## Title

Associations between cytokine gene variations and self-reported sleep disturbance in women following breast cancer surgery.



## Journal

European journal of oncology nursing : the official journal of European Oncology Nursing Society, 18(1)

## ISSN

1462-3889

## Authors

Alfaro, Emely
Dhruva, Anand
Langford, Dale J
et al.

## Publication Date

2014-02-01

## DOI

10.1016/j.ejon.2013.08.004

Peer reviewed

Published in final edited form as:
*Eur J Oncol Nurs*. 2014 February ; 18(1): 85–93. doi:10.1016/j.ejon.2013.08.004.

# ASSOCIATIONS BETWEEN CYTOKINE GENE VARIATIONS AND SELF-REPORTED SLEEP DISTURBANCE IN WOMEN FOLLOWING BREAST CANCER SURGERY

**Emily Alfaro, RN, MS**[1], **Anand Dhruva, MD**[2], **Dale J. Langford, PhD**[1], **Theresa Koetters, RN, MS**[1], **John D. Merriman, RN, PhD(c)**[1], **Claudia West, RN, MS**[1], **Laura B. Dunn, MD**[2], **Steven M. Paul, PhD**[1], **Bruce Cooper, PhD**[1], **Janine Cataldo, RN, PhD**[1], **Deborah Hamolsky, RN, MS**[1], **Charles Elboim, MD**[3], **Kord Kober, PhD**[1], **Bradley E. Aouizerat, PhD, MAS**[1,4], and **Christine Miaskowski, RN, PhD**[1]

[1]School of Nursing, University of California, San Francisco, CA

[2]School of Medicine, University of California, San Francisco, CA

[4]Institute for Human Genetics, University of California, San Francisco, CA

[3]Redwood Regional Medical Group, Santa Rosa, CA

## Abstract

**Purpose of the research—**To attempt to replicate the associations found in our previous study of patients and family caregivers between interleukin 6 (IL6) and nuclear factor kappa beta 2 (NFKB2) and sleep disturbance and to identify additional genetic associations in a larger sample of patients with breast cancer.

**Methods and sample—**Patients with breast cancer (n=398) were recruited prior to surgery and followed for six months. Patients completed a self-report measure of sleep disturbance and provided a blood sample for genomic analyses. Growth mixture modeling was used to identify distinct latent classes of patients with higher and lower levels of sleep disturbance.

**Key results—**Patients who were younger and who had higher comorbidity and lower functional status were more likely to be in the high sustained sleep disturbance class. Variations in three cytokine genes (i.e., IL1 receptor 2 (IL1R2), IL13, NFKB2) predicted latent class membership.

**Conclusions—**Polymorphisms in cytokine genes may partially explain inter-individual variability in sleep disturbance. Determination of high risk phenotypes and associated molecular markers may allow for earlier identification of patients at higher risk for developing sleep disturbance and lead to the development of more targeted clinical interventions.

### Keywords

sleep disturbance; breast cancer; cytokine genes; growth mixture modeling; symptom trajectories; insomnia



Address correspondence to: Christine Miaskowski, RN, PhD, FAAN, Professor and Associate Dean, Department of Physiological Nursing, University of California, 2 Koret Way – N631Y, San Francisco, CA 94143-0610, 415-476-9407 (phone), 415-476-8899 (fax), chris.miaskowski@nursing.ucsf.edu.

## INTRODUCTION

Findings from several studies suggest that women with breast cancer experience a significant amount of sleep disturbance or insomnia (Davidson et al., 2002; Palesh et al., 2010; Savard et al., 2011; Savard et al., 2009). For example, in one large cross sectional study that evaluated the prevalence of sleep disturbance in patients with a variety of cancer diagnoses (Davidson et al., 2002), patients with breast cancer reported the highest rate of insomnia (37.8%). In another study of a heterogeneous sample of oncology patients receiving chemotherapy (CTX) (Palesh et al., 2010), breast cancer patients had the highest rates of insomnia (i.e., 84% reported insomnia symptoms). Of the patients who reported insomnia symptoms, 45% met the diagnostic criteria for insomnia.

Recent work from our research team used growth mixture modeling (GMM) to identify subgroups of patients with distinct self-reported sleep disturbance trajectories prior to and for six months following breast cancer surgery (Van Onselen et al., 2012). Three distinct latent classes of patients were identified (i.e., high sustained (55.0%), low sustained (39.7%), and decreasing (5.3%) levels of sleep disturbance). Women in the high sustained class were significantly younger and had more comorbidities and poorer functional status than women in the low sustained class. These findings suggest that GMM can be used to identify subgroups of patients with distinct sleep disturbance trajectories, as well as specific phenotypic characteristics associated with increased risk for higher levels of sleep disturbance.

While an evaluation of differences in phenotypic characteristics is important to identify patients at highest risk for sleep disturbance before and during cancer treatment, an equally important consideration is whether genomic markers can distinguish among these patient subgroups. As noted by Cirelli (2009), specific candidate genes are associated with sleep regulation and sleep disorders like restless leg syndrome and narcolepsy. In addition, recent evidence suggests that cytokine dysregulation is associated with sleep disturbance in humans (for reviews see Cirelli (2009) and Sehgal and Mignot (2011)).

However, only a limited number of studies have evaluated the association between cytokine gene polymorphisms and sleep disturbance. For example, in one study that examined single nucleotide polymorphisms (SNPs) in interleukin 6 (IL6), IL1, and tumor necrosis factor alpha (TNFA) in patients newly diagnosed with obstructive sleep apnea syndrome (OSAS) (Popko et al., 2008), the only cytokine gene that was associated with OSAS was a polymorphism located in the promoter region of IL6 (rs1800795). In addition, this association was found only in male patients with OSAS compared to unaffected males. A higher percentage of men with OSAS (35.1%) were homozygous for the rare C allele compared to men in the control group (10.3%; p=.004). Recent work from our research team found associations between IL6 rs35610689 and nuclear factor kappa beta (NFKB2 rs7897947) and self-reported sleep disturbance in patients and family caregivers prior to and following radiation treatment. Carrying one or two doses of the rare allele for these two SNPs was associated with a decreased odds of belonging to the higher sleep disturbance class (Miaskowski et al., 2012b).

The purpose of the current study was to attempt to replicate the associations between IL6 and NFKB2 and sleep disturbance found in our previous study (Miaskowski et al., 2012b) and to identify additional associations in a larger sample of patients with breast cancer. To achieve this objective, we evaluated for differences in phenotypic and genotypic characteristics between breast cancer patients who were classified into the high sustained (58.1%) and low sustained (41.9%) GMM classes (Figure 1). Patients in the decreasing class

were not included in this analysis because the sample size (n= 21) was too small to allow for meaningful comparisons among the three latent classes (Miaskowski et al., 2012b).

## MATERIALS AND METHODS

### Patients and Settings

This analysis is part of a larger, longitudinal study that evaluated neuropathic pain and lymphedema in women who underwent breast cancer surgery (McCann et al., 2012; Miaskowski et al., 2012a; Miaskowski et al., 2013; Van Onselen et al., 2013). 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 adult women (≥18 years) who were scheduled to 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, 410 were enrolled (response rate 79.5%), and 398 completed the baseline assessment. The most common 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, marital status, education, ethnicity, employment status, and living situation. The Karnofsky Performance Status (KPS) scale is widely used to evaluate functional status in patients with cancer and has well established validity and reliability (Karnofsky et al., 1948). 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).

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 (Sangha et al., 2003). 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 the SCQ contains 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 (Brunner et al., 2008; Cieza et al., 2006).

The 21-item General Sleep Disturbance Scale (GSDS) was used to evaluate self-reported sleep disturbance during the past week. Each item is rated on a scale that ranges from 0 (never) to 7 (everyday). The total GSDS score can range from 0 (no disturbance) to 147 (extreme sleep disturbance). A total GSDS score of ≥43 indicates a clinically meaningful level of sleep disturbance (Fletcher et al., 2008; Lee, 1992). Cronbach’s alpha for the GSDS total score was 0.86.

## 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 and determined the patient's 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 consent, patients completed the enrollment questionnaires an average of 4 days prior to surgery. Patients completed the GSDS at enrollment and monthly for 6 months (i.e., 7 assessments). Medical records were reviewed for disease and treatment information.

## Genomic analyses

**Gene selection—**Cytokines and their receptors are classes of polypeptides that mediate inflammatory processes. Cytokine dysregulation is associated with sleep disturbance (Cirelli, 2009; Sehgal and Mignot, 2011). These polypeptides are divided into pro- and anti-inflammatory cytokines. Pro-inflammatory cytokines promote systemic inflammation and include: interferon gamma (IFNG), IFNG receptor 1(IFNGR1), IL1R1, IL2, IL8, IL17A, NFKB1, NFKB2, and TNFA. Anti-inflammatory cytokines suppress the activity of pro-inflammatory cytokines and include: IL1R2, IL4, IL10, and IL13. Of note, IFNG1, IL1B, and IL6 possess pro- and anti-inflammatory functions (Seruga et al., 2008).

**Blood collection and genotyping—**Of the 398 patients who completed the baseline assessment, 310 provided a blood sample from which DNA could be isolated from peripheral blood mononuclear cells (PBMCs). Genomic DNA was extracted from PBMCs using the PUREGene DNA Isolation System (Invitrogen, Carlsbad, CA). DNA was quantitated with a Nanodrop Spectrophotometer (ND-1000) 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 SNP. Disagreements were adjudicated by a third reviewer.

**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-values of <.001 were excluded. As shown in Table 1, a total of 82 SNPs among the 15 candidate genes (IFNG1: 5 SNPs, IFNGR1: 1 SNP; IL1B: 12 SNPs; IL1R1: 4 SNPs; IL1R2: 3 SNPs; IL2: 3 SNPs; IL4: 2 SNPs; IL6: 9 SNPs; IL8: 3 SNPs; IL10: 7 SNPs; IL13: 4 SNPs; IL17A: 5 SNPs; NFKB1: 11 SNPs; NFKB2: 4 SNPs; TNFA: 9 SNPs) passed all of the quality control filters and were included in the genetic association analyses. Potential functional roles of SNPs associated with sleep disturbance were examined using PUPASuite 2.0 (Conde et al., 2006), 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 19 (SPSS, 2010) and STATA Version 9 (StataCorp, 2005). Descriptive statistics and frequency distributions were generated for sample

characteristics. Independent sample t-tests (for continuous variables), Mann-Whitney U tests (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 the two latent classes. 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.

Unconditional GMM with robust maximum likelihood estimation was carried out to identify latent classes with distinct sleep disturbance trajectories using Mplus Version 5.21. These methods are described in detail elsewhere (Van Onselen et al., 2012). In brief, a single growth curve that represented the "average" change trajectory was estimated for the whole sample. Then, the number of latent growth classes that best fit the data was identified using guidelines recommended in the literature (Jung and Wickrama, 2008; Nylund et al., 2007; Tofighi and Enders, 2008).

## Statistical Analyses for the Genetic Data

Allele and genotype frequencies were determined by gene counting. Hardy-Weinberg equilibrium was assessed by the Chi-square or Fisher Exact tests. Measures of linkage disequilibrium ((LD), i.e., D' and $r^2$) were computed from the participants' genotypes with Haploview 4.2. The LD-based haplotype block definition was based on D' confidence interval (Gabriel et al., 2002).

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 (Stephens et al., 2001). 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 ≥.85, across the five iterations, were retained for downstream analyses. Only inferred haplotypes that occurred with a frequency estimate of ≥15% were included in the association analyses, assuming a dosage model (i.e., analogous to the additive model).

Ancestry informative markers (AIMs) were used to minimize confounding due to population stratification (Halder et al., 2008; Hoggart et al., 2003; Tian et al., 2008). Homogeneity in ancestry among patients was verified by principal component analysis (Price et al., 2006), using HelixTree (GoldenHelix, 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 discernable 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 them in all logistic 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 association between genotype and sleep disturbance class membership. Only those genetic associations identified as significant from the bivariate analyses were evaluated in the multivariate analyses. A backwards stepwise approach was used to create the most 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 9 (StataCorp, 2005).

As was done in our previous studies (Dunn et al., 2013; Illi et al., 2012; Miaskowski et al., 2012b), based on the recommendations of Rothman (Rothman, 1990), the implementation of rigorous quality controls for genomic data, the non-independence of SNPs/haplotypes in LD, and the exploratory nature of the analyses, adjustments were not made for multiple testing. In addition, significant SNPs identified in the bivariate analyses were evaluated further using logistic regression analyses that controlled for differences in phenotypic characteristics, potential confounding due to population stratification, and variations in other SNPs/haplotypes within the same gene. Only those SNPs that remained significant were included in the final presentation of the results. Therefore, the significant independent associations reported are unlikely to be due solely to chance. Unadjusted (bivariate) associations are reported for all of the SNPs that passed quality control criteria in Table 1, to allow for subsequent comparisons and meta-analyses.

## RESULTS

### Differences in Demographic and Clinical Characteristics

As summarized in Table 2, no differences were found between the low sustained and high sustained sleep disturbance classes for the majority of the demographic and clinical characteristics. However, patients in the high sustained class were significantly younger, had a lower KPS score, and a higher SCQ score (all $p < .0001$). In addition, a lower percentage of patients in the high sustained class were employed ($p = .04$) and had undergone a sentinel lymph node biopsy (SLNB; $p = .053$). A higher percentage of patients in the high sustained class had received adjuvant CTX during the first 6 months after breast cancer surgery ($p = .02$).

### Candidate gene analyses of the two GMM classes

As summarized in Table 1, the minor allele frequency was significantly different between the two latent classes for 5 SNPs and one haplotype: IL1R2 haplotype (HapA2), IL6 rs35610689, IL10 rs1878672, IL13 rs1881457, IL13 rs1800925, and NFKB2 rs1056890. For IL6 rs35610689 ($p = .037$) and IL10 rs1878672 ($p = .043$), a recessive model fit the data best. For IL13 rs1881457 ($p = .011$), IL13 rs1800925 ($p = .002$), and NFKB2 rs1056890 ($p = .025$), a dominant model fit the data best. Dosage of the IL1R2 haplotype (composed of rs11674595-rs7570441) was found to be significantly different between the two latent classes ($p = .037$).

### Regression analyses for IL1R2, IL13, and NFKB2 genotypes and low sustained versus high sustained sleep disturbance classes

In order to better estimate the magnitude (i.e., odds ratio, OR) and precision (95% confidence interval, CI) of genotype on the odds of belonging to the high sustained as compared to the low sustained sleep disturbance class, multivariate logistic regression models were fit. In these regression analyses that included genomic estimates of and self-reported race/ethnicity, the phenotypic characteristics that remained significant in the multivariate model were: age (in 5 year increments), KPS score (in 10 point increments), SCQ score, receipt of adjuvant CTX in the six months following breast cancer surgery, and having undergone a sentinel node biopsy (SLNB).

The only genetic associations that remained significant in the multivariate logistic regression analyses were for IL1R2 Hap A2, IL13 rs1800925, and NFKB2 rs1056890 (Table 3, Figures 2 and 3). In the regression analysis for IL1R2 Hap A2, that is composed of alleles at two SNPs (i.e., rs11674595 [T major allele], rs7570441 [A rare allele]), each additional dose of

IL1R2 Hap A2 was associated with a 2.08-fold increase in the odds of belonging to the high sustained sleep disturbance class (p=.024).

In the regression analysis for IL13 rs1800925 (see Figure 3A), carrying one or two doses of the rare T allele (i.e., CC versus CT+TT) was associated with a 2.21-fold increase in the odds of belonging to the high sustained sleep disturbance class (p=.005). In the regression analysis for NFKB2 rs1056890 (see Figure 3B), carrying one or two doses of the rare T allele (i.e., CC versus CT+TT) was associated with a 47% decrease in the odds of belonging to the high sustained sleep disturbance class (p=.028).

## DISCUSSION

This study is the first to evaluate associations between a number of phenotypic characteristics and variations in cytokine genes and sleep disturbance in breast cancer patients following surgery. Consistent with previous reports of sleep disturbance in patients with breast cancer, women in the high sustained class were younger (Davidson et al., 2002) and reported higher levels of comorbidity and lower levels of function (Foley et al., 2010). In addition, consistent with work by Berger and colleagues (2010), the receipt of adjuvant CTX was associated with being classified into the higher sustained sleep disturbance class. Of note, decreased age and poorer functional status were associated with membership in the high sleep disturbance class in our previous study of oncology patients and family caregivers (Miaskowski et al., 2012b). Taken together, these findings suggest that younger age, lower functional status, and higher levels of comorbidity place individuals at higher risk for sleep disturbance.

A primary aim of this study was to replicate the genetic associations identified in our previous study (Miaskowski et al., 2012b). In our previous study, carrying one or two doses of the rare allele for NFKB2 rs7897947 was associated with a 74% decrease in the odds of belonging to the higher sleep disturbance class. In the current study, an association was found in the same cytokine gene, but with a different SNP. Women who carried one or two doses of the rare allele in NFKB2 rs106890 had a 47% decrease in their odds of belonging to the high sustained sleep disturbance class. While the SNPs in NFKB2, in both of our studies, were different, carriers of the rare allele were less likely to be classified in the higher sleep disturbance class. The differences in the SNP associations identified may be related to differences in sample sizes and/or phenotypic characteristics (e.g., gender) between the two studies. Finally, the SNPs identified in our prior and current studies may be in LD with an unmeasured causal SNP(s) in NFKB2.

NFKB2 is a cytokine that belongs to the nuclear factor-kappa beta family that is made up of transcription factors that regulate various biological processes including immunity, stress responses, apoptosis, and cellular differentiation (Oeckinghaus et al., 2011). Inappropriate activation of NFKB is linked to inflammatory processes such as autoimmune arthritis, asthma, lung fibrosis, and septic shock (Oeckinghaus et al., 2011). Prior to our recent work (Miaskowski et al., 2012b), polymorphisms in NFKB2 were not linked directly to sleep disturbance. The SNP identified in the current study (rs1056890) has no known function. It is located in the 3’ untranslated region that occurs in an evolutionarily conserved region of the gene. Findings from our two studies suggest a role for NFKB2 in inflammatory processes that are associated with the development of sleep disturbance.

In this study, variation in IL13 rs1800925 was associated with sleep disturbance class membership. Carrying one or two doses of the rare allele was associated with a 2.21-fold increase in the odds of belonging to the high sustained sleep disturbance class. This SNP is located in the promoter region of IL13. While it occurs in an evolutionarily conserved region

of the gene, this SNP has no known function (e.g., is not predicted to alter known transcripton factor binding). However, it was associated with the development of psoriasis (Chang et al., 2008). In addition, variations in IL13 are associated with a number of inflammatory conditions including asthma and eczema (Chen et al., 1999; Zhu et al., 2004). Why this association was not identified in our previous study (Miaskowski et al., 2012b) warrants investigation in future studies with larger samples of oncology patients.

The third association identified in this study was between sleep disturbance and the IL1R2 HapA2 that is composed of two SNPs (i.e., rs11674595, rs7570441). Each additional dose of IL1R2 HapA2 was associated with a 2.08 increase in the odds of belonging to the high sustained sleep disturbance class. Prior to this study, no associations were found between this haplotype and sleep disturbance. However in another study from our research team (Dunn et al., 2013), a different haplotype in the same region (i.e., a 3-SNP haplotype composed of the rare C allele of rs4141134, the common T allele of rs11674595, and the rare A allele of rs7570441) was associated with a 2-fold increase in the odds of belonging to the class with a higher level of depressive symptoms.

While the functions of each of the individual SNPs in the haplotype are not known, both of these two SNPs in IL1R2 HapA2 (rs11674595 and rs7570441) are located in introns in regions of the gene that are evolutionarily conserved. IL1R2 is an anti-inflammatory cytokine that blocks inflammatory signaling and inhibits pro-inflammatory IL1 activity by acting as a decoy receptor (Colotta et al., 1993). Therefore, IL1R2 plays a role in the regulation of inflammatory pathways and its association with sleep disturbance requires further study.

Lastly, we did not replicate the association found in our previous study between IL6 and sleep disturbance. In our prior study (Miaskowski et al., 2012b), carrying one or two doses of the rare G allele in IL6 rs35610689 was associated with a 78% decrease in the odds of belonging to the higher sleep disturbance class. In our current study, while an association between IL6 rs35610689 and sleep disturbance group membership was found in the bivariate analysis ($p=.037$), it was no longer significant after adjusting for covariates. Given this preliminary association, as well as recent findings that higher serum levels of IL6 are associated with sleep disturbance in patients with ovarian cancer (Clevenger et al., 2012), additional research is warranted on the associations between genetic variations in IL6 and sleep disturbance.

A number of limitations need to be acknowledged. While our sample size was adequate, future studies with larger sample sizes are needed to confirm these findings and identify additional latent classes and/or significant phenotypic predictors. Differences in demographic (e.g., gender) and clinical (e.g., homogenous versus heterogeneous cancer diagnoses) characteristics between participants in this study and our previous study (Miaskowski et al., 2012b) may partially explain why some of our findings were not replicated. While, the genotypic findings were somewhat consistent, additional investigations are warranted on the associations between variations in candidate genes and sleep disturbance.

Despite these limitations, these findings provide evidence to support distinct sleep disturbance phenotypes in breast cancer patients prior to and following surgery. In addition, in this sample, the higher risk phenotype was associated with higher levels of depression and fatigue prior to surgery (Van Onselen et al., 2013). It is important that these higher risk patients be identified early in order to evaluate the need for pre-emptive and ongoing treatment. As part of their initial and ongoing assessments, oncology nurses need to systematically evaluate for the co-occurrence of sleep disturbance, fatigue, and depression.

Women with high levels of all three symptoms need a more comprehensive evaluation to determine if pharmacologic and nonpharmacologic interventions are warranted. The findings of associations between cytokine genes and sleep disturbance trajectories suggest a role for inflammation in the development and maintenance of sleep disturbance in patients prior to and following surgery for breast cancer. If the genetic associations are replicated in an independent cohort, these findings may be used to identify patients who are at higher risk for the development of sleep disturbance.

## Acknowledgments

This study was funded by grants from the National Cancer Institute (CA107091 and CA118658). Dr. Christine Miaskowski is an American Cancer Society Clinical Research Professor. Dr. Dhruva is funded through NIH Mentored Patient-Oriented Research Career Development Award (K23 AT005340). Dr. Langford is supported by a Department of Defense Breast Cancer Research Program Postdoctoral Fellowship. Mr. Merriman is supported by an NINR fellowship (F31 NR012604), an ACS Doctoral Degree Scholarship (DSCN-10-087), an Oncology Nursing Foundation Doctoral Scholarship, and a UCSF Nursing Alumni Association Scholarship. This project is supported by NIH/NCRR UCSF-CTSI Grant Number UL1 RR024131. Its contents are solely the responsibility of the authors and do not necessarily represent the official views of the NIH.

## References

Berger AM, Wielgus K, Hertzog M, Fischer P, Farr L. Patterns of circadian activity rhythms and their relationships with fatigue and anxiety/depression in women treated with breast cancer adjuvant chemotherapy. Support Care IN Cancer. 2010; 18:105–114.

Brunner F, Bachmann LM, Weber U, Kessels AG, Perez RS, Marinus J, et al. Complex regional pain syndrome 1--the Swiss cohort study. BMC Musculoskeletal Disorders. 2008; 9:92. [PubMed: 18573212]

Chang M, Li Y, Yan C, Callis-Duffin KP, Matsunami N, Garcia VE, et al. Variants in the 5q31 cytokine gene cluster are associated with psoriasis. Genes and Immunity. 2008; 9:176–181. [PubMed: 18075513]

Chen F, Castranova V, Shi X, Demers LM. New insights into the role of nuclear factor-kappaB, a ubiquitous transcription factor in the initiation of diseases. Clinical Chemistry. 1999; 45:7–17. [PubMed: 9895331]

Cieza A, Geyh S, Chatterji S, Kostanjsek N, Ustun BT, Stucki G. Identification of candidate categories of the International Classification of Functioning Disability and Health (ICF) for a Generic ICF Core Set based on regression modelling. BMC Medical Research Methodolology. 2006; 6:36.

Cirelli C. The genetic and molecular regulation of sleep: from fruit flies to humans. Nature Reviews Neuroscience. 2009; 10:549–560.

Clevenger L, Schrepf A, Christensen D, DeGeest K, Bender D, Ahmed A, et al. Sleep disturbance, cytokines, and fatigue in women with ovarian cancer. Brain Behavior and Immunity. 2012; 26:1037–1044.

Colotta F, Re F, Muzio M, Bertini R, Polentarutti N, Sironi M, et al. Interleukin-1 type II receptor: a decoy target for IL-1 that is regulated by IL-4. Science. 1993; 261:472–475. [PubMed: 8332913]

Conde L, Vaquerizas JM, Dopazo H, Arbiza L, Reumers J, Rousseau F, et al. PupaSuite: finding functional single nucleotide polymorphisms for large-scale genotyping purposes. Nucleic Acids Research. 2006; 34:W621–625. [PubMed: 16845085]

Davidson JR, MacLean AW, Brundage MD, Schulze K. Sleep disturbance in cancer patients. Social Science and Medicine. 2002; 54:1309–1321. [PubMed: 12058848]

Dunn LB, Aouizerat BE, Langford DJ, Cooper BA, Dhruva A, Cataldo JK, et al. Cytokine gene variation is associated with depressive symptom trajectories in oncology patients and family caregivers. European Journal of Oncology Nursing. 2013; 17:346–353. [PubMed: 23187335]

Fletcher BS, Paul SM, Dodd MJ, Schumacher K, West C, Cooper B, et al. Prevalence, severity, and impact of symptoms on female family caregivers of patients at the initiation of radiation therapy for prostate cancer. Journal of Clincal Oncology. 2008; 26:599–605.

Foley KA, Sarsour K, Kalsekar A, Walsh JK. Subtypes of sleep disturbance: associations among symptoms, comorbidities, treatment, and medical costs. Behavioral Sleep Medicine. 2010; 8:90–104. [PubMed: 20352545]

Gabriel SB, Schaffner SF, Nguyen H, Moore JM, Roy J, Blumenstiel B, et al. The structure of haplotype blocks in the human genome. Science. 2002; 296(5576):2225–2229. [PubMed: 12029063]

Halder I, Shriver M, Thomas M, Fernandez JR, Frudakis T. A panel of ancestry informative markers for estimating individual biogeographical ancestry and admixture from four continents: utility and applications. Human Mutation. 2008; 29:648–658. [PubMed: 18286470]

Hoggart CJ, Parra EJ, Shriver MD, Bonilla C, Kittles RA, Clayton DG, McKeigue PM. Control of confounding of genetic associations in stratified populations. American Journal of Human Genetics. 2003; 72:1492–1504. [PubMed: 12817591]

Illi J, Miaskowski C, Cooper B, Levine JD, Dunn L, West C, et al. Association between pro- and anti-inflammatory cytokine genes and a symptom cluster of pain, fatigue, sleep disturbance, and depression. Cytokine. 2012; 58:437–447. [PubMed: 22450224]

Jung T, Wickrama KAS. An introduction to latent class growth analysis and growth mixture modeling. Social and Personality Psychology Compass. 2008; 2:302–317.

Karnofsky D, Abelmann WH, Craver LV, Burchenal JH. The use of nitrogen mustards in the palliative treatment of carcinoma. Cancer. 1948; 1:634–656.

Lee KA. Self-reported sleep disturbances in employed women. Sleep. 1992; 15:493–498. [PubMed: 1475563]

McCann B, Miaskowski C, Koetters T, Baggott C, West C, Levine JD, Elboim C, et al. Associations between pro- and anti-inflammatory cytokine genes and breast pain in women prior to breast cancer surgery. Journal of Pain. 2012; 13:425–437. [PubMed: 22515947]

Miaskowski C, Cooper B, Paul SM, West C, Langford D, Levine JD, et al. Identification of patient subgroups and risk factors for persistent breast pain following breast cancer surgery. Journal of Pain. 2012a; 13:1172–1187. [PubMed: 23182226]

Miaskowski C, Cooper BA, Dhruva A, Dunn LB, Langford DJ, Cataldo JK, et al. Evidence of associations between cytokine genes and subjective reports of sleep disturbance in oncology patients and their family caregivers. PLoS One. 2012b; 7:e40560. [PubMed: 22844404]

Miaskowski C, Dodd M, Paul SM, West C, Hamolsky D, Abrams G, et al. Lymphatic and angiogenic candidate genes predict the development of secondary lymphedema following breast cancer surgery. PLoS One. 2013; 8:e60164. [PubMed: 23613720]

Nylund KL, Asparouhov T, Muthen BO. Deciding on the number of classes in latent class analysis and growth mixture modeling: A Monte Carlo simulation study. Structural Equation Modeling. 2007; 14:535–569.

Oeckinghaus A, Hayden MS, Ghosh S. Crosstalk in NF-kappaB signaling pathways. Nature Immunology. 2011; 12:695–708. [PubMed: 21772278]

Palesh OG, Roscoe JA, Mustian KM, Roth T, Savard J, Ancoli-Israel S, et al. Prevalence, demographics, and psychological associations of sleep disruption in patients with cancer: University of Rochester Cancer Center-Community Clinical Oncology Program. Journal of Clinical Oncology. 2010; 28:292–298. [PubMed: 19933917]

Popko K, Gorska E, Potapinska O, Wasik M, Stoklosa A, Plywaczewski R, et al. Frequency of distribution of inflammatory cytokines IL-1, IL-6 and TNF-alpha gene polymorphism in patients with obstructive sleep apnea. Journal of Physiology and Pharmacology 59 Supplement. 2008; 6:607–614.

Price AL, Patterson NJ, Plenge RM, Weinblatt ME, Shadick NA, Reich D. Principal components analysis corrects for stratification in genome-wide association studies. Nature Genetics. 2006; 38:904–909. [PubMed: 16862161]

Rothman KJ. No adjustments are needed for multiple comparisons. Epidemiology. 1990; 1:43–46. [PubMed: 2081237]

Sangha O, Stucki G, Liang MH, Fossel AH, Katz JN. The Self-Administered Comorbidity Questionnaire: a new method to assess comorbidity for clinical and health services research. Arthritis and Rheumatism. 2003; 49:156–163. [PubMed: 12687505]

Savard J, Ivers H, Villa J, Caplette-Gingras A, Morin CM. Natural course of insomnia comorbid with cancer: an 18-month longitudinal study. Journal of Clinical Oncology. 2011; 29:3580–3586. [PubMed: 21825267]

Savard J, Villa J, Ivers H, Simard S, Morin CM. Prevalence, natural course, and risk factors of insomnia comorbid with cancer over a 2-month period. Journal of Clinical Oncology. 2009; 27:5233–5239. [PubMed: 19738124]

Sehgal A, Mignot E. Genetics of sleep and sleep disorders. Cell. 2011; 146:194–207. [PubMed: 21784243]

Seruga B, Zhang H, Bernstein LJ, Tannock IF. Cytokines and their relationship to the symptoms and outcome of cancer. Nature Reviews Cancer. 2008; 8:887–899.

SPSS. IBM SPSS for Windows (Version 19). SPSS, Inc; Chicago, Illinois: 2010.

StataCorp. Stata Statistical Software: Release 9. Stata Corporation; College Station, Texas: 2005.

Stephens M, Smith NJ, Donnelly P. A new statistical method for haplotype reconstruction from population data. Americal Journal of Human Genetics. 2001; 68:978–989.

Tian C, Gregersen PK, Seldin MF. Accounting for ancestry: population substructure and genome-wide association studies. Human Molecular Genetics. 2008; 17:R143–150. [PubMed: 18852203]

Tofighi, D.; Enders, CK. Identifying the correct number of classes in growth mixture models. Information Age Publishing; Charlotte, NC: 2008.

Van Onselen C, Aouizerat BE, Dunn LB, Paul SM, West C, Hamolsky D, et al. Differences in sleep disturbance, fatigue and energy levels between women with and without breast pain prior to breast cancer surgery. Breast. 2013; 22:273–276. [PubMed: 22858121]

Van Onselen C, Cooper BA, Lee K, Dunn L, Aouizerat BE, West C, et al. Identification of distinct subgroups of breast cancer patients based on self-reported changes in sleep disturbance. Supportive Care in Cancer. 2012; 20:2611–2619. [PubMed: 22290719]

Zhu Z, Zheng T, Homer RJ, Kim YK, Chen NY, Cohn L, et al. Acidic mammalian chitinase in asthmatic Th2 inflammation and IL-13 pathway activation. Science. 2004; 304:1678–1682. [PubMed: 15192232]

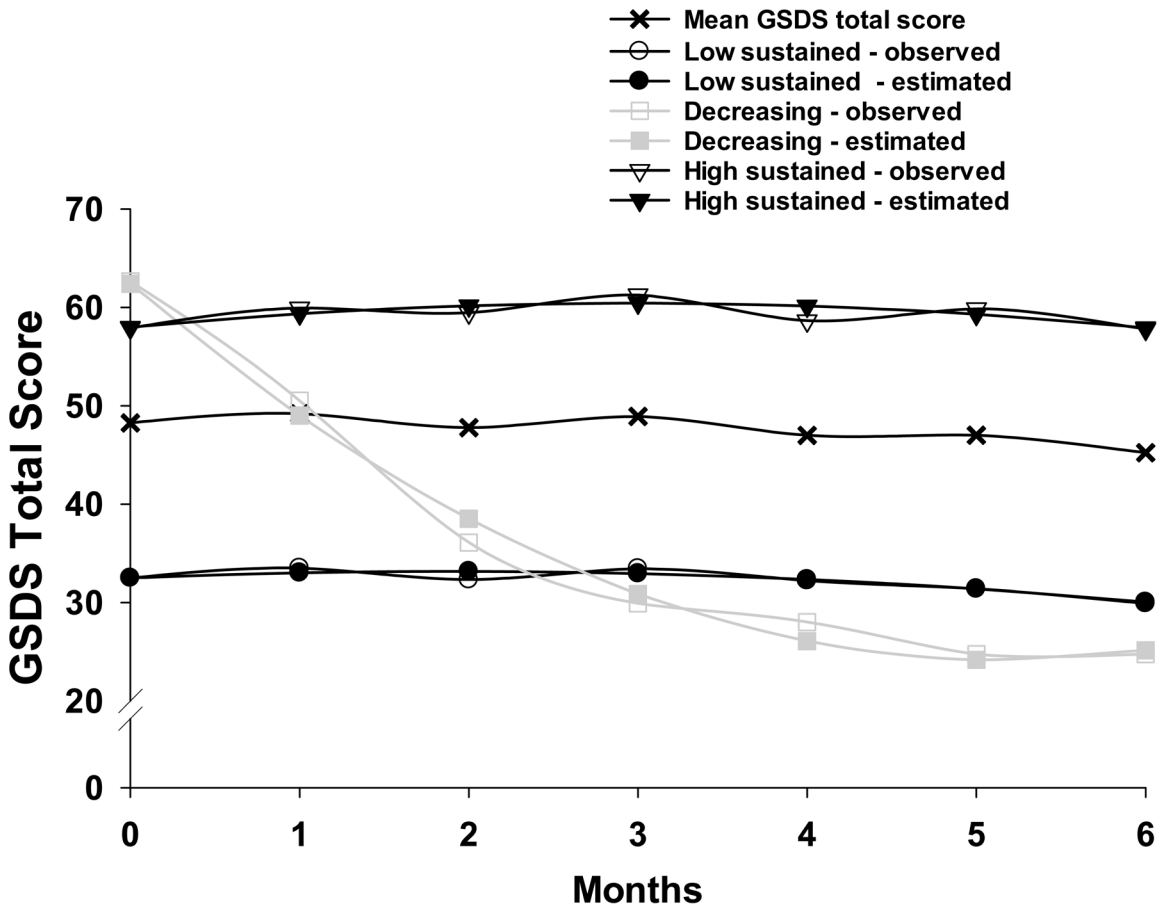
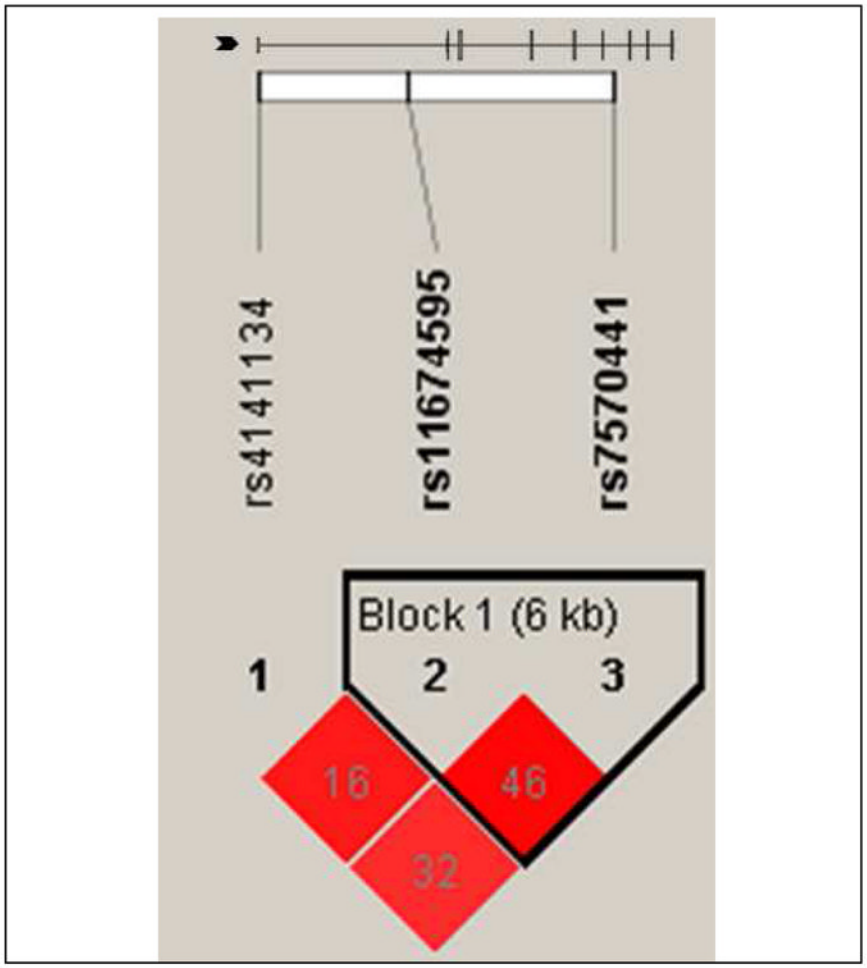



**Figure 1.**
Observed and estimated General Sleep Disturbance Scale (GSDS) trajectories for patients in each of the latent classes, as well as the mean GSDS scores for the total sample (Adapted from Van Onselen et al., 2012).




| Haplotype | Low Sustained | High Sustained |
|---|---|---|
| A1: T-G | 157 (59.5%) | 183 (57.5%) |
| ***A2: T-A*** | ***37 (14.0%)*** | ***63 (19.7%)*** |
| A3: C-G | 1 (<0.0%) | 0 (0.0%) |
| A4: C-A | 69 (26.1%) | 74 (23.1%) |

**Figure 2.**

IL1R2 linkage disequilibrium-based heatmap and haplotype analysis. In the figure embedded in the top row of the table, an ideogram of interleukin 1 receptor 2 (IL1R2) is presented above the white bar that represents the physical distance along human chromosome 2 (position 31, 96,370,336 to 96,380,807; genome build 36.3, contig NT_022171.14). Exons are represented as tick marks. Gray lines connecting the exons represent introns. The black chevron indicates the direction of gene transcription. 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 to render the pairwise linkage disequilibrium (LD) estimates that were

calculated and visualized with Haploview 4.2. The gene structure for IL1R2 (i.e., reference sequence NM_004633) 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 the D' confidence interval method. 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 color, with darker red 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 two SNPs that compose the haplotype (i.e., rs11674595, rs7570441) and both the count (n) and frequency (%) of each haplotype observed in the two GMM Sleep Disturbance groups. The T-A haplotype identified in the bivariate analyses (Table 1) that remained significant after controlling for relevant confounders is rendered in bold and italicized.

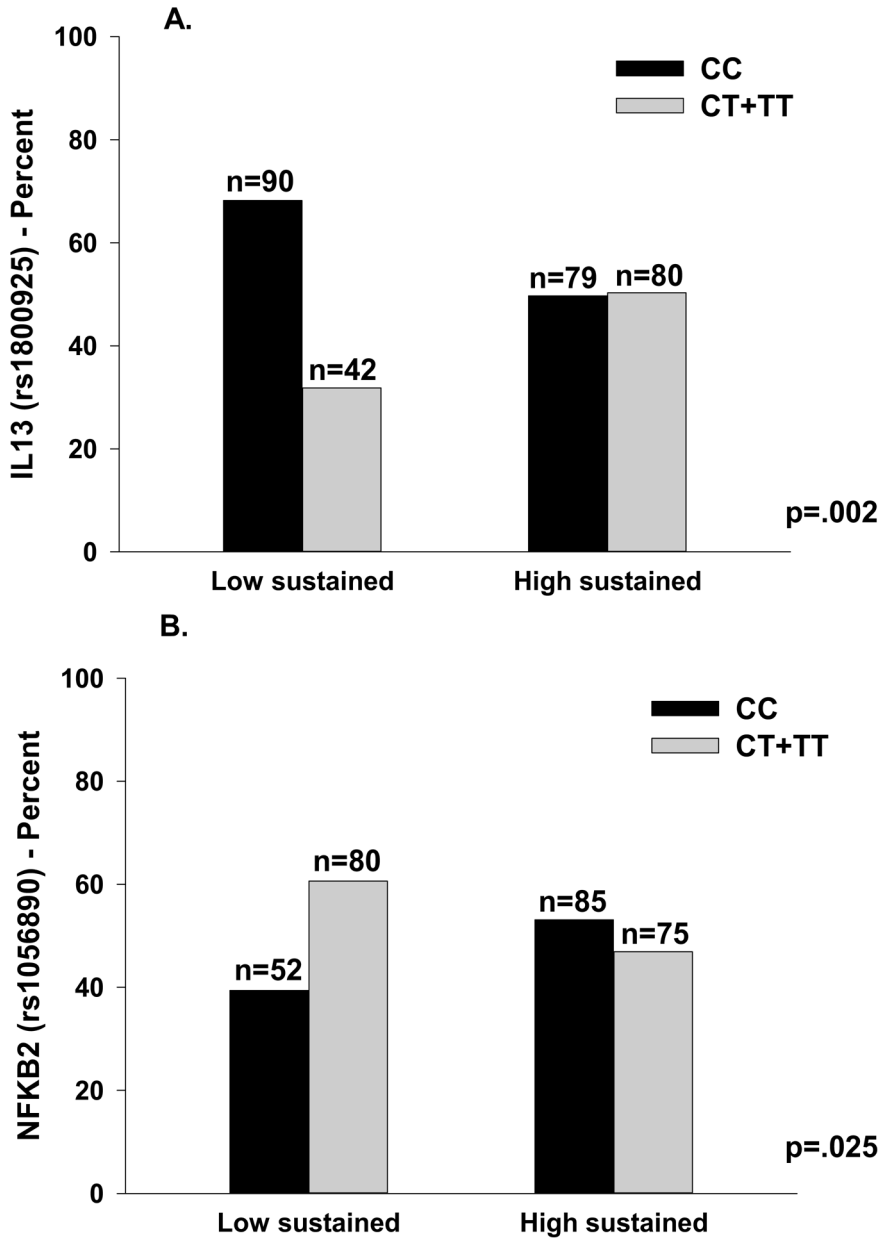



**Figure 3.**

Figure 3A. Differences between the latent classes in the percentages of patients who were homozygous for the common allele (CC) or heterozygous or homozygous for the minor allele (CT+TT) for rs1800925 in interleukin 13 (IL13). Values are plotted as unadjusted proportions with corresponding p-value.

Figure 3B - Differences between the latent classes in the percentages of patients who were homozygous for the common allele (CC) or heterozygous or homozygous for the minor allele (CT+TT) for rs1056890 in nuclear factor kappa beta 2 (NFKB2). Values are plotted as unadjusted proportions with corresponding p-value.

**Table 1**

Summary of Single Nucleotide Polymorphisms Analyzed for Pro- and Anti-Inflammatory Cytokine Genes and Self-reported Sleep Disturbance

| Gene | SNP | Position | Chr | MAF | Alleles | Chi Square | p-value | Model |
|---|---|---|---|---|---|---|---|---|
| IFNG1 | rs2069728 | 66834051 | 12 | .079 | G>A | 0.737 | .692 | A |
| IFNG1 | rs2069727 | 66834490 | 12 | .411 | A>G | 0.729 | .694 | A |
| IFNG1 | rs2069718 | 66836429 | 12 | .442 | C>T | 0.454 | .797 | A |
| IFNG1 | rs1861493 | 66837463 | 12 | .264 | A>G | 1.866 | .393 | A |
| IFNG1 | rs1861494 | 66837676 | 12 | .279 | T>C | 1.892 | .388 | A |
| IFNG1 | rs2069709 | 66839970 | 12 | .008 | G>T | n/a | n/a | n/a |
| IFNG1 | HapA3 | | | | | 1.837 | .399 | |
| IFNG1 | HapA5 | | | | | 0.812 | .666 | |
| IFNGR1 | rs9376268 | 137574444 | 6 | .246 | G>A | 0.597 | .742 | A |
| IL1B | rs1071676 | 106042060 | 2 | .189 | G>C | 0.859 | .651 | A |
| IL1B | rs1143643 | 106042929 | 2 | .383 | G>A | 0.086 | .958 | A |
| IL1B | rs1143642 | 106043180 | 2 | .082 | C>T | 3.049 | .218 | A |
| IL1B | rs1143634 | 106045017 | 2 | .187 | C>T | 0.871 | .647 | A |
| IL1B | rs1143633 | 106045094 | 2 | .392 | G>A | 0.283 | .868 | A |
| IL1B | rs1143630 | 106046282 | 2 | .115 | C>A | 3.951 | .139 | A |
| IL1B | rs3917356 | 106046990 | 2 | .450 | G>A | 0.355 | .837 | A |
| IL1B | rs1143629 | 106048145 | 2 | .389 | T>C | 1.749 | .417 | A |
| IL1B | rs1143627 | 106049014 | 2 | .397 | T>C | 1.828 | .401 | A |
| IL1B | rs16944 | 106049494 | 2 | .386 | G>A | 2.266 | .322 | A |
| IL1B | rs1143623 | 106050452 | 2 | .277 | G>C | 0.021 | .990 | A |
| IL1B | rs13032029 | 106055022 | 2 | .448 | C>T | 0.809 | .667 | A |
| IL1B | HapA1 | | | | | 0.379 | .827 | |
| IL1B | HapA4 | | | | | 0.106 | .948 | |
| IL1B | HapA6 | | | | | 0.875 | .645 | |
| IL1B | HapB1 | | | | | 1.452 | .484 | |
| IL1B | HapB6 | | | | | 0.301 | .860 | |
| IL1B | HapB8 | | | | | 0.427 | .808 | |

| Gene | SNP | Position | Chr | MAF | Alleles | Chi Square | p-value | Model |
|---|---|---|---|---|---|---|---|---|
| IL1R1 | rs949963 | 96533648 | 2 | .223 | G>A | 3.293 | .193 | A |
| IL1R1 | rs2228139 | 96545511 | 2 | .053 | C>G | 2.659 | .265 | A |
| IL1R1 | rs3917320 | 96556738 | 2 | .047 | A>C | n/a | n/a | n/a |
| IL1R1 | rs2110726 | 96558145 | 2 | .317 | C>T | 1.118 | .572 | A |
| IL1R1 | rs3917332 | 96560387 | 2 | .187 | A>T | 1.514 | .469 | A |
| IL1R1 | HapA1 | | | | | 0.059 | .971 | |
| IL1R1 | HapA2 | | | | | 0.329 | .848 | |
| IL1R1 | HapA3 | | | | | 1.426 | .490 | |
| IL1R2 | rs4141134 | 96370336 | 2 | .362 | T>C | 0.011 | .995 | A |
| IL1R2 | rs11674595 | 96374804 | 2 | .247 | T>C | 0.993 | .609 | A |
| IL1R2 | rs7570441 | 96380807 | 2 | .408 | G>A | 1.357 | .507 | A |
| IL1R2 | HapA1 | | | | | 1.572 | .456 | |
| IL1R2 | HapA2 | | | | | | .037 | |
| IL1R2 | HapA4 | | | | | 0.544 | .762 | |
| IL2 | rs1479923 | 119096993 | 4 | .308 | C>T | 0.391 | .822 | A |
| IL2 | rs2069776 | 119098582 | 4 | .184 | T>C | n/a | n/a | n/a |
| IL2 | rs2069772 | 119099739 | 4 | .241 | A>G | 0.222 | .895 | A |
| IL2 | rs2069777 | 119103043 | 4 | .047 | C>T | n/a | n/a | n/a |
| IL2 | rs2069763 | 119104088 | 4 | .277 | T>G | 1.565 | .457 | A |
| IL2 | HapA1 | | | | | 0.580 | .748 | |
| IL2 | HapA2 | | | | | 1.613 | .446 | |
| IL2 | HapA3 | | | | | 0.222 | .895 | |
| IL4 | rs2243248 | 127200946 | 5 | .086 | T>G | 2.604 | .272 | 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 | 1.503 | .472 | A |
| IL4 | rs2243266 | 127206091 | 5 | .237 | G>A | n/a | n/a | n/a |

| Gene | SNP | Position | Chr | MAF | Alleles | Chi Square | p-value | Model |
|---|---|---|---|---|---|---|---|---|
| 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 |
| IL4 | HapA1 | | | | | 0.390 | .823 | |
| IL4 | HapA3 | | | | | 0.454 | .797 | |
| IL4 | HapX1 | | | | | 2.939 | .230 | |
| IL6 | rs4719714 | 22643793 | 7 | .255 | A>T | 3.053 | .217 | A |
| IL6 | rs2069827 | 22648536 | 7 | .069 | G>T | 4.431 | .109 | A |
| IL6 | rs1800796 | 22649326 | 7 | .134 | C>G | n/a | n/a | n/a |
| IL6 | rs1800795 | 22649725 | 7 | .285 | C>G | 1.583 | .453 | A |
| IL6 | rs2069835 | 22650951 | 7 | .130 | T>C | n/a | n/a | n/a |
| IL6 | rs2066992 | 22651329 | 7 | .091 | G>T | 0.889 | .641 | A |
| IL6 | rs2069840 | 22651652 | 7 | .333 | C>G | 4.107 | .128 | A |
| IL6 | rs1554606 | 22651787 | 7 | .319 | G>T | 0.187 | .911 | A |
| IL6 | rs2069845 | 22653229 | 7 | .319 | A>G | 0.187 | .911 | A |
| IL6 | rs2069849 | 22654236 | 7 | .024 | C>T | n/a | n/a | n/a |
| IL6 | rs2069861 | 22654734 | 7 | .056 | C>T | 2.473 | .290 | A |
| IL6 | rs35610689 | 22656903 | 7 | .259 | A>G | FE | .037 | R |
| IL6 | HapA1 | | | | | 1.280 | .527 | |
| IL6 | HapA5 | | | | | 3.623 | .163 | |
| IL6 | HapA8 | | | | | 1.471 | .479 | |
| IL8 | rs4073 | 70417508 | 4 | .455 | T>A | 0.888 | .642 | A |
| IL8 | rs2227306 | 70418539 | 4 | .366 | C>T | 2.088 | .352 | A |
| IL8 | rs2227543 | 70419394 | 4 | .368 | C>T | 1.847 | .397 | A |
| IL8 | HapA1 | | | | | 0.888 | .642 | |
| IL8 | HapA4 | | | | | 1.903 | .386 | |
| IL10 | rs3024505 | 177638230 | 1 | .129 | C>T | 1.987 | .370 | A |
| IL10 | rs3024498 | 177639855 | 1 | .204 | A>G | 0.259 | .878 | A |
| IL10 | rs3024496 | 177640190 | 1 | .421 | T>C | 5.439 | .066 | A |
| IL10 | rs1878672 | 177642039 | 1 | .416 | G>C | FE | .043 | R |

| Gene | SNP | Position | Chr | MAF | Alleles | Chi Square | p-value | Model |
|---|---|---|---|---|---|---|---|---|
| IL10 | rs3024492 | 177642438 | 1 | .161 | T>A | n/a | n/a | n/a |
| IL10 | rs1518111 | 177642971 | 1 | .303 | G>A | 2.561 | .278 | A |
| IL10 | rs1518110 | 177643187 | 1 | .301 | G>T | 2.213 | .331 | A |
| IL10 | rs3024491 | 177643372 | 1 | .408 | G>T | 4.998 | .082 | A |
| IL10 | HapA1 | | | | | 2.493 | .287 | |
| IL10 | HapA2 | | | | | 2.930 | .231 | |
| IL10 | HapA8 | | | | | 0.329 | .849 | |
| IL13 | rs1881457 | 127184713 | 5 | .210 | A>C | FE | .011 | D |
| IL13 | rs1800925 | 127185113 | 5 | .233 | C>T | FE | .002 | D |
| IL13 | rs2069743 | 127185579 | 5 | .019 | A>G | n/a | n/a | n/a |
| IL13 | rs1295686 | 127188147 | 5 | .265 | G>A | 2.902 | .234 | A |
| IL13 | rs20541 | 127188268 | 5 | .212 | C>T | 1.010 | .604 | A |
| IL13 | HapA1 | | | | | 3.247 | .197 | |
| IL13 | HapA4 | | | | | 0.727 | .695 | |
| IL17A | rs4711998 | 51881422 | 6 | .346 | G>A | 2.290 | .318 | A |
| IL17A | rs8193036 | 51881562 | 6 | .327 | T>C | 4.927 | .085 | A |
| IL17A | rs3819024 | 51881855 | 6 | .372 | A>G | 1.002 | .606 | A |
| IL17A | rs2275913 | 51882102 | 6 | .361 | G>A | 2.172 | .338 | A |
| IL17A | rs3804513 | 51884266 | 6 | .023 | A>T | n/a | n/a | n/a |
| IL17A | rs7747909 | 51885318 | 6 | .217 | G>A | 1.470 | .479 | A |
| NFKB1 | rs3774933 | 103645369 | 4 | .409 | T>C | 2.139 | .343 | A |
| NFKB1 | rs170731 | 103667933 | 4 | .397 | A>T | 1.457 | .483 | A |
| NFKB1 | rs17032779 | 103685279 | 4 | .023 | T>C | n/a | n/a | n/a |
| NFKB1 | rs230510 | 103695201 | 4 | .366 | T>A | 0.249 | .883 | A |
| NFKB1 | rs230494 | 103706005 | 4 | .477 | A>G | 0.772 | .680 | A |
| NFKB1 | rs4648016 | 103708706 | 4 | .017 | C>T | n/a | n/a | n/a |
| NFKB1 | rs4648018 | 103709236 | 4 | .025 | G>C | n/a | n/a | n/a |
| NFKB1 | rs3774956 | 103727564 | 4 | .479 | C>T | 0.502 | .778 | A |
| NFKB1 | rs10489114 | 103730426 | 4 | .025 | A>G | n/a | n/a | n/a |

| Gene | SNP | Position | Chr | MAF | Alleles | Chi Square | p-value | Model |
|---|---|---|---|---|---|---|---|---|
| NFKB1 | rs4648068 | 103737343 | 4 | .366 | A>G | 0.752 | .687 | A |
| NFKB1 | rs4648095 | 103746914 | 4 | .052 | T>C | FE | 1.000 | A |
| NFKB1 | rs4648110 | 103752867 | 4 | .205 | T>A | 2.612 | .271 | A |
| NFKB1 | rs4648135 | 103755716 | 4 | .060 | A>G | FE | 1.000 | A |
| NFKB1 | rs4648141 | 103755947 | 4 | .188 | G>A | 4.570 | .102 | A |
| NFKB1 | rs1609798 | 103756488 | 4 | .337 | C>T | 1.531 | .465 | A |
| NFKB1 | HapA1 | | | | | 0.156 | .925 | |
| NFKB1 | HapA9 | | | | | 1.261 | .532 | |
| NFKB2 | rs12772374 | 104146901 | 10 | .157 | A>G | 1.312 | .519 | A |
| NFKB2 | rs7897947 | 104147701 | 10 | .229 | T>G | 0.554 | .758 | A |
| NFKB2 | rs11574849 | 104149686 | 10 | .085 | G>A | 1.360 | .507 | A |
| NFKB2 | rs1056890 | 104152760 | 10 | .317 | C>T | FE | .025 | D |
| TNFA | rs2857602 | 31533378 | 6 | .341 | T>C | 0.864 | .649 | A |
| TNFA | rs1800683 | 31540071 | 6 | .390 | G>A | 0.119 | .942 | A |
| TNFA | rs2239704 | 31540141 | 6 | .335 | G>T | 1.020 | .601 | A |
| TNFA | rs2229094 | 31540556 | 6 | .278 | T>C | 1.562 | .458 | A |
| TNFA | rs1041981 | 31540784 | 6 | .386 | C>A | 0.076 | .963 | A |
| TNFA | rs1799964 | 31542308 | 6 | .224 | T>C | 4.349 | .114 | A |
| TNFA | rs1800750 | 31542963 | 6 | .016 | G>A | n/a | n/a | n/a |
| TNFA | rs1800629 | 31543031 | 6 | .149 | G>A | 1.219 | .544 | A |
| TNFA | rs1800610 | 31543827 | 6 | .100 | C>T | 1.258 | .533 | A |
| TNFA | rs3093662 | 31544189 | 6 | .074 | A>G | 2.396 | .302 | A |
| TNFA | HapA1 | | | | | 3.187 | .203 | |
| TNFA | HapA5 | | | | | 2.905 | .234 | |
| TNFA | HapA6 | | | | | 2.526 | .283 | |

A = additive model, Chr = chromosome, D = dominant model, IFNG = interferon gamma, IL = interleukin, MAF = minor allele frequency, n/a = not assayed because SNP violated Hardy-Weinberg expectations, NFKB = nuclear factor kappa beta, R = recessive model, SNP= single nucleotide polymorphism, TNFA = tumor necrosis factor alpha

## Table 2

Differences in Demographic and Clinical Characteristics Between Low (n=158) and High (n=219) Sustained Sleep Disturbance Classes

| Characteristic | Low Sustained n=158 (41.9%) Mean (SD) | High Sustained n=219 (58.1%) Mean (SD) | Statistic and p-value |
|---|---|---|---|
| Age (years) | 57.7 (12.1) | 53.0 (10.9) | t=3.93, p<0.0001 |
| Education (years) | 15.5 (2.6) | 15.9 (2.7) | t=−1.34, p=0.18 |
| Karnofsky Performance Status score | 96.5 (6.8) | 90.9 (11.7) | t=5.76, p<0.0001 |
| Self-administered Comorbidity Questionnaire score | 3.7 (2.4) | 4.8 (3.1) | t=−3.86, p<0.0001 |
| Total number of breast biopsies in the past year | 1.5 (0.9) | 1.5 (0.8) | U, p=0.62 |
| | N (%) | N (%) | |
| Ethnicity | | | |
| White | 102 (65) | 136 (62.4) | |
| Black | 11 (7.0) | 28 (12.8) | $\chi^2$=4.46, p=0.22 |
| Asian/Pacific Islander | 24 (15.3) | 24 (11.0) | |
| Hispanic/Mixed ethnic background/Other | 20 (12.7) | 30 (13.8) | |
| Married/partnered (% yes) | 62 (39.5) | 92 (42.6) | FE p=0.60 |
| Lives alone (% yes) | 37 (23.7) | 50 (23.1) | FE p=0.90 |
| Working for pay (% yes) | 86 (54.4) | 93 (43.1) | FE p=0.04 |
| Stage of disease at diagnosis | | | |
| Stage 0 | 25 (15.8) | 40 (18.3) | |
| Stage I | 72 (45.6) | 74 (33.8) | |
| Stage IIA and IIB | 50 (31.6) | 84 (38.4) | U p=0.24 |
| Stage IIIA, IIIB, IIIC, and IV | 11 (7.0) | 21 (9.6) | |
| Type of Surgery | | | |
| Breast Conservation | 131 (82.9) | 177 (80.8) | FE p=0.69 |
| Mastectomy | 27 (17.1) | 42 (19.2) | |
| Gone through menopause (% yes) | 104 (68.0) | 130 (61.0) | FE p=0.19 |
| Sentinel node biopsy (% yes) | 138 (87.3) | 174 (79.5) | FE p=0.053 |
| Axillary lymph node dissection (% yes) | 52 (32.9) | 92 (42.2) | FE p=0.07 |
| Breast reconstruction at time of surgery (% yes) | 30 (19.1) | 45 (20.5) | FE p=0.79 |
| Neoadjuvant chemotherapy (% yes) | 27 (17.1%) | 48 (22.0) | FE p=0.30 |
| Radiation therapy during first 6 months (% yes) | 99 (62.7%) | 117 (53.4) | FE p=0.09 |

| Characteristic | Low Sustained n=158 (41.9%) Mean (SD) | High Sustained n=219 (58.1%) Mean (SD) | Statistic and p-value |
|---|---|---|---|
| Chemotherapy during first 6 months (% yes) | 43 (27.2) | 86 (39.3) | FE p=0.02 |

Abbreviations: FE = Fisher's Exact test, SD = standard deviation, U = Mann Whitney U test

**Table 3**

Multiple Logistic Regression Analyses for IL1R2, IL13, and NFKB2 candidate gene markers

| Predictor | Odds Ratio | Standard Error | 95% CI | Z | p-value |
|---|---|---|---|---|---|
| IL1R2 haplotype | 2.08 | 0.673 | 1.101, 3.921 | 2.26 | 0.024 |
| Age | 0.85 | 0.055 | 0.747, 0.964 | −2.52 | 0.012 |
| KPS score | 0.52 | 0.095 | 0.362, 0.744 | −3.57 | <0.001 |
| SCQ score | 1.15 | 0.069 | 1.025, 1.298 | 2.38 | 0.006 |
| Adjuvant CTX | 2.43 | 0.745 | 1.330, 4.427 | 2.89 | 0.004 |
| SLNB | 0.31 | 0.126 | 0.141, 0.690 | −2.88 | 0.004 |
| Overall model fit: $\chi^2 = 60.40$, p <.0001 $R^2 = 0.1548$ | | | | | |
| IL13 rs1800925 | 2.21 | 0.619 | 1.277, 3.827 | 2.83 | 0.005 |
| Age | 0.85 | 0.056 | 0.743, 0.963 | −2.54 | 0.011 |
| KPS score | 0.55 | 0.098 | 0.384, 0.776 | −3.37 | 0.001 |
| SCQ score | 1.17 | 0.070 | 1.036, 1.311 | 2.54 | 0.011 |
| Adjuvant CTX | 2.19 | 0.670 | 1.203, 3.987 | 2.56 | 0.010 |
| SLNB | 0.38 | 0.152 | 0.171, 0.829 | −2.42 | 0.015 |
| Overall model fit: $\chi^2 = 63.34$, p <.0001 $R^2 = 0.1624$ | | | | | |
| NFKB2 rs1056890 | 0.53 | 0.153 | 0.306, 0.935 | −2.19 | 0.028 |
| Age | 0.84 | 0.056 | 0.739, 0.958 | −2.61 | 0.009 |
| KPS score | 0.54 | 0.098 | 0.378, 0.769 | −3.41 | 0.001 |
| SCQ score | 1.16 | 0.070 | 1.034, 1.309 | 2.51 | 0.012 |
| Adjuvant CTX | 2.32 | 0.706 | 1.274, 4.208 | 2.75 | 0.006 |
| SLNB | 0.35 | 0.140 | 0.161, 0.769 | −2.62 | 0.009 |
| Overall model fit: $\chi^2 = 60.05$, p <.0001 $R^2 = 0.1539$ | | | | | |

Multiple logistic regression analysis of candidate gene associations with lower sustained versus higher sustained sleep disturbance groups. 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 haplotype A2 composed of rs11674595-rs7570441: zero, one, or two doses of the T-A haplotype); IL13 rs1800925: CC versus CT + TT; NFKB2 rs1056890: CC versus CT + TT), age (in 5 year increments), KPS score (in 10 point increments), SCQ score, receiving adjuvant chemotherapy in the six months following surgery for breast cancer, and having undergone a SLNB.

Abbreviations; CI =confidence interval; CTX = chemotherapy; IL13 = interleukin 13; IL1R2= interleukin 1 receptor 2; KPS = Karnofsky Performance Status; NFKB2 = nuclear factor kappa beta 2; SCQ = Self-administered Comorbidity Questionnaire; SLNB = sentinel lymph node biopsy