad.mit.edu/mpg/haploview/). Linkage disequilibrium (LD)-based haplotype block definition was based on D' confidence interval.[20]

For SNPs that were members of the same haploblock (i.e., IL1R1, IL13), 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.[59] In order to improve the stability of haplotype inference, the haplotype construction procedure was repeated 5 times using different seed numbers with each cycle. Only haplotypes that were inferred with probability estimates of ≥ 0.9 were retained for downstream analyses. Haplotypes with frequency estimates of $\leq 1\%$ were grouped into a single category.

For association tests, three genetic models were assessed for each SNP: additive, dominant, and recessive. Barring trivial improvements (delta<10%), the genetic model that best fit the data, by maximizing the significance of the p-value was selected for each SNP. Both un-adjusted and adjusted associations were calculated. Logistic regression was used to control for covariates (i.e., age in years, ethnicity (i.e., white versus nonwhite), functional status (i.e., KPS score), menopausal status, report of breastfeeding, and

number of biopsies). Genetic model fit and both unadjusted and adjusted odds ratios were estimated using the STATA software package, version 9 (STATA Corp).

RESULTS

Differences in demographic and clinical characteristics between the pain groups

Of the 398 who completed the baseline assessment, 390 (98%) completed the BSQ at enrollment. One hundred and ten women (28.2%) reported pain in their breast prior to surgery. As shown in Table 2, no between group differences were found in education, marital status, or living arrangements. However, women who reported pain were significantly younger ($p < 0.001$) and a higher percentage of them were non-white ($p= 0.018$). In terms of clinical characteristics (Table 2), women in the pain group reported significantly lower KPS scores ($p = 0.008$); were less likely to have gone through menopause ($p = 0.012$), and had had significantly more biopsies (Mann Whitney $U = 12887.0$; $p=0.006$).

Pain Characteristics

As illustrated in Figure 1, women reported an average pain intensity score of 2.23 (standard deviation (SD) = 2.12) and a worst pain intensity score of 3.58 (SD=2.39). Women reported significant amounts of pain (i.e., pain that interfered with their mood or function) for an average of 6.16 (SD=7.90) hours per day, on an average of 2.86 (SD=2.75) days per week.

Patients' ratings of pain interference with routine activities and specific upper extremity functions are illustrated in Figure 2. Interference ratings ranged from 2.37 (SD=2.91) (for mood) to 0.51 (SD=1.60) (for ability to write). The mean interference score was 1.67 (SD=2.23). Patients' ratings on the PQAS are summarized in Table 3. The

five descriptors with the highest ratings were tender, intense, dull, unpleasant, and aching.

Patients with and without pain completed Part 1 of the BSQ. As shown in Figure 3, a significantly higher percentage of women with breast pain prior to surgery reported swelling (20.0% versus 3.9%), numbness (15.5% versus 0.7%), strange sensations (55.5% versus 15.4%), and hardness (36.4% versus 12.1%; all $p<0.0001$) in their affected breast.

Candidate Gene Analysis for the Occurrence of Pre-Operative Breast Pain

Tag SNPs in the genes for IL1β, IL1R1, IL1R2, IL2, IL4, IL6, IL8, IL10, IL13, IL17A, and TNF-α were chosen for analysis. Of those SNPs chosen, all had minor allele frequencies (MAF) that met Hardy-Weinberg expectations with the exception of one each in IL2 and IL10, two in IL6, and seven in IL4. Because these SNPs did not meet this quality control criterion, they were not utilized in subsequent analyses. Statistically significant differences in minor allele distribution between the pain and no pain groups were found for rs2110726 ($p = 0.007$) in IL1R1 and rs1295686 ($p= 0.019$) in IL13. While they did not reach the pre-specified level of significance, some SNPs had p-values that approached significance: rs2069777 ($p= 0.07$) in IL2, rs2069840 ($p= 0.08$) in IL6, rs1800925 ($p=0.08$) in IL13, and rs4711998 ($p= 0.08$) in IL17A.

Of note, the observation that 6 of the 8 tag SNPs selected to measure the common variability at the IL4 gene locus failed to meet Hardy-Weinberg expectations (i.e., rs2243250, rs2070874, rs2227284, rs2227282, rs2243266, rs2243267, rs2243274) suggested that the allele frequencies in these SNPs might vary among the major ethnic groups found in our sample (i.e., White, African American, Asian). In fact, the minor

allele frequencies of all 6 of these SNPs did vary among the ethnic groups (data not shown). However, no evidence of association was found between these IL4 SNPs and the occurrence of pre-operative pain within or across the population subgroups.

Statistically significant differences in the presence of pain were found for SNPs within the genes for IL1R1, IL4, and IL13. Following multivariate regression analyses that controlled for age in years, ethnicity (i.e., white versus nonwhite), functional status (i.e., KPS score), menopausal status, report of breastfeeding, and number of biopsies, differences in pain remained significant for two of these SNPs, namely rs2110726 in IL1R1 ($p= 0.007$) and rs1295686 in IL13 ($p= 0.019$).

As shown in Figure 4, in the model fitted for pain prior to surgery for IL1R1 (rs2110726), genotype and age were the only predictors retained in the final model ($p<0.0001$). After controlling for age, carriers of the minor allele (i.e., CT + TT) had a 51% decrease in the odds of reporting pain prior to surgery (95% CI: 16.2%, 70.7%, $p=0.009$). After controlling for IL1R1 genotype, for every 5-year increase in age, the odds of reporting pain prior to surgery decreased by 22% (95% CI: 10.8%, 30.6%, $p<0.001$). In the model for IL1R1, age and genotype accounted for only 6.5% of the variance in the odds of reporting pain prior to surgery. Of note, the association between report of pain prior to surgery and the IL1R1 two-SNP haplotype (i.e., rs2110726, rs3917332) was collinear with the association observed with rs2110726.

As shown in Figure 5, in the model fitted for pain prior to surgery for IL13 (rs1295686), genotype and age were the only predictors retained in the final model ($p<0.0001$). After controlling for age, each dose of the minor allele was associated with a 70% increase in the odds of reporting pain prior to surgery (95% CI: 1.170, 2.494,

p=0.006). After controlling for IL13 genotype, for every 5-year increase in age, the odds of reporting pain prior to surgery decreased by 11% (95% CI: 11.3%, 31.1%, p<0.001). In the model for IL13, age and genotype only accounted for 6.6% of the variance in odds of reporting pain prior to surgery. Of note, the association between report of pain prior to surgery and the IL13 two-SNP haplotype (i.e., rs1295686, rs20541) was collinear with the association observed with rs1295686.

DISCUSSION

This study is the first to describe the characteristics of preoperative breast pain in a sample of women prior to breast cancer surgery and to evaluate for genetic variations in pro- and anti- inflammatory genes in women who did and did not report pain. Consistent with previous studies,[14,43,60-62] over one quarter of these patients experienced pain prior to surgery. This number is not insignificant given that in 2010 an estimated 208,000 new cases of breast cancer were diagnosed in the United States.[29] While the worst pain scores were in the mild to moderate range, a large amount of inter-individual variability was noted in this sample. In fact, 36.7% of the women who reported pain reported a worst pain score of ≥ 4. In addition, women with pain reported that pain interfered with their activities or mood on approximately 3 days per week for about 6 hours per day. In terms of level of interference (Figure 2), this pain had the largest effect on patients' mood, sleep, enjoyment of life, and ability to sleep on the affected side. Again, a large amount of inter-individual variability was noted in patients' interference ratings. Taken together these findings suggest that preoperative breast pain is a significant problem for a subset of women.

In terms of differences in pain group membership, consistent with previous reports women who reported pain were more likely to be younger.[1] In addition, consistent with previous studies of chronic cancer pain,[10,38,39,66] patients in the pain group reported lower mean KPS scores than the no pain group (90.9 versus 94.0). However, while these differences were statistically significant, both groups of women reported high levels of function.

Another interesting but not easily explained finding is that a higher percentage of non-white women reported breast pain prior to surgery. While findings from several studies suggest that members of minority groups report higher rates of chronic pain[16-17] and increased sensitivity to painful stimuli,[6-8,46] other studies have not demonstrated ethnic differences.[17-37] One potential reason for the ethnic differences found in this study is that a higher percentage of non-white women were diagnosed with more advanced disease (61% versus 41%, p=0.035). However, stage of disease was not associated with the occurrence of pain in this study. The potential link between ethnicity, stage of disease, and pain warrants investigation in future studies. Finally, women in the pain group were less likely to have gone through menopause, which is consistent with the younger age of this group, and the potential effects of the menstrual cycle and estrogen upon nociception.[19,41] These demographic and clinical characteristics suggest a profile of women who are at higher risk for pain prior to surgery.

Possible contributors to pretreatment pain are tissue injury or nerve damage associated with tumor growth, or the number of biopsies performed prior to surgery. These mechanical injuries could result in the release of inflammatory mediators. This hypothesis is supported by several findings. First, women in the pain group reported a

significantly higher number of biopsies. While the total number of biopsies was not normally distributed, 48% of the women in the pain group compared to only 29% in the no pain group had more than one biopsy. Unfortunately, data are not available on the type of biopsy performed, nor when the last biopsy was performed in relationship to completion of the baseline questionnaire. A higher percentage of patients in the pain group reported swelling, numbness, strange sensations and hardness in their breasts (Figure 3). In addition, the pain qualities reported by study participants are suggestive of nociceptive pain rather than primarily neuropathic pain.[69] These phenotypic findings support the data from the genomic analyses that suggest that some innate differences in inflammatory responses may be influencing the development of pre-surgical pain in breast cancer patients.

The results of the SNP analyses suggest that variation in inflammatory pathways involving IL1R1 and IL13 are involved in preoperative pain. In this study, carriers of the minor allele for IL1R1 (rs2110726) had a 51% decrease in the odds of reporting preoperative breast pain. This finding is consistent with studies of IL1 function in which removal of IL1R function or blockade of IL1 led to a decrease in inflammation and pain behaviors in mice.[63] Additional functional studies will need to determine if the minor allele of rs2110726 is associated with a decrease in IL1R1 function and therefore a decrease in the pro-inflammatory effects of IL1. The rs2110726 is in the 3' untranslated region of the IL1R1 gene.[27]

SNP analysis data from this study support the hypothesis that genetic variation in anti-inflammatory cytokines may be involved in the development of pain prior to surgery. IL13, unlike IL1R1, is a cytokine with anti-inflammatory activity. Therefore, its role in

pain may be as a moderator of the inflammatory response. IL4,[67] IL10,[67] and IL13[32,67] are known to have antinociceptive effects in mice, independent of endogenous opioid release, possibly through inhibition of TNFα and IL1β release. Furthermore, patients with chronic widespread pain syndrome have reduced levels of anti-inflammatory cytokines IL2, IL4, IL8, and IL10, which suggests a relationship between sustained pain and deficiencies in anti-inflammatory mediators.[65] Because the minor allele of rs1295686 was associated with increased reporting of pain, it seems plausible that this allele is associated with a decrease in the effect of IL13, either through decreased expression, or compromised conformation, or some other mechanism. The SNP rs1295686 is located in intron 3 of the IL13 gene.[54] Given that neither tag SNP is in a coding region of the gene nor predicted to impact gene function (i.e., splicing, alteration of transcription factor binding sites), it is likely that each SNP is in linkage disequilibrium with a functional SNP(s).

Several study limitations need to be acknowledged. No direct measurements of systemic levels of inflammatory markers or physical examination for signs of inflammation at the site were performed to provide additional data on the underlying mechanisms for the preoperative pain. In addition, type of biopsy, needle size, and time since biopsy were not obtained which would have provided additional information on the pain phenotype. While proportions of African Americans, Asian/Pacific Islanders, and Caucasians were more representative of the United States population than previous studies on pretreatment breast cancer pain,[43,60-62] the relatively small number of non-whites (36%) may have limited our ability to detect genotypic differences among the various ethnic groups. Finally, future studies with a larger sample size, would increase the power to detect differences in the other cytokine genes. This hypothesis might be true

for those SNPs in this study where genotypic differences approached statistical significance.

In conclusion, findings from this study and others[43,60-62] suggest that preoperative breast pain affects a significant proportion of patients. In addition, the genomic data support the hypothesis that this pain problem involves inflammatory processes. This information may help to identify women who are at greater risk for preoperative breast pain. Subsequent studies will need to confirm these findings and evaluate whether preoperative pain influences the severity of post-operative pain and/or the development of chronic pain following breast cancer surgery.

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Table 1. Genes and Single Nucleotide Polymorphisms Analyzed for Pain versus No Pain in Women Prior to Breast Cancer Surgery

Gene	SNP	Position	Chr	MAF	Alleles	Chi Square	p-value	Model
IL1B	rs1071676	106042060	2	.189	G>C	0.30	.863	A
IL1B	rs1143643	106042929	2	.383	G>A	1.51	.469	A
IL1B	rs1143642	106043180	2	.082	C>T	2.87	.238	A
IL1B	rs1143634	106045017	2	.187	C>T	0.51	.774	A
IL1B	rs1143633	106045094	2	.392	G>A	2.85	.241	A
IL1B	rs1143630	106046282	2	.115	C>A	0.64	.728	A
IL1B	rs3917356	106046990	2	.450	G>A	0.29	.864	A
IL1B	rs1143629	106048145	2	.389	A>G	1.03	.599	A
IL1B	rs1143627	106049014	2	.397	A>G	1.15	.562	A
IL1B	rs16944	106049494	2	.386	G>A	0.64	.726	A
IL1B	rs1143623	106050452	2	.277	C>G	2.10	.350	A
IL1B	rs13032029	106055022	2	.448	C>T	0.09	.958	A
IL1R1	rs949963	96533648	2	.223	G>A	1.94	.379	A
IL1R1	rs2228139	96545511	2	.053	C>G	1.66	.436	A
IL1R1	rs3917320	96556738	2	.047	A>C	0.90	.637	A
IL1R1	rs2110726	96558145	2	.317	C>T	**FE**	**.007**	**D**
IL1R1	rs3917332	96560387	2	.187	A>T	2.25	.324	A
IL1R2	rs4141134	96370336	2	.362	A>G	0.77	.680	A
IL1R2	rs11674595	96374804	2	.247	A>G	1.36	.507	A
IL1R2	rs7570441	96380807	2	.408	G>A	1.70	.428	A
IL2	rs1479923	119096993	4	.308	C>T	1.89	.388	A
IL2	rs2069776	119098582	4	*.184*	A>G	n/a	n/a	n/a
IL2	rs2069772	119099739	4	.241	A>G	0.19	.911	A
IL2	rs2069777	119103043	4	.047	C>T	5.21	.074	A
IL2	rs2069763	119104088	4	.277	A>C	0.85	.653	A
IL4	rs2243248	127200946	5	.086	A>C	1.06	.588	A
IL4	rs2243250	127201455	5	*.269*	C>T	n/a	n/a	n/a
IL4	rs2070874	127202011	5	*.245*	C>T	n/a	n/a	n/a
IL4	rs2227284	127205027	5	*.387*	C>A	n/a	n/a	n/a
IL4	rs2227282	127205481	5	*.390*	C>G	n/a	n/a	n/a
IL4	rs2243263	127205601	5	.124	C>G	1.90	.386	A
IL4	rs2243266	127206091	5	*.237*	G>A	n/a	n/a	n/a
IL4	rs2243267	127206188	5	*.237*	G>C	n/a	n/a	n/a
IL4	rs2243274	127207134	5	*.261*	G>A	n/a	n/a	n/a
IL6	rs4719714	22643793	7	.255	A>T	1.59	.452	A
IL6	rs2069827	22648536	7	.069	G>T	0.84	.658	A
IL6	rs1800796	22649326	7	*.134*	C>G	n/a	n/a	n/a
IL6	rs1800795	22649725	7	.285	C>G	3.35	.187	A
IL6	rs2069835	22650951	7	*.130*	A>G	n/a	n/a	n/a
IL6	rs2066992	22651329	7	.091	G>T	2.37	.306	A
IL6	rs2069840	22651652	7	.333	C>G	3.19	.203	A
IL6	rs1554606	22651787	7	.319	A>C	1.36	.507	A
IL6	rs2069845	22653229	7	.319	A>G	1.36	.507	A
IL6	rs2069849	22654236	7	.024	C>T	2.65	.266	A
IL6	rs2069861	22654734	7	.056	C>T	2.06	.357	A
IL6	rs35610689	22656903	7	.259	A>G	2.03	.363	A
IL8	rs4073	70417508	4	.455	A>T	0.35	.838	A
IL8	rs2227306	70418539	4	.366	C>T	1.06	.588	A
IL8	rs2227543	70419394	4	.368	C>T	0.61	.738	A
IL10	rs3024505	177638230	1	.129	C>T	2.85	.241	A
IL10	rs3024498	177639855	1	.204	A>G	0.86	.650	A
IL10	rs3024496	177640190	1	.421	A>G	0.79	.674	A
IL10	rs1878672	177642039	1	.416	C>G	0.08	.960	A
IL10	rs3024492	177642438	1	*.161*	A>T	n/a	n/a	n/a
IL10	rs1518111	177642971	1	.303	G>A	2.04	.361	A
IL10	rs1518110	177643187	1	.301	G>T	1.82	.402	A
IL10	rs3024491	177643372	1	.408	A>C	0.08	.961	A
IL13	rs1881457	127184713	5	.210	A>C	2.20	.332	A
IL13	rs1800925	127185113	5	.233	C>T	5.14	.077	A
IL13	rs2069743	127185579	5	.019	A>G	2.62	.270	A
IL13	rs1295686	127188147	5	.265	G>A	**7.89**	**.019**	**A**
IL13	rs20541	127188268	5	.212	C>T	2.18	.337	A
IL17A	rs4711998	51881422	6	.346	G>A	5.02	.081	A
IL17A	rs8193036	51881562	6	.327	A>G	1.77	.412	A

IL17A	rs3819024	51881855	6	.372	A>G	0.52	.772	A
IL17A	rs2275913	51882102	6	.361	G>A	1.29	.525	A
IL17A	rs3804513	51884266	6	.023	A>T	FE	.544	A
IL17A	rs7747909	51885318	6	.217	G>A	2.70	.259	A
TNFA	rs2857602	31533378	6	.341	A>G	0.69	.708	A
TNFA	rs1800683	31540071	6	.390	G>A	1.85	.397	A
TNFA	rs2239704	31540141	6	.335	G>T	0.42	.810	A
TNFA	rs2229094	31540556	6	.278	A>G	1.71	.426	A
TNFA	rs1041981	31540784	6	.386	C>A	1.68	.433	A
TNFA	rs1799964	31542308	6	.224	A>G	2.34	.311	A
TNFA	rs1800750	31542963	6	.016	G>A	FE	.712	A
TNFA	rs1800629	31543031	6	.149	G>A	3.29	.193	A
TNFA	rs1800610	31543827	6	.100	C>T	0.71	.702	A
TNFA	rs3093662	31544189	6	.074	A>G	0.68	.712	A

A = Additive model, chr = chromosome, D = Dominant model, MAF = minor allele frequency, n/a = not assayed because SNP violated Hardy-Weinberg expections (p,0.001), R = Recessive model

Single nucleotide polymorphisms (SNPs) that violated Hardy-Weinberg expectations are denoted in italics in the MAF column

Table 2. Differences in Demographic and Clinical Characteristics Between Patients With (n=110) and Without (n= 280) Breast Pain

Characteristic	No pain	Pain	Statistic and p-value
	mean (SD)	mean (SD)	
Age (years)	56.5 (11.8)	50.9 (9.8)	t= 4.81; p< 0.001
Education (years)	15.8 (2.7)	15.4 (2.6)	t= 1.42; p= 0.16
Self-administered Comorbidity Questionnaire score	4.3 (2.8)	4.2 (3.1)	t= 0.40; p= 0.69
Karnofsky Performance Status score	94.0 (10.3)	90.9 (10.1)	t= 2.66; p= 0.008
Number biopsies in past year	1.5 (0.8)	1.6 (0.8)	U= 12887.0, p< 0.01
	% (N)	% (N)	
Married	41.9 (117)	43.0 (46)	FE; p= 0.91
Employed	48.4 (134)	50.0 (55)	FE; p= 0.82
Lives alone	24.1 (67)	25.2 (27)	FE; p= 0.90
Non-white	31.9 (89)	45.0 (49)	FE; p= 0.018
Stage at diagnosis 0 I IIA, IIB IIIA, IIIB, IIIC, IV	17.7 (47) 39.6 (105) 34.7 (92) 7.9 (21)	16.0 (17) 31.1 (33) 40.6 (43) 12.3 (13)	X^2= 3.86; p= 0.28
Gone through menopause	67.9 (186)	53.8 (57)	FE; p= 0.012
Mastitis	11.6 (32)	14.0 (15)	FE; p= 0.49
Fibrocystic or cystic breast disease	17.8 (48)	22.9 (24)	FE; p= 0.31
Breastfed	49.6 (138)	39.1 (43)	FE; p= 0.07
Injury to affected arm	26.1 (72)	20.2 (22)	FE; p= 0.24
Injury to affected hand	22.3 (62)	27.4 (29)	FE; p= 0.35
Non-cancer surgery on the affected breast	12.9 (36)	16.5 (18)	FE; p= 0.41
Non-cancer surgery on the affected arm	6.1 (17)	6.5 (7)	FE; p= 1.00
Non-cancer surgery on the affected hand	8.7 (24)	8.3 (9)	FE; p= 1.00

Table 3. Individual Item Scores* and Subscale Scores for the Pain Qualities Assessment Scale

Descriptor	Mean (SD)	Range
tender	3.62 (3.20)	0-10
intense	2.82 (2.49)	0-10
dull	2.80 (2.61)	0-10
unpleasant	2.72 (2.49)	0-10
aching	2.64 (2.83)	0-10
shooting	2.49 (2.91)	0-10
sharp	2.35 (2.77)	0-10
sensitive	1.86 (2.75)	0-10
radiating	1.67 (2.42)	0-10
heavy	1.66 (2.69)	0-10
electrical	1.62 (2.60)	0-10
throbbing	1.63 (2.63)	0-10
hot	1.52 (2.55)	0-10
itchy	1.38 (2.54)	0-10
tingling	1.35 (2.49)	0-10
cramping	1.16 (2.41)	0-10
numb	0.99 (1.99)	0-8
cold	0.36 (1.29)	0-8
Intense surface pain	2.15 (2.58)	0-10
Intense deep pain	2.92 (2.58)	0-10
PQAS subscale scores		
Surface pain subscale	1.19 (1.72)	
Paroxysmal pain subscale	1.95 (2.22)	
Deep pain subscale	1.99 (2.07)	

*Individual item scores are listed in descending order

Figure 1. Ratings of pain intensity and time of breast pain interference with mood and/or activities

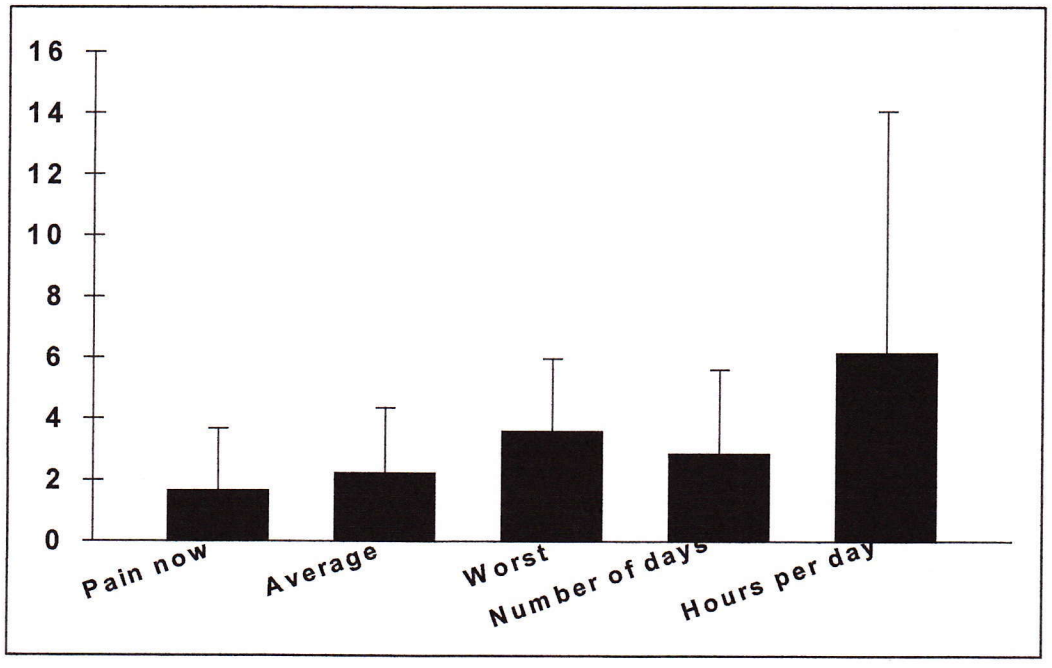


Figure 2. Pain interference items ratings (means)

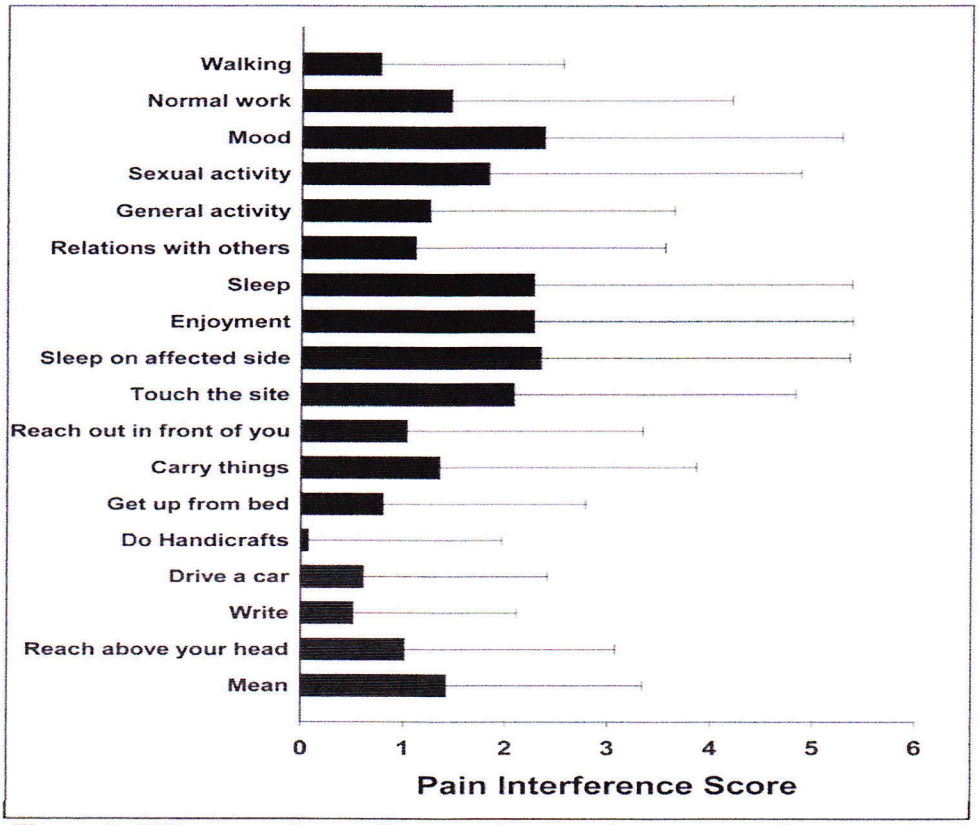


Figure 3. Differences in swelling, numbness, strange sensations and hardness reported in the affected breast by pain grouping

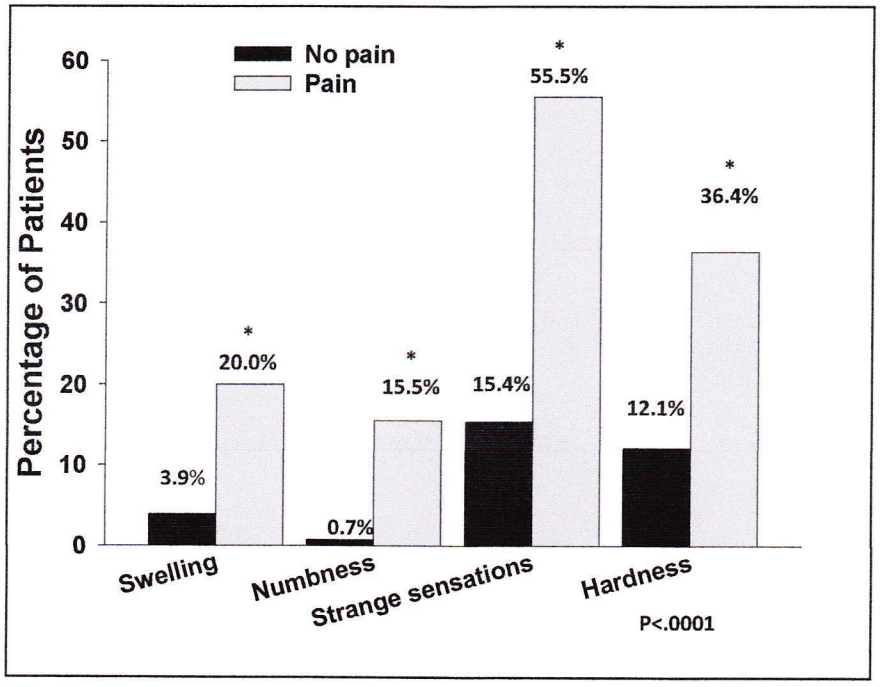


Figure 4. Differences in pain and no pain by rs2110726 genotype in IL1RI

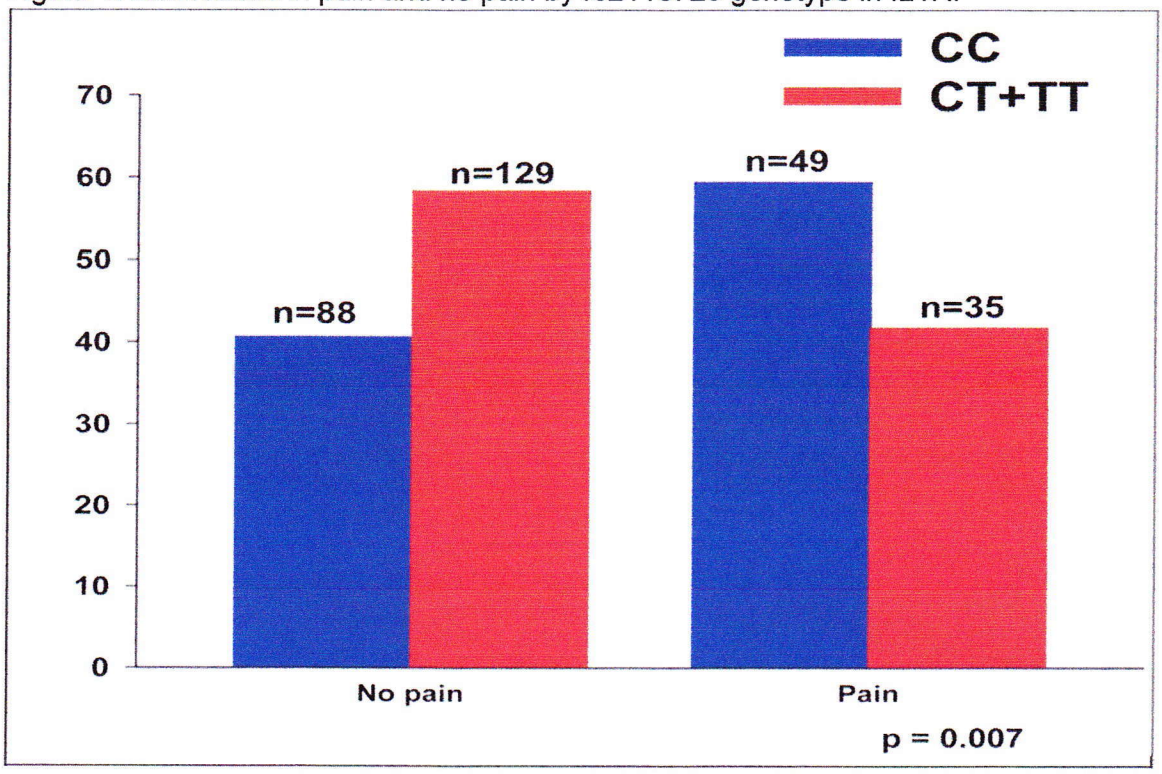
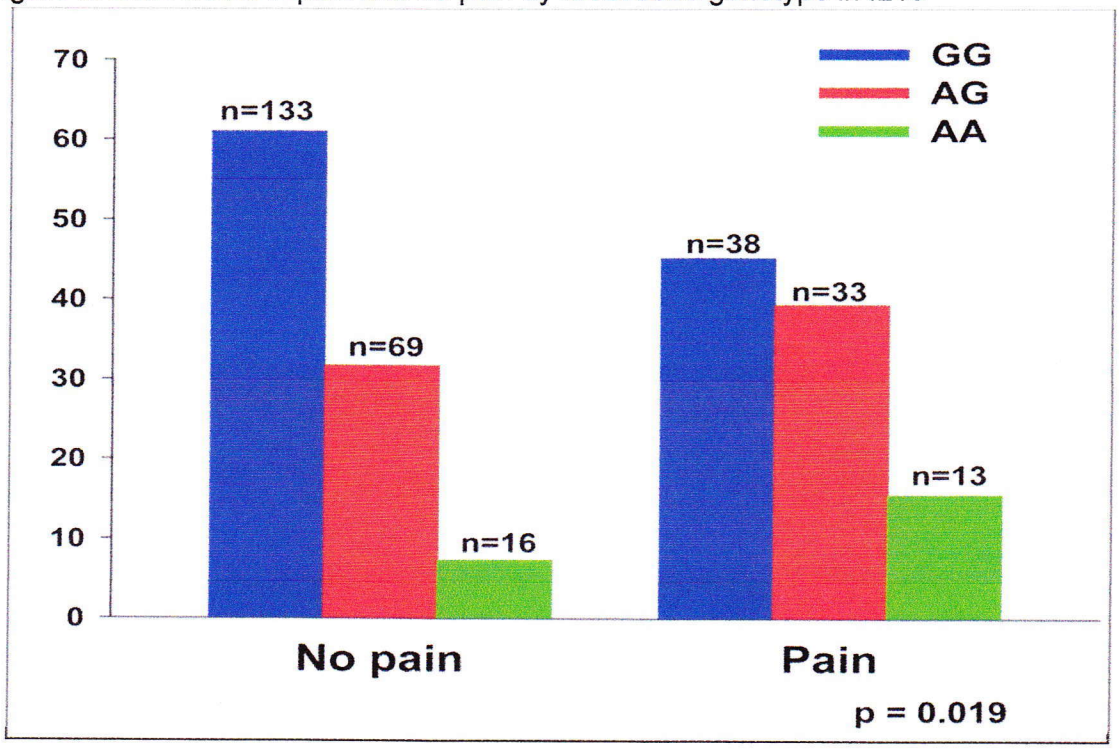


Figure 5. Differences in pain and no pain by rs1295686 genotype in IL13



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