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Publication Date
2011

Peer reviewed|Thesis/dissertation

Evidence of an Association Between Pro- and Anti-Inflammatory Cytokine Genes
and a Symptom Cluster of Pain, Fatigue, Sleep Disturbance, and Depression

by

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THESIS

Submitted in partial satisfaction of the requirements for the degree of

MASTER OF SCIENCE

in

Nursing

in the

GRADUATE DIVISION

of the

ACKNOWLEDGEMENTS

TITLE: Evidence of an Association Between Pro- and Anti-Inflammatory Cytokine Genes and a Symptom Cluster of Pain, Fatigue, Sleep Disturbance, and Depression

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ABSTRACT

The experience of multiple symptoms related to 'sickness behavior' (e.g., pain, fatigue, sleep disturbance, and depression) can have a negative impact on an individual's functional status and quality of life (QOL). A greater understanding of the mechanisms that underlie inter-individual variability in this symptom experience is needed. The purposes of this study were to determine if distinct classes of individuals could be identified based on their experience with the symptom cluster of pain, fatigue, sleep disturbance and depression; to determine if these classes differed on demographic and clinical characteristics; and to determine if variations in a number of pro- and anti- inflammatory cytokine genes were associated with latent class membership.

This descriptive, correlational study used self report measures of pain, fatigue, sleep disturbance, and depression to evaluate symptoms in 168 outpatients with breast, prostate, lung, or brain cancer and 85 family caregivers (FCs). Three relatively distinct classes were identified using latent class profile analysis (LCPA): those who reported low depression and low pain (83.0%), those who reported high depression and low pain (4.7%), and those who reported high levels of all four symptoms (12.3%). The minor allele of IL4 rs2243248 was associated with membership in the "All high" class along with lower Karnofsky

Performance Status (KPS) score, higher number of comorbid conditions, being a patient (versus a FC), and being female. The minor allele of IL8 rs2227306 was associated with membership in the "High depression and low pain" class.

Findings suggest that LCPA can be used to differentiate distinct phenotypes based on a symptom cluster associated with sickness behavior. Identification of this distinct phenotype reveals new evidence for the role of IL4 and IL8 in the modulation of a sickness behavior symptom cluster in oncology patients and their FCs.

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INTRODUCTION

Oncology patients and their family caregivers (FC) commonly report experiencing pain, fatigue, sleep disturbance, and depression. While these symptoms can occur singly, they often co-occur and have significant deleterious effects on an individual's functional status and quality of life (QOL) (Desai et al., 2007; Dodd et al., 2011; Dodd et al., 2001; Kim et al., 2009; Fletcher et al., 2008; Granda-Cameron et al., 2008; Gwede et al., 2008).

In 2001, Dodd, Miaskowski, and Paul defined the concept of a symptom cluster as three or more concurrent symptoms that are related to each other. In addition, they were the first to suggest that specific symptom clusters could influence patient outcomes. In a subsequent publication, Miaskowski and colleagues (2007) described two conceptual approaches to guide symptom cluster research. One approach focuses on the "de novo" identification of symptom clusters using multidimensional symptom inventories. The second approach focuses on the identification of distinct subgroups of individuals based on their experience with a specific symptom cluster (e.g., pain, fatigue, sleep disturbance, depression). Since then, a number of studies have confirmed the feasibility of creating distinct subgroups of individuals based on their symptom experiences (Dodd et al., 2010; Dodd et al., 2011; Gwede et al., 2008; Miaskowski et al., 2006; Pud et al., 2008). This approach highlights the high level of variability in an individual's innate susceptibility to experiencing symptoms with different levels of severity.

Traditional epidemiologic approaches identify subgroups of individuals at

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higher risk for symptom clusters based on gender, age, ethnicity, or occurrence of comorbid conditions. However, it is now understood that molecular epidemiology, which integrates the use of biological markers that measure events at the physiological, cellular, and molecular levels may provide additional information that explains inter-individual variability in symptom severity (Reyes-Gibby et al., 2008). While the molecular mechanisms that underlie inter-individual variability in symptom experiences are not yet well-defined, recent reviews suggest that part of this variability may be the result of an individual's genetically determined ability to respond to physical and psychological stressors through changes in pro- and anti-inflammatory cytokines (Cleeland et al., 2003; Dantzer and Kelley, 2007; Miaskowski and Aouizerat, 2007).

Studies of 'sickness behavior' in animal models support the hypothesis that cytokines are key players in modulating the symptom experience of patients and their FCs. Sickness behavior, defined as a coordinated set of adaptive behavioral changes that develop in individuals during the course of an infection (Hart, 1988), was observed in individuals following the administration of inflammatory agents or specific pro-inflammatory cytokines (Dantzer, 2001; Dantzer et al., 1998; Konsman et al., 2002; Watkins and Maier, 2000; Watkins et al., 1995). Symptoms of sickness behavior include (but are not limited to) lethargy, anorexia, depression, anxiety, sleepiness, and hyperalgesia. For oncology patients, both the cancer and its treatment may trigger the release of pro-inflammatory cytokines that result in the symptoms associated with sickness behavior (Cleeland et al., 2003; Myers, 2008). In addition, for patients and their

FCs, both physical and psychosocial stressors may modulate the release of pro-inflammatory cytokines (Brydon et al., 2009). Therefore, the sickness behavior model provides a framework for evaluation of symptoms in oncology patients and their FCs (Barsevick, 2007).

The associations between common symptoms and specific pro-inflammatory cytokines are the focus of an increasing number of studies (Seruga et al., 2008). For example, over 20 pro-inflammatory cytokines and chemokines may cause hyperexcitability in pain transmission neurons or modify the activity of nociceptors that contribute to pain hypersensitivity (Maier and Watkins, 2003; Oh et al., 2001). In fact, individuals with a painful neuropathy had twofold higher pro-inflammatory interleukin (IL)-2 and tumor necrosis factor (TNF)-α mRNA levels and twofold lower mRNA levels for anti-inflammatory IL-10 than individuals with a painless neuropathy (Uceyler et al., 2007). In addition, intrathecal administration of IL-10 was shown to prevent or reverse neuropathic pain in animal models (Milligan et al., 2005). In another study, the preoperative use of pentoxifylline, which inhibits TNF-α production and leukotriene synthesis in immune cells, decreased the release of pro-inflammatory cytokines and reduced post surgical morphine consumption in patients with colorectal cancer (Lu et al., 2004).

Similar to the experience of pain, fatigue severity is associated with changes in serum concentrations of cytokines (Kurzrock, 2001). For instance, a systematic review of 18 studies showed a positive association between cancer-related fatigue and circulating levels of IL6 and IL1 receptor antagonist in patients with various cancer diagnoses (Schubert et al., 2007). Another study found

higher concentrations of tumor growth factor (TGF)-α to be associated with increased fatigue in individuals with colorectal cancer (Rich et al., 2005). Associations between single nucleotide polymorphisms (SNPs) in the promoter regions of IL6 and TNFA and increased levels of fatigue and sleep disturbance were shown in a sample of patients with cancer and their FCs (Aouizerat et al., 2009; Miaskowski et al., 2010). Additionally, a SNP in the promoter region of IL1B was found to be associated with persistent fatigue in breast cancer survivors.(Collado-Hidalgo et al., 2008)

In terms of depressive symptoms, several studies including a meta-analysis of 24 studies found significant elevations in circulating levels of pro-inflammatory cytokines, particularly IL6 and TNF-α, in patients with major depression (Alesci et al., 2005; Brambilla and Maggioni, 1998; Dowlati et al., 2010; Kiecolt-Glaser and Glaser, 2002; Lutgendorf et al., 1999; Maes et al., 1997; Maes et al., 1995). Furthermore, depressive behaviors and mood alterations including sadness, apathy, depressed mood, and suicidal ideation were observed in patients who received repeated injections of recombinant cytokines, mainly IL2 or interferon (INF)-α, for the treatment of autoimmune disease, viral infections (e.g., hepatitis C), or cancer (Capuron et al., 2004; De La Garza, 2005).

While the knowledge base continues to grow regarding the ways in which pro-inflammatory cytokines may influence individuals' experiences with single symptoms, less is known about the association between cytokines and symptom clusters. In addition, little is known about the role of anti-inflammatory cytokines

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in this phenomenon. Reyes-Gibby et al. (2008) advanced a hypothesis-driven, pathway-based approach that assessed SNPs in both pro- and anti-inflammatory cytokine genes as potential markers for genetic susceptibility for individual cancer-related symptoms. Yet, no studies have evaluated this hypothesis using a comprehensive panel of pro- and anti-inflammatory cytokines in participants who were categorized based on their experiences with the symptom cluster of pain, fatigue, sleep disturbance, and depression. Therefore, the purposes of this study were to determine if distinct latent classes of oncology patients and their FCs could be identified based on their experience with the symptom cluster of pain, fatigue, sleep disturbance, and depression and to determine if these classes differed on demographic and clinical characteristics. In addition, the study sought to determine if genetic variations in a number of pro- and anti- inflammatory cytokines were associated with latent class membership.

METHODS

Participants and Settings: This descriptive, correlational study is part of a larger, longitudinal study that evaluated multiple symptoms in both patients who underwent primary or adjuvant radiotherapy (RT) and their FCs. Although it is difficult to determine when a family member assumes the role of a caregiver, in most studies of symptoms in FCs, the caregiver role is linked to the trajectory of the patient's treatment (Swore Fletcher et al., 2008). Therefore, to obtain a "baseline" assessment of symptoms, FCs were recruited with patients before the initiation of RT. Patients and their FCs were recruited from two RT departments located in a Comprehensive Cancer Center and a community-based oncology

program at the time of the patient's simulation visit.

Patients were eligible to participate if they: were ≥18 years of age; were scheduled to receive primary or adjuvant RT for one of four cancer diagnoses (i.e., breast, prostate, lung, brain); were able to read, write, and understand English; gave written informed consent; and had a Karnofsky Performance Status (KPS) score of ≥ 60. Patients were excluded if they had: metastatic disease; more than one cancer diagnosis; or a diagnosed sleep disorder.

FCs were eligible to participate if they: were an adult (≥18years of age); were able to read, write, and understand English; gave written informed consent; had a KPS ≥ 60; were living with the patient; and did not have a diagnosed sleep disorder.

Instruments: The study instruments included a demographic questionnaire, the KPS scale (Karnofsky et al., 1948), the General Sleep Disturbance Scale (GSDS) (Lee, 1992), the Lee Fatigue Scale (LFS) (Lee et al., 1991), and the Center for Epidemiologic Studies-Depression (CES-D) Scale (Radloff, 1977). Pain was evaluated using an adaptation of the Brief Pain Inventory (BPI) (Daut et al., 1983) that obtained information on causes, intensity, locations, descriptors, and interference associated with pain.

The demographic questionnaire obtained information on age, gender, marital status, co-habitation status, education, ethnicity, employment status, and the presence of a number of comorbid conditions.

The GSDS consists of 21 items designed to assess the quality of sleep in the past week. Each item was rated on a 0 (never) to 7 (everyday) numeric rating

scale (NRS). The GSDS total score is the sum of the seven subscale scores (i.e., quality of sleep, quantity of sleep, sleep onset latency, mid-sleep awakenings, early awakenings, medications for sleep, excessive daytime sleepiness) that can range from 0 (no disturbance) to 147 (extreme sleep disturbance). Each mean subscale score can range from 0 to 7. Higher total and subscale scores indicated higher levels of sleep disturbance. Subscales scores of $\geq$ 3 and a GSDS total score of $\geq$ 43 indicate a significant level of sleep disturbance (Fletcher et al., 2008). The GSDS has well-established validity and reliability in shift workers, pregnant women, and patients with cancer and HIV (Barsevick et al.; Lee, 1992; Lee and DeJoseph, 1992; Miaskowski and Lee, 1999). In the current study, the Cronbach's alphas for the GSDS total score for patients and FCs were 0.84 and 0.79, respectively.

The LFS consists of 13 items designed to assess <u>physical</u> fatigue. (Lee, et al., 1991) Each item was rated on a 0 to 10 NRS. A total fatigue score was calculated as the mean of the 13 fatigue items, with higher scores indicating greater fatigue severity. Respondents were asked to rate each item based on how they felt "right now," within 30 minutes of going to bed (evening fatigue). The LFS has been used with healthy individuals (Gay et al., 2004; Lee et al., 1991) and with patients with cancer and HIV (Lee et al., 1999; Miaskowski et al., 2006; Miaskowski and Lee, 1999; Miaskowski et al., 2008). A cutoff score of $\geq$5.6 indicates clinically significant levels of evening fatigue (Fletcher et al., 2008). The LFS was chosen for this study because it is relatively short, easy to administer,

and has well-established validity and reliability. In this study, Cronbach's alphas for evening fatigue for patients and FCs were 0.96 and 0.95, respectively.

The CES-D consists of 20 items selected to represent the major symptoms in the clinical syndrome of depression. Scores can range from 0 to 60. Scores of $\geq$ 16 are considered clinically significant and indicate the need for individuals to seek clinical evaluation for major depression. The CES-D has well-established concurrent and construct validity (Carpenter et al., 1998; Radloff, 1977; Sheehan et al., 1995). In the current study, the Cronbach's alphas for the CES-D for patients and FCs were 0.88 and 0.84, respectively.

Multiple dimensions of pain were evaluated using a modified version of the BPI (Daut et al., 1983). Participants who responded yes to the question of having pain other than every day kinds of pain were asked to indicate the cause of their pain and to rate its intensity (i.e., now, least, average, and worst) using 0 (no pain) to 10 (worst pain imaginable) NRSs (Jensen, 2003). In addition, participants were asked to complete a body map of pain locations, to rate the quality of their pain, and to rate the pain's level of interference with function.

Study Procedures: This study was approved by the Committee on Human Research at the University of California, San Francisco and the Institutional Review Board (IRB) at the second site. At the time of the simulation visit (i.e., approximately one week prior to the initiation of RT), patients were approached by a research nurse to discuss participation in the study. After obtaining written informed consent, patients completed the demographic questionnaire, KPS scale, LFS, GSDS, CES-D, and the BPI. After recruitment, patients were asked

to identify the person most involved in their care (i.e., their FC). If the FC was with the patient, the research nurse explained the study and obtained written informed consent from the FC. FCs who were not with the patient were contacted by phone to determine their interest in study participation. The research nurse visited those FCs at home, obtained written informed consent, and had FCs complete the study questionnaires. Both patients and FCs had blood drawn for genomic analysis. In addition, height and weight were obtained for patients and their medical records were reviewed for disease and treatment information.

Methods of Analysis for Clinical 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; sometimes called latent class cluster analysis), a type of finite mixture model (Muthen and Shedden, 1999; Vermunt and Magdison, 2002) was used to identify participants with similar symptom profiles (i.e., latent classes). Based on our previous studies of the symptom cluster of pain, fatigue, sleep disturbance, and depression (Miaskowski et al., 2006; Pud et al., 2008), ratings of these symptoms from patients and their FCs were used in the LCA. Conceptually similar to cluster analysis (Everitt et al., 2001), LCA identifies latent classes based on an observed response pattern (Collins and Lanza, 2010; Nylund et al., 2007a).

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 help decide the number of classes. The final number of latent classes is typically identified by evaluating the Bayesian Information Criterion (BIC), the Vuong-Lo-Mendell-Rubin likelihood ratio test (VLMR), the parametric bootstrapped likelihood ratio test (BLRT), and entropy (the consistency between model-based latent classes and the classes to which observations are assigned). The model that fits the data best has the lowest BIC and a VLMR and/or BLRT that indicates that the estimated model is a better fit than the model with one fewer class (Nylund et al., 2007b). In addition, better-fitting models should produce higher entropy values (Celeux and Soromenho, 1996). Well fitting models have loglikelihood values that are replicated in analyses with multiple "random starts," which indicates that the solution is not based on a local maximum for the loglikelihood. 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 (Nylund et al., 2007b).

Often, latent class models use categorical variables (Collins and Wugalter, 1992; Lanza et al., 2003). In this study, continuous variables were analyzed. When continuous variables are used, LCA is called latent class profile analysis (LCPA). One of the variables, namely "worst pain," which was reported on a 0 to 10 NRS, had a large number of zeros because many of the patients and FCs did not report any pain. 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) (Muthen and Muthen, 1998-2010b; Olsen and Schafer, 2001).

An additional consideration in this analysis was that 65% of the participants were in patient-caregiver dyads. Although no differences were found in the severity of symptoms between patients and FCs within dyads, we chose to accommodate even minor dependency due to dyads by carrying out our analyses, treating the sample as a "complex" sample, clustered by dyads. In addition, because information on whether the observations were of patient or FCs could "inform" the latent class solution, "role" (i.e., patient or FC) was included as part of the model.

The LCPA was performed using Mplus^{TM} Version 6 (Muthen and Muthen, 1998-2010a, b). Estimation was carried out with robust maximum-likelihood ratio (MLR) and the expectation-maximization (EM) algorithm (Muthen and Shedden, 1999). Due to the inclusion of a categorical variable (the binary variable for the occurrence of pain versus no pain), Gauss-Hermite adaptive quadrature with 20 integration points was employed. Although the BLRT is usually employed to assist in the identification of the model that best fits the data, it could not be used in this analysis because the BLRT cannot be completed for analyses that use complex samples. Therefore, only the VLMR for the test of the K versus K-1

class models is reported. Subsequent analyses of differences among the identified classes were carried out with SPSS for Windows (SPSS, 2010).

Methods of Analysis for Genomic Data

Gene Selection: Cytokines and their receptors are classes of polypeptides that exercise a major influence on the inflammatory process. Their dysregulation is hypothesized to induce symptoms associated with "sickness behavior" (Dantzer et al., 2008; Dantzer and Kelley, 2007). These polypeptides are divided into pro- and anti-inflammatory cytokines. Pro-inflammatory cytokines promote systemic inflammation and include IL1R1, IL1R2, IL2, IL8, IL17A, and TNF-α. Anti-inflammatory cytokines suppress the activity of pro-inflammatory cytokines and include: IL4, IL10, and IL13. Of note, IL1β and IL6 possess pro- and anti-inflammatory functions.

Blood collection and genotyping: Genomic DNA was extracted from archived buffy coats maintained by the UCSF Genomic Markers of Symptoms Tissue Bank using the PUREGene DNA Isolation System (Invitrogen, Carlsbad, CA). Of the 287 participants recruited, DNA could be recovered from the archived buffy coats of 253 (i.e., 168 patients and 85 FCs). No differences were found in any demographic or clinical characteristics between individuals who did and did not choose to participate in the study or in those participants for whom DNA could not be recovered from archived specimens.

Genotyping was performed blinded to clinical status and positive and negative controls were included. DNA samples were quantitated with a Nanodrop Spectrophotometer (ND-1000) and normalized to a concentration of 50 ng/µL

(diluted in 10 mM Tris/1 mM EDTA). Samples were genotyped using the 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 SNP were visually inspected by two blinded reviewers. Disagreements were adjudicated by a third reviewer.

SNP Selection: A combination of tagging SNPs and literature driven SNPs (i.e., SNPs 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 of <95% or Hardy-Weinberg p-values of <.001 were excluded.

As shown in Table 1, 78 SNPs among the 11 candidate genes (IL1B: 12 SNPs; IL1R1: 5 SNPs; IL1R2: 3 SNPs; IL2: 5 SNPs; IL4: 9 SNPs; IL6: 12 SNPs; IL8: 3 SNPs; IL10: 8 SNPs; IL13: 5 SNPs; IL17A: 6 SNPs; TNFA: 10 SNPs) passed all quality control filters and were included in the genetic association analyses. Potential functional roles for SNPs associated with specific symptoms were examined using PUPASuite 2.0, (Conde et al., 2006) a comprehensive search engine that screens for 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: Allele and genotype frequencies were determined by gene counting. Hardy-Weinberg equilibrium was assessed by the Chi-square exact test. Measures of linkage disequilibrium (i.e., D' and r^2) were computed from the participants' genotypes with Haploview 4.1. LD-based haplotype block definition was based on the D' confidence interval method (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 five times using different seed numbers with each cycle. Only haplotypes that were inferred with probability estimates of ≥ 0.9 across the five iterations were retained for downstream analyses. Haplotypes with frequency estimates of $\leq 1\%$ were grouped into a single category.

For association tests, three genetic models were assessed for each SNP (i.e., additive, dominant, recessive). Barring trivial improvements (delta<10%), the genetic model that best fit the data, by maximizing the significance of the p-value was selected for each SNP. Both un-adjusted and adjusted associations were calculated. Logistic regression was used to control for covariates (i.e., age, gender, ethnicity, patient/ FC status, functional status (KPS score), number of comorbidities, genotype). Unadjusted and covariate-adjusted odds ratios were estimated using the STATA software package, version 9 (STATA Corp).

RESULTS

Participant characteristics: As summarized in Table 2, the majority of the participants were Caucasian, well educated, and married/partnered. Patients made up 66.4% of the total sample. The mean age of the total sample was 61.5 years. The average participant had greater than four comorbid conditions and a mean KPS score of 92. Gender was evenly represented within the total sample with 46.2% male and 53.8% female participants.

Results of LCPA: Three distinct latent classes of individuals were identified, based on their experiences with the symptoms of pain, fatigue, sleep disturbance, and depression using LCPA. The fit indices for the candidate models are shown in Table 3. As summarized in Table 4, the largest percentage of participants (83.0%) was classified in the "Low depression and low pain" class and had mean scores for all four symptoms that were below clinically meaningful cutoff scores. A second group, that comprised 4.7% of participants, was classified as the "High depression and low pain" class. High levels of depression, average levels of fatigue, and low levels of pain and sleep disturbance characterized this class. The third class, comprised of 12.3% of participants was classified as the "All high" class. Clinically meaningful levels of all four symptoms characterized this group.

Differences in demographic and clