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Associations Between Pro- and Anti-Inflammatory Cytokine Genes
and a Symptom Cluster of Pain, Fatigue, Sleep Disturbance, and
Depression in Patients Prior to Surgery for Breast Cancer

by

Sy-Huey Doong

THESIS

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by

Sy-Huey Doong

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Associations Between Cytokine Genes and a Symptom Cluster of Pain, Fatigue, Sleep
Disturbance, and Depression in Patients Prior to Breast Cancer Surgery

Sy-Huey Doong, RN, MS

Abstract

Pain, fatigue, sleep disturbance, and depression are common and frequently occurring symptoms in oncology patients. This symptom cluster is often attributed to the release of pro-inflammatory cytokines. The purposes of this study were to determine whether distinct latent classes of patients with breast cancer (n=398) could be identified based on their experience with this symptom cluster; determine whether patients in these latent classes differed on demographic and clinical characteristics; and to determine whether variations in cytokine genes were associated with latent class membership. Three distinct latent classes were identified: (All Low (61.0%), Low Pain and High Fatigue (31.6%), All High (7.1%)). Compared to patients in the All Low class, patients in the ALL High class were significantly younger, had less education, were more likely to be Non-White, had a lower annual income, were more likely to live alone, had a lower functional status, had a higher comorbidity score, and had more advanced disease. Significant associations were found between interleukin (IL) 6 rs2069845, IL13 rs1295686, and tumor necrosis factor alpha rs18800610 and latent class membership. Findings suggest that variations in pro- and anti-inflammatory cytokine genes are associated with this symptom cluster in breast cancer patients.

Key words: pain; fatigue; sleep disturbance; depression; symptom cluster, cytokines, candidate genes; interleukin, tumor necrosis factor alpha

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1. INTRODUCTION

Pain, fatigue, sleep disturbance, and depression are common and frequently co-occurring symptoms reported by oncology patients [1-4]. Recent studies identified that these symptoms are related to each other and can form a symptom cluster [5-9]. This symptom cluster is associated with significant decrements in oncology patients' functional status and quality of life [10-13]. Evidence suggests that the model of cytokine-induced sickness behavior may be a potential mechanism to explain the occurrence of this symptom cluster in oncology patients [14-16].

Sickness behavior refers to the physiologic changes and associated behaviors (e.g., depression, sleep disturbance, hyperalgesia, drowsiness, anorexia, cognitive impairment, decreased social interaction) that develop in individuals with an infection [17,18] or through the administration of agents that trigger an inflammatory response [19-21]. Sickness behavior occurs as a result of the release of pro-inflammatory cytokines from the central nervous system. A growing body of evidence suggests that some of the most common symptoms reported by oncology patients are associated with changes in pro- and anti-inflammatory cytokines [22,23].

For example, in recent studies of oncology patients, sleep disturbance and increased fatigue were associated with changes in a number of serum interleukins [24-27]. Recent work from our research team identified associations between a polymorphism in tumor necrosis factor alpha (TNFA) and increased levels of fatigue and sleep disturbance in oncology patients and their family caregivers [28]. In addition, higher levels of interleukin 6 (IL6) and TNF α [29-31] or polymorphisms in interleukin 1 receptor 2 (IL1R2), interleukin 10 (IL10), and TNFA [32] were associated with depressive symptoms. Finally, elevated plasma levels of pro-inflammatory cytokines, including IL1- β , IL6, and TNF α , were associated with the development and maintenance of

neuropathic pain [33]. Collectively, these findings suggest that variations in cytokine genes may influence the symptom experience of oncology patients.

In a recent study of oncology patients and their family caregivers, we used latent class analysis (LCA) to identify subgroups of participants with distinct symptom profiles and evaluated for associations between polymorphisms in a number of cytokine genes and participants who reported high levels of pain, fatigue, sleep disturbance, and depression. A significant association was found in that individuals who were homozygous for the rare allele in IL4 rs2243248 were more likely to be classified into the “all high” symptom group [34]. However, this heterogeneous and relatively small sample limited our ability to identify additional genetic associations. In this study, we sought to replicate this finding in a larger and more homogeneous sample of patients with breast cancer.

While surgery can trigger the release of cytokines, several studies found that fatigue, pain, sleep disturbance, and depression can occur prior to and may trigger higher levels of cancer-related symptoms after breast cancer surgery [35-37]. For example, in one study, patients with chronic preoperative pain were three times more likely to report postoperative pain compared to patients without preoperative pain [38]. In addition, patients who developed chronic pain after breast cancer surgery reported higher preoperative levels of depression and anxiety [36,39].

To our knowledge, no studies have evaluated associations between the symptom cluster of pain, fatigue, sleep disturbance, and depression and variations in cytokine genes in patients prior to breast cancer surgery. Therefore, the purposes of this study were to: determine whether distinct latent classes of patients with breast cancer could be identified based on their experience with the symptom cluster of pain, fatigue, sleep disturbance, and depression; determine whether patients in these latent classes differed

on demographic and clinical characteristics; and determine whether genetic variations in pro- and anti- inflammatory cytokines were associated with latent class membership.

2. METHODS

2.1 Patients and Settings

This longitudinal study is part of a larger study that evaluated for neuropathic pain and lymphedema in a sample of women who underwent breast cancer surgery [39-42]. Patients were recruited from Breast Care Centers located in a Comprehensive Cancer Center, two public hospitals, and four community practices. Patients were eligible to participate if they were an adult woman (≥ 18 years) who would undergo breast cancer surgery on one breast; were able to read, write, and understand English; agreed to participate; and gave written informed consent. Patients were excluded if they were having breast cancer surgery on both breasts and/or had distant metastasis at the time of diagnosis. A total of 516 patients were approached to participate, 410 were enrolled in the study (response rate 79.5%), and 398 completed the study questionnaires. The major reasons for refusal were: too busy, overwhelmed with the cancer diagnosis, or insufficient time available to do baseline assessment prior to surgery.

2.2 Instruments

The demographic questionnaire obtained information on age, education, ethnicity, marital status, employment status, living situation, and financial status. The Karnofsky Performance Status (KPS) scale is widely used to evaluate functional status in patients with cancer and has well established validity and reliability [43,44]. Patients rated their functional status using the KPS scale that ranged from 30 (I feel severely disabled and need to be hospitalized) to 100 (I feel normal; I have no complaints or symptoms). Patients were asked to indicate if they exercised on a regular basis (yes/no format).

The Self-Administered Comorbidity Questionnaire (SCQ) is a short and easily understood instrument that was developed to measure comorbidity in clinical and health service research settings [45]. 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 [45-49].

Patients were asked to rate the intensity of their average and worst pain, in the past week, using a 0 (no pain) to 10 (worst imaginable pain) numeric rating scale (NRS). The NRS is a valid and reliable measure of pain intensity [50].

The Center for Epidemiologic Studies-Depression (CES-D) scale consists of 20 items selected to represent the major symptoms in the clinical syndrome of depression. Scores can range from 0 to 60, with scores of ≥ 16 indicating the need for individuals to seek clinical evaluation for major depression. The CES-D has well established concurrent and construct validity [51-53]. Cronbach’s alpha for the CES-D was .90.

The General Sleep Disturbance Scale (GSDS) consists of 21 items designed to assess sleep disturbance in the past week. Each item was rated on a 0 (never) to 7 (everyday) NRS. The GSDS total score is the sum of the 21 items that can range from 0 (no disturbance) to 147 (extreme sleep disturbance). A GSDS total score of ≥ 43 indicates a significant level of sleep disturbance [11,54-56]. The GSDS has well-established validity and reliability in shift workers, pregnant women, and patients with cancer and HIV [57-59]. Cronbach’s alpha for the GSDS total score was .86.

The Lee Fatigue Scale (LFS) consists of 18 items designed to assess physical fatigue and energy.[60] Each item was rated on a 0 to 10 NRS. Total fatigue and energy scores were calculated as the mean of the 13 fatigue items and the 5 energy items, with higher scores indicating greater fatigue severity and higher levels of energy. Respondents were asked to rate each item based on how they felt “right now”. The LFS has been used with healthy individuals [60,61] and in patients with cancer and HIV [11,59,62,63]. A cutoff score of ≥ 4.4 indicates high levels of fatigue [64]. A cutoff score of ≤ 4.8 indicates low levels of energy [64]. The LFS has well established validity and reliability. Cronbach’s alphas for fatigue and energy were .96 and .93, respectively.

2.3 Study Procedures

The study was approved by the Committee on Human Research at the University of California, San Francisco and by the Institutional Review Boards at each of the study sites. During the patient’s preoperative visit, a clinician explained the study to the patient, determined her willingness to participate, and introduced the patient to the research nurse. The research nurse determined eligibility and obtained written informed consent prior to surgery. After obtaining written informed consent, patients completed the enrollment questionnaires.

2.4 Methods of Analysis for Phenotypic Data

Descriptive statistics and frequency distributions were generated for the sample characteristics and symptom data. 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 across groups. A p-value of $<.05$ is considered statistically significant.

Latent class analysis (LCA) was used to identify subgroups of patients with similar experiences with the symptom cluster of pain, fatigue, sleep disturbance, and

depression (i.e., latent classes) [65,66]. Conceptually similar to cluster analysis [67], LCA identifies latent classes based on an observed response pattern [68,69].

LCA has several advantages over cluster analysis. LCA is model-based and generates probabilities for group membership. In addition, statistical fit indices are used to assess model fit and to determine the number of classes. The final number of latent classes is identified by evaluating the Bayesian Information Criterion (BIC), the parametric bootstrapped likelihood ratio test (BLRT), and entropy. The model that fits the data best has the lowest BIC and/or BLRT which indicates that the estimated model is a better fit than the model with one fewer class [70]. In addition, better-fitting models should produce higher entropy values [71]. Finally, well-fitting models “make sense” conceptually and the estimated classes differ as might be expected on variables not used in the generation of the model [70].

Latent class models often use categorical variables [72,73]. As in this study, when continuous variables are analyzed (i.e., pain fatigue, sleep disturbance, and depression scores), LCA is called latent class profile analysis (LCPA). However, in this study one of the continuous variables, namely “worst pain,” which was reported on a 0 to 10 NRS, had a large number of zeros because a number of the patients did not report pain prior to surgery. Therefore, the number of zeros was accommodated by modeling worst pain as a “two part” variable. In this type of model, the variable is examined with one “part” representing the difference between those who reported no pain compared to those who reported any pain, and with the second “part” differentiating among those who reported any pain on the remaining portion of the NRS (i.e., the 1 to 10 part of the NRS) [74, 75].

The LCPA was performed using MplusTM Version 6 [75,76]. Estimation was carried out with robust Maximum-Likelihood (MLR) and the Expectation-Maximization (EM) algorithm [65]. Due to the inclusion of a categorical variable (i.e., the binary

variable for the occurrence of pain versus no pain), Gauss-Hermite adaptive numeric integration with 20 integration points was employed. Subsequent analyses of differences among the identified classes were carried out with the Statistical Package for the Social Sciences (SPSS) Version 19 for Windows™ [77].

2.5 Methods of Analysis for Genomic Data

2.5.1 Gene Selection - Cytokines and their receptors are classes of polypeptides that mediate inflammatory processes [78]. These polypeptides are divided into pro- and anti-inflammatory cytokines. Pro-inflammatory cytokines promote systemic inflammation and include: interferon gamma (IFNG) 1, IFNG receptor 1 (IFNGR1), IL1R1, IL2, IL8, IL17A, nuclear factor kappa beta 1 (NFKB1), NFKB2, and TNFA [23,78]. Anti-inflammatory cytokines suppress the activity of pro-inflammatory cytokines and include: IL1R2, IL4, IL10, and IL13 [23,78]. Of note, IFNG1, IL1B, and IL6 possess pro- and anti-inflammatory functions [23].

2.5.2 Blood collection and genotyping - Of the 398 patients who completed the baseline assessment, 302 provided a blood sample. Genomic deoxyribonucleic acid (DNA) was extracted from archived buffy coats using the PUREGene DNA Isolation System (Invitrogen, Carlsbad, CA). Genotyping was performed blinded to LCPA status and positive and negative controls were included. 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). Samples were genotyped using the GoldenGate genotyping platform (Illumina, San Diego, CA) and processed according to the standard protocol using GenomeStudio (Illumina, San Diego, CA). Signal intensity profiles and resulting genotype calls for each single nucleotide polymorphism (SNP) were visually inspected by two blinded reviewers. Disagreements were adjudicated by a third reviewer.

2.5.3 SNP Selection - A combination of tagging SNPs and literature driven SNPs were selected for analysis. Tagging SNPs were required to be common (i.e.,

estimated to have a minor allele frequency $\geq .05$) 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 of $<95\%$ 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 quality control filters and were included in the genetic association analyses. Potential functional roles of SNPs associated with the symptoms of pain, fatigue, sleep disturbance, and depression were examined using PUPASuite 2.0 [79], 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).

2.5.4 Statistical Analyses - 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 patients' genotypes with Haploview 4.2. LD-based haplotype block definition was based on D' confidence interval [80].

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 [81]. In order to improve the stability of haplotype inference, the haplotype construction procedure was repeated five times using different seed numbers with each cycle. Only haplotypes that were inferred with probability estimates of $\geq .85$, across the five iterations, were retained for

downstream analyses. Haplotypes were evaluated assuming a dosage model (i.e., analogous to the additive model).

Ancestry informative markers (AIMS) were used to minimize confounding due to population stratification [82-84]. Homogeneity in ancestry among patients was verified by principal component analysis [85], using Helix Tree (Golden Helix, Bozeman, MT). Briefly, the number of principal components (PCs) was sought which distinguished the major racial/ethnic groups in the sample by visual inspection of scatter plots of orthogonal PCs (i.e., PC 1 versus PC2, PC2 versus PC3). This procedure was repeated until no discernible clustering of patients by their self-reported race/ethnicity was possible (data not shown). One hundred and six AIMS were included in the analysis. The first three PCs were selected to adjust for potential confounding due to population substructure (i.e., race/ethnicity) by including the three covariates in all regression models.

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 relationship between genotype and LCPA group membership. A backwards stepwise approach was used to create the most parsimonious model. Genetic model fit and both unadjusted and covariate-adjusted odds ratios were estimated using STATA version 9 [86].

As was done in our previous studies [34,40,87], based on recommendations in the literature [88,89], 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 regression analyses that controlled for differences in phenotypic characteristics, potential confounding due to population stratification, and variation in other SNPs/haplotypes within the same gene. Only those SNPs that remained significant 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 SNPs passing quality control criteria in Table 1 to allow for subsequent comparisons and meta-analyses.

3. RESULTS

3.1 Results of the Latent Class Analysis

Using LCPA, three distinct classes of patients were identified based on their experiences with the symptoms of pain, fatigue, sleep disturbance, and depression. The fit indices for the candidate models are shown in Table 2. The three class solution was selected because its BIC was lower than the BIC for both the 2- and 4-class solutions. As summarized in Table 3, the largest percentage of patients (61.0%) was classified in the “All Low” class and had mean scores for all symptoms that were below the clinically meaningful cutoff scores. A second group, that comprised 7.1% of the patients, was classified as the “All High” class. All four of the symptom scores were above the clinically meaningful cutoff scores. The third class, comprised of 31.6% of the sample, was classified as the “Low Pain and High Fatigue” class.

3.2 Differences in demographic and clinical characteristics among the three latent classes

As shown in Table 4, significant differences were found among the three latent classes in age, years of education, ethnicity, living arrangements, annual income, KPS score, estrogen and progesterone receptor status, and receipt of neoadjuvant CTX.

3.3 Differences in demographics and clinical characteristics between the All Low and the All High latent classes

Because the subsequent genomic analyses were done using an extreme phenotype approach,[90] differences in demographic and clinical characteristics between the All Low and the All High latent classes are summarized in Table 4. Compared to the All Low class, patients in the All High class were significantly younger, had fewer years of education, were more likely to be nonwhite, were more likely to have a lower annual household income, and were more likely to live alone. In addition, compared to the All Low class, patients in the All High class had a lower functional

status score, a higher comorbidity score, and a more advanced stage of disease at the time of diagnosis.

3.4 Candidate gene analyses for the two latent classes

As summarized in Table 1, the genotype frequency was significantly different between the two latent classes for nine SNPs and two haplotypes spanning 7 genes (i.e., IL1R1, IL6, IL13, IL17A, NFKB1, NFKB2, TNFA). One haplotype (HapA2, $p=.010$) was identified in IL1R1. For the two SNPs identified in IL6 (rs1554606, rs2069845), a recessive model (both, $p<.04$) fit the data best. Two SNPs (rs1295686, rs20541) and one haplotype (HapA1, $p<.0001$) were identified in IL13. For these two SNPs, a dominant model fit the data best ($p<.0001$, $p=.041$, respectively). For the SNP in IL17A (rs2275913), a dominant model fit the data best ($p=.048$). For the two SNPs identified in NFKB1 (rs4648110, rs4648141), an additive model fit the data best ($p=.009$, $p=.031$, respectively). For the one SNP identified in NFKB2 (rs1056890), a dominant model fit the data best ($p=.026$). For the one SNP identified in TNFA (rs1800610), a dominant model fit the data best ($p=.039$).

3.5 Regression analyses of IL1R1, IL6, IL13, IL17A, NFKB1, NFKB2, and TNFA genotypes and haplotypes and latent class membership

In order to better estimate the magnitude (i.e., odds ratio, OR) and precision (95% confidence interval, CI) of the association between genotype and latent class membership, multivariate logistic regression models were fit. In addition to genotype, the phenotypic variables included in the regression models were genomic estimates of and self-reported race/ethnicity (i.e., White, Black, Asian, Hispanic/Mixed Ethnic Background/other), age, KPS score, and living arrangements.

The only genetic associations that remained significant in the multivariate regression analyses were for IL6 rs2069845, IL13 rs1295686, and TNFA rs1800610 (Table 5). In the regression analysis for IL6 rs2069845, carrying two doses of the rare G

allele (i.e., AA+AG versus GG) was associated with a 14-fold increase in the odds of being in the All High latent class. Of note, IL6 rs1554606 and IL6 rs2069845 were completely collinear (i.e., mutual surrogates). Therefore, only one of the two SNPs (i.e., rs2069845) was selected to represent these two SNPs.

In the regression analysis for IL13 rs1295686, carrying one or two doses of the rare A allele (i.e., GG versus GA+AA) was associated with a 29-fold increase in the odds of being in the All High latent class. In the regression analysis for TNFA rs1800610, carrying one or two doses of the rare T allele (i.e., CC versus CT+TT) was associated with a 5-fold increase in the odds of being in the All High latent class.

4. DISCUSSION

To the best of our knowledge, this study is the first to identify distinct subgroups of women prior to breast cancer surgery based on their experience with the symptom cluster of pain, fatigue, sleep disturbance, and depression. The LCPA identified three relatively distinct latent classes: patients who reported low levels of all four symptoms (i.e., All Low, 61%); those who reported high levels of all four symptoms (i.e., All High, 7.1%); and those who reported low levels of pain and high levels of fatigue (31.6%). While our previous study of oncology patients and their FCs identified three distinct latent classes [34], only the All High classes, in both studies, reported similar symptom severity scores and constituted a similar percentage of the study participants (i.e., 12.3% in previous study versus 7.1% in the current study). While some of the differences in the symptom severity scores associated with latent classes may be attributable to differences in sample characteristics, two characteristics were associated with membership in the All High classes. In both studies, compared to participants in the other classes, participants in the All High class were younger and reported lower KPS scores. An emerging and consistent finding in our research [10,34,87] and others [91,92] is that younger age and poorer functional status are associated with a higher symptom burden.

Three SNPs, among three cytokine genes (i.e., IL6, IL13, TNFA), were associated with latent class membership. Taken together, these findings support our hypothesis that cytokine genes are involved in the symptom cluster of pain, fatigue, sleep disturbance, and depression that mimics sickness behavior in animals and humans [18,93].

In this study, carriers who were homozygous for the rare allele in IL6 rs2069845 had an increased odds of belonging to the All High class. While this SNP is located in the intronic region of the IL6 gene, it resides in region that is a likely ribosomal binding

protein (RBP) binding site for two RBP [94]. In addition, recent findings suggest that it plays a role in the modulation of inflammatory responses. In one study [95], carriers of the rare allele were at higher risk for the occurrence of leprosy Type 2 reactions. These Type 2 reactions present as disseminated painful erythematous tender nodules with or without neuritis, usually followed by systemic symptoms (e.g., fever, malaise). This Type 2 reaction was associated with elevated levels of TNF α . In another study [96], the minor allele was associated with an increased risk for childhood obesity. Interestingly, a systemic inflammatory response appears to occur in children with obesity that is related to elevated levels of inflammatory cytokines being produced by excessive amounts of adipose tissue [97]. In another study of patients with myocardial infarction [98], the rare allele was associated with increased circulating levels of IL-6. Finally, findings from our research group and others suggest that additional SNPs in IL6 are associated with the symptoms of fatigue [99], sleep disturbance [87,99], and depression [30,31]. Taken together, these findings suggest that variations in the IL6 gene contribute to inflammatory responses and symptoms associated with sickness behavior.

In terms of TNFA, patients who were homozygous for the rare allele in rs1800610 were more likely to be in All High class. This SNP is located in an intronic region of the gene. Multiple lines of evidence suggest that IL6 rs1800610 is involved in gene regulation. It resides in a RPB site, a transcription factor binding site, and a regulation sensitive region of the TNFA gene [94]. No studies were identified that evaluated the association between variations in this SNP and common symptoms experienced by oncology patients. However, TNF α is an inducer of a diverse number of processes that initiate and perpetuate inflammatory responses. A number of lines of evidence suggested that changes in TNF α levels are associated with pain, fatigue, sleep disturbance, and depression. For example, the expression of this pro-inflammatory cytokine increases in neurons following a painful stimulus [100]. In addition, a recent

meta-analysis reported significantly higher serum concentrations of $\text{TNF}\alpha$ in patients with major depression [30].

In a previous study of oncology patients and their FCs [28], we found an association between a different SNP in TNFA (rs1800629) and the severity of sleep disturbance and morning fatigue. Compared to participants who were homozygous for the common allele, participants who were heterozygous or homozygous for the rare allele reported lower levels of sleep disturbance and morning fatigue. In addition, in the same sample, participants with one or two doses of the rare allele were less likely to be categorized into a subsyndromal depression class (i.e., CES-D scores of ~ 15) compared to patients in the resilient class (i.e., CES-D scores of ~ 5) [32]. While in vitro evidence suggests that TNFA rs1800629 is a functional SNP, studies of the magnitude and direction of this SNPs' gene's expression have yielded conflicting results. In the current study, it is not clear why this SNP was not associated with membership in the All High Class. Research is needed to identify additional SNPs in TNFA that are associated with higher and lower levels of pain, fatigue, sleep disturbance, and depression in oncology patients.

In this study, a SNP in an anti-inflammatory cytokine gene was associated with membership in the All High class. Patients who were heterozygous or homozygous for the rare allele in IL13 rs1295686 were 29 times more likely to be classified in the All High class. It is not readily apparent why this SNP was not associated with membership in the All High class in our previous study [34]. However, in this same sample of women with breast cancer, we found that each dose of the rare A allele in IL13 rs1295686 was associated with a 1.6 fold increase in the odds of reporting pain in the breast prior to surgery [40].

While rs1295686 is located in intron 3 of the IL13 gene, it resides in a region of the IL13 gene that is a binding site for the NF κ B transcription factor [101,102] and it

occurs within a region of IL13 that undergoes DNA methylation. Preclinical studies suggest that IL13 has antinociceptive effects in mice. This effect may be mediated through inhibition of the release of TNF α [103] and IL1 β [104]. In addition, in a preclinical study by Karam and colleagues [104], mice that were infected with a low dose of *Leishmania* (that induces a sustained hyperalgesia) and received concomitant administration of IL-13 (that reduced the hyperalgesic response), produced increased levels of IL6. Karam and colleagues concluded that the anti-hyperalgesia effect of IL13 was independent of its effect on IL6 release. Given the associations between latent class membership and variations in IL6 and IL13 found in the current study, additional research with larger samples is warranted to examine the relationships between these two molecular markers and the severity of pain, fatigue, sleep disturbance, and depression not only as a symptom cluster but as individual symptoms.

In terms of clinical studies, associations between IL13 rs1295686 and Immunoglobulin E (IgE) dysfunction were evaluated in the Framingham Heart study [105] and in a study that evaluated total IgE concentrations in cord blood samples [106]. In both of these studies, the rare allele in IL13 rs1295686 was associated with decreases in IgE concentrations. While no additional clinical studies were found on associations between this SNP and other symptoms, the preclinical and clinical findings suggest that additional research is warranted on the role of the IL13 gene, in conjunction with other pro- and anti-inflammatory cytokine genes in the symptom experience of oncology patients.

In our previous study of oncology patients and their family caregivers [34], an association was found with another anti-inflammatory cytokine gene (i.e., IL4 rs2243248) and membership in the All High symptom class. Participants who were heterozygous or homozygous for the rare allele were 6 times more likely to be classified in the All High class. In the current study, no association was found between this SNP and the All High

class. The reasons why this SNP was not associated with latent class membership in the current study may be related to differences in demographic (e.g., gender) and clinical (e.g., cancer diagnoses) characteristics between the two samples, as well as differences in the in the symptom characteristics of the latent classes in the two studies. Additional research is needed with larger samples to explore the role of variations in IL4 and common symptoms in oncology patients.

Several limitations need to be acknowledged. First, most of the patients were Caucasian, middle-aged, well-educated, mildly overweight, married, and postmenopausal. This relatively homogenous sample of women limits the generalizability of the study findings. Second, the exact etiologies for the symptom cluster of pain, fatigue, sleep disturbance and depression warrants additional investigation. In this study, we hypothesized that sickness behavior associated with inflammation was the potential mechanism for this symptom cluster. However, the specific interactions that occur among IL-6, IL-13, and $TNF\alpha$ and how they trigger these four symptoms remain to be determined. In addition, each cytokine may contribute in a differential manner to the development of these four symptoms. Therefore, additional research is warranted to evaluate each single gene's role in the development of sickness behavior and to evaluate for interactions among multiple genes, in order to understand the causes of symptom clusters. Third, patients in the current study reported a comorbidity score between four and five. Therefore, we cannot rule out the fact that some of the associations identified in this study might be attributable to other chronic conditions. Finally, it is possible that some SNPs in cytokine genes were not identified due to the small sample size in the All High class. A larger sample is needed to increase the power to detect differences in other cytokine genes in future studies.

5. CONCLUSIONS

In conclusion, findings from this study provide new evidence of genetic variations associated with the symptom cluster of pain, fatigue, sleep disturbance, and depression. The association between pro- and anti-inflammatory cytokine genes and this symptom cluster provides additional support for the role of inflammatory mechanisms in the development of these symptoms.

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Table 1. Pro- and anti-inflammatory cytokine genes and single nucleotide polymorphisms analyzed for All Low versus All High latent classes

Gene	SNP	Position	Chr	MAF	Alleles	Chi Square	p-value	Model
IFNG1	rs2069728	66834051	12	.110	G>A	5.651	.059	A
IFNG1	rs2069727	66834490	12	.384	A>G	0.690	.708	A
IFNG1	rs2069718	66836429	12	.494	C>T	0.541	.763	A
IFNG1	rs1861493	66837463	12	.266	A>G	3.496	.174	A
IFNG1	rs1861494	66837676	12	.273	T>C	2.358	.308	A
IFNG1	rs2069709	66839970	12	.003	G>T	n/a	n/a	n/a
IFNG1	HapA3					3.496	.174	
IFNG1	HapA5					0.690	.708	
IFNGR1	rs9376268	137574444	6	.254	G>A	1.262	.532	A
IL1B	rs1071676	106042060	2	.189	G>C	0.673	.714	A
IL1B	rs1143643	106042929	2	.383	G>A	0.626	.731	A
IL1B	rs1143642	106043180	2	.082	C>T	0.784	.676	A
IL1B	rs1143634	106045017	2	.187	C>T	0.695	.706	A
IL1B	rs1143633	106045094	2	.392	G>A	0.622	.733	A
IL1B	rs1143630	106046282	2	.115	C>A	3.475	.176	A
IL1B	rs3917356	106046990	2	.450	G>A	2.080	.353	A
IL1B	rs1143629	106048145	2	.389	T>C	3.944	.139	A
IL1B	rs1143627	106049014	2	.397	T>C	3.816	.148	A
IL1B	rs16944	106049494	2	.386	G>A	4.091	.129	A
IL1B	rs1143623	106050452	2	.277	G>C	0.748	.688	A
IL1B	rs13032029	106055022	2	.448	C>T	2.017	.365	A
IL1B	HapA1					3.359	.186	
IL1B	HapA4					0.626	.731	
IL1B	HapA6					0.691	.708	
IL1B	HapB1					0.547	.761	
IL1B	HapB6					1.324	.516	
IL1B	HapB8					2.000	.368	
IL1R1	rs949963	96533648	2	.223	G>A	0.235	.889	A
IL1R1	rs2228139	96545511	2	.053	C>G	FE	.184	A
IL1R1	rs3917320	96556738	2	.047	A>C	n/a	n/a	n/a
IL1R1	rs2110726	96558145	2	.317	C>T	1.805	.405	A
IL1R1	rs3917332	96560387	2	.187	A>T	2.839	.242	A
IL1R1	HapA1					5.700	.058	
IL1R1	HapA2					9.175	.010	
IL1R1	HapA3					2.839	.242	
IL1R2	rs4141134	96370336	2	.362	T>C	0.843	.656	A
IL1R2	rs11674595	96374804	2	.258	T>C	1.500	.472	A
IL1R2	rs7570441	96380807	2	.408	G>A	3.098	.212	A
IL1R2	HapA1					3.098	.212	
IL1R2	HapA2					FE	.062	
IL1R2	HapA4					1.627	.443	
IL2	rs1479923	119096993	4	.308	C>T	1.315	.518	A
IL2	rs2069776	119098582	4	.184	T>C	n/a	n/a	n/a
IL2	rs2069772	119099739	4	.241	A>G	0.883	.643	A

IL2	rs2069777	119103043	4	.047	C>T	n/a	n/a	n/a
IL2	rs2069763	119104088	4	.277	T>G	0.911	.634	A
IL2	HapA1					0.621	.733	
IL2	HapA2					0.911	.634	
IL2	HapA3					0.883	.643	
IL4	rs2243248	127200946	5	.086	T>G	0.237	.888	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	0.516	.773	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
IL4	HapA1					0.253	.881	
IL4	HapA3					0.374	.829	
IL4	HapX1					1.994	.369	
IL6	rs4719714	22643793	7	.255	A>T	1.121	.571	A
IL6	rs2069827	22648536	7	.069	G>T	FE	.398	A
IL6	rs1800796	22649326	7	.134	C>G	n/a	n/a	n/a
IL6	rs1800795	22649725	7	.285	C>G	0.191	.909	A
IL6	rs2069835	22650951	7	.061	T>C	n/a	n/a	n/a
IL6	rs2066992	22651329	7	.049	G>T	3.849	.146	A
IL6	rs2069840	22651652	7	.333	C>G	1.219	.544	A
IL6	rs1554606	22651787	7	.319	G>T	FE	.044	R
IL6	rs2069845	22653229	7	.319	A>G	FE	.044	R
IL6	rs2069849	22654236	7	.024	C>T	n/a	n/a	n/a
IL6	rs2069861	22654734	7	.056	C>T	0.288	.866	A
IL6	rs35610689	22656903	7	.259	A>G	0.137	.934	A
IL6	HapA1					0.611	.737	
IL6	HapA5					1.519	.468	
IL6	HapA8					0.379	.827	
IL8	rs4073	70417508	4	.455	T>A	3.086	.214	A
IL8	rs2227306	70418539	4	.366	C>T	1.284	.526	A
IL8	rs2227543	70419394	4	.368	C>T	0.557	.757	A
IL8	HapA1					3.086	.214	
IL8	HapA4					1.280	.527	
IL10	rs3024505	177638230	1	.129	C>T	0.434	.805	A
IL10	rs3024498	177639855	1	.204	A>G	0.708	.702	A
IL10	rs3024496	177640190	1	.421	T>C	0.302	.860	A
IL10	rs1878672	177642039	1	.416	G>C	1.378	.502	A
IL10	rs3024492	177642438	1	.190	T>A	n/a	n/a	n/a
IL10	rs1518111	177642971	1	.303	G>A	2.252	.324	A
IL10	rs1518110	177643187	1	.301	G>T	2.205	.332	A
IL10	rs3024491	177643372	1	.408	G>T	1.221	.543	A
IL10	HapA1					2.260	.323	
IL10	HapA2					1.869	.393	
IL10	HapA8					0.708	.702	
IL13	rs1881457	127184713	5	.210	A>C	1.717	.424	A

IL13	rs1800925	127185113	5	.233	C>T	4.793	.091	A
IL13	rs2069743	127185579	5	.019	A>G	n/a	n/a	n/a
IL13	rs1295686	127188147	5	.265	G>A	FE	<.0001	D
IL13	rs20541	127188268	5	.212	C>T	FE	.041	D
IL13	HapA1					15.648	<.0001	
IL13	HapA4					5.261	.072	
IL17A	rs4711998	51881422	6	.346	G>A	4.458	.108	A
IL17A	rs8193036	51881562	6	.327	T>C	3.209	.201	A
IL17A	rs3819024	51881855	6	.372	A>G	4.362	.113	A
IL17A	rs2275913	51882102	6	.361	G>A	FE	.048	D
IL17A	rs3804513	51884266	6	.023	A>T	n/a	n/a	n/a
IL17A	rs7747909	51885318	6	.217	G>A	2.664	.264	A
NFKB1	rs3774933	103645369	4	.409	T>C	2.605	.272	A
NFKB1	rs170731	103667933	4	.358	A>T	0.952	.621	A
NFKB1	rs17032779	103685279	4	.011	T>C	n/a	n/a	n/a
NFKB1	rs230510	103695201	4	.410	T>A	3.672	.159	A
NFKB1	rs230494	103706005	4	.434	A>G	0.917	.632	A
NFKB1	rs4648016	103708706	4	.010	C>T	n/a	n/a	n/a
NFKB1	rs4648018	103709236	4	.018	G>C	n/a	n/a	n/a
NFKB1	rs3774956	103727564	4	.435	C>T	1.911	.385	A
NFKB1	rs10489114	103730426	4	.018	A>G	n/a	n/a	n/a
NFKB1	rs4648068	103737343	4	.363	A>G	0.923	.630	A
NFKB1	rs4648095	103746914	4	.052	T>C	FE	.619	A
NFKB1	rs4648110	103752867	4	.170	T>A	9.344	.009	A
NFKB1	rs4648135t	103755716	4	.061	A>G	FE	1.000	A
NFKB1	rs4648141	103755947	4	.180	G>A	6.921	.031	A
NFKB1	rs1609798	103756488	4	.337	C>T	0.068	.967	A
NFKB1	HapA1					3.869	.144	
NFKB1	HapA9					1.423	.491	
NFKB2	rs12772374	104146901	10	.168	A>G	3.655	.161	A
NFKB2	rs7897947	104147701	10	.221	T>G	2.060	.357	A
NFKB2	rs11574849	104149686	10	.070	G>A	1.956	.376	A
NFKB2	rs1056890	104152760	10	.305	C>T	FE	.026	D
TNFA	rs2857602	31533378	6	.341	T>C	2.311	.315	A
TNFA	rs1800683	31540071	6	.390	G>A	0.930	.628	A
TNFA	rs2239704	31540141	6	.335	G>T	2.793	.248	A
TNFA	rs2229094	31540556	6	.278	T>C	1.580	.454	A
TNFA	rs1041981	31540784	6	.386	C>A	0.876	.645	A
TNFA	rs1799964	31542308	6	.224	T>C	1.118	.572	A
TNFA	rs1800750	31542963	6	.016	G>A	n/a	n/a	n/a
TNFA	rs1800629	31543031	6	.149	G>A	0.655	.721	A
TNFA	rs1800610	31543827	6	.100	C>T	FE	.039	D
TNFA	rs3093662	31544189	6	.074	A>G	0.299	.861	A
TNFA	HapA1					3.423	.181	
TNFA	HapA5					1.320	.517	
TNFA	HapA6					2.561	.278	

A = additive model, Chr = chromosome, D = dominant model, Hap = haplotype, IFNG = interferon gamma, IL = interleukin, MAF = minor allele frequency, n/a = not assayed because SNP violated Hardy-Weinberg expectations ($p < 0.001$) or because MAF was

<.05, NFkB = nuclear factor kappa beta, R = recessive model, SNP= single nucleotide polymorphism, TNFA = tumor necrosis factor alpha

Table 2. Fit indices for two- through four- latent class solutions

Model	LL	AIC	BIC	BLRT	Entropy
2 Class	-2487.97	5017.95	5101.40	69.83 ⁺	.84
3 Class ^a	-2469.03	4992.06	5099.35	37.89 ⁺	.75
4 Class	-2455.45	4976.89	5108.03	27.17 [*]	.81

*p < .05; ⁺p<.0001

^aThe three class solution was selected because the BIC for that solution was lower than the BIC for both the 2 and 4 class solutions.

Abbreviations: AIC = Akaike Information Criterion; BIC = Bayesian Information Criterion; BLRT = parametric bootstrapped likelihood ratio test for the K vs. K-1 model; LL = log-likelihood.

Table 3. Differences in symptom severity scores at enrollment among the three latent classes

Symptom score	All Low (1) n=241 (61.3%)	Low Pain and High Fatigue (2) n=124 (31.6%)	All High (3) n=28 (7.1%)	Omnibus test p-value and Sidak post hoc contrasts
	Mean (SD)	Mean (SD)	Mean (SD)	
Global Sleep Disturbance Scale score	40.1 (18.4)	59.8 (19.9)	67.6 (19.7)	p < .00005 1 < 2 and 3
Center for Epidemiological Studies – Depression Scale score	10.7 (7.7)	16.5 (9.0)	28.0 (12.6)	p < .00005 1 < 2 < 3
Worst Pain Severity score (0-10) ^b	2.4 (1.1)	2.9 (1.2)	7.6 (1.6)	p < .00005 1 and 2 < 3
Lee Fatigue Scale score	1.5 (1.2)	5.8 (1.3)	4.6 (1.7)	p < .00005 2 > 3 > 1

^aPredicted classes based on the most likely latent class membership.

^bMeans and tests are only for patients who reported pain: Class 1 (N=20), Class 2 (N=31), Class 3 (N= 48).

Table 4. Differences in demographic and clinical characteristics among the latent classes

Characteristic	All Low (1) n = 241 (61%)	Low Pain and High Fatigue (2) n = 124 (31.6%)	All High (3) n = 28 (7.1%)	Statistic and p-value for the comparisons among the three latent classes	Statistic and p-value for the comparisons between the All Low and the All High latent classes
	Mean (SD)	Mean (SD)	Mean (SD)		
Age (years)	56.9 (12.1)	52.0 (9.4)	50.7 (9.4)	F(2,390) = 9.72, p < .0001 1 > 2 and 3	t = 2.60; p = .01
Education (years)	15.7 (2.7)	16.0 (2.6)	14.0 (2.6)	F(2,385) = 6.5, p = .002 1 and 2 > 3	t = 3.18; p = .002
Number biopsies in past year	1.5 (0.7)	1.5 (0.9)	1.7 (1.1)	NS	NS
Karnofsky Performance Status Score	95.6 (8.2)	90.4 (11.8)	84.4 (12.5)	F(2,284) = 23.0, p < .0001 1 > 2 > 3	t = 4.53; p < .0001
Self-administered Comorbidity Questionnaire Score	4.1 (2.7)	4.5 (3.1)	5.1 (2.7)	NS	t = -2.00; p = .047
Body mass index (kilograms/meter ²)	26.5 (6.1)	27.5 (6.2)	26.9 (6.7)	NS	NS
	% (N)	% (N)	% (N)		
Lives alone	22.9 (55)	23.0 (28)	46.2 (12)	X ² = 7.08, p = .029	FE; p = .016
Married/partnered	42.3 (102)	38.2 (47)	60.0 (15)	NS	NS
Ethnicity					
White	65.4 (157)	69.9 (86)	32.1 (9)	X ² = 24.3, p < .0001	X ² = 17.55; p = .001
Black	8.8 (21)	7.3 (9)	28.6 (8)		
Asian/Pacific Islander	13.3 (32)	8.1 (10)	28.6 (8)		
Hispanic/Mixed	12.5 (30)	14.6 (18)	10.7 (3)		
Income					
<\$30,000	17.7 (35)	21.9 (23)	57.1 (12)	X ² = 19.5, p = .001	X ² = 17.89; p < .0001
\$30,000 to \$99,000	43.9 (87)	35.2 (37)	28.6 (6)		

>\$100,000	38.4 (76)	42.9 (45)	14.3 (3)		
Stage of disease					
Stage 0	19.9 (48)	16.9 (21)	14.3 (4)	NS	MW = .018
Stage I	38.2 (92)	40.3 (50)	21.4 (6)		
Stage IIA and IIB	35.3 (85)	33.9 (42)	42.9 (12)		
Stage IIIA, IIIB, IIIC, and IV	6.6 (16)	8.9 (11)	21.4 (6)		
Estrogen receptor positive	82.1 (197)	69.4 (86)	67.9 (19)	$\chi^2 = 8.9, p=.012$	NS
Progesterone receptor positive	75.4 (181)	61.3 (76)	64.3 (18)	$\chi^2 = 8.3, p=.016$	NS
HER2/neu receptor positive	13.1 (28)	21.9 (25)	18.5 (5)	NS	NS
Gone through menopause	65.8 (158)	60.2 (118)	64.0 (16)	NS	NS
Received neoadjuvant therapy	16.3 (39)	27.4 (34)	21.4 (6)	$\chi^2 = 6.4, p=.041$	NS
Received hormone replacement therapy prior to cancer diagnosis	17.5 (42)	17.1 (21)	10.7 (3)	NS	NS

Abbreviations: MW = Mann Whitney U, NS = not significant, SD = standard deviation

Table 5 - Multiple Logistic Regression Analyses for IL6, IL13, and TNFA genotype

LCPA class comparison	Predictor	Odds Ratio	Standard Error	95% CI	Z	p-value
All Low versus All High (n=196)	IL6 genotype	14.22	15.125	1.768, 114.378	2.50	0.013
	Age	0.72	0.131	0.505, 1.030	-1.80	0.072
	KPS score	0.36	0.120	0.186, 0.689	-3.07	0.002
	Lives alone	9.12	7.567	1.793, 46.375	2.66	0.008
	Overall model fit: $\chi^2 = 36.05$, $p = 0.001$, $R^2 = 0.3768$					
All Low versus All High (n=196)	IL13 genotype	28.53	38.358	2.045, 397.932	2.49	0.013
	Age	0.68	0.148	0.444, 1.040	-1.78	0.075
	KPS score	0.44	0.135	0.242, 0.805	-2.67	0.008
	Lives alone	10.89	9.960	1.815, 65.376	2.61	0.009
	Overall model fit: $\chi^2 = 40.32$, $p < 0.001$, $R^2 = 0.4215$					
All Low versus All High (n=196)	TNFA genotype	4.97	3.871	1.079, 22.879	2.06	0.040
	Age	0.71	0.130	0.500, 1.026	-1.84	0.065
	KPS score	0.45	0.133	0.253, 0.806	-2.69	0.007
	Lives alone	10.77	8.864	2.145, 54.050	2.89	0.004
	Overall model fit: $\chi^2 = 33.50$, $p = 0.002$, $R^2 = 0.3502$					

For each model, the first three principal components identified from the analysis of ancestry informative markers as well as self-report race/ethnicity (i.e., White, Black, Asian/Pacific Islander, Hispanic/Mixed ethnic background/Other) 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 (IL6 rs2069845: AA + AG versus GG; IL13 rs1295686: GG versus GA + AA; TNFA rs1800610: CC versus CT + TT), age (in 5 year increments), KPS score (in 10 point increments), and if the patient lives alone (No/Yes).

Abbreviations: CI = confidence interval; KPS = Karnofsky Performance Status; IL = interleukin; LCPA = latent class profile analysis; TNFA = tumor necrosis factor alpha

Figure legend

Figure 1A - Differences between the latent classes in the percentages of patients who were homozygous or heterozygous for the common allele (AA+AG) or homozygous for the rare allele (GG) for rs2069845 in interleukin 6 (IL6). Values are plotted as unadjusted proportions with the corresponding p-value.

Figure 1B - Differences between the latent classes in the percentages of patients who were homozygous for the common allele (GG) or heterozygous or homozygous for the rare allele (GA+AA) for rs1295686 in IL13. Values are plotted as unadjusted proportions with the corresponding p-value.

Figure 1C - Differences between the latent classes in the percentages of patients who were homozygous for the common allele (CC) or heterozygous or homozygous for the rare allele (CT+TT) for rs1800610 in tumor necrosis factor alpha (TNFA). Values are plotted as unadjusted proportions with the corresponding p-value.

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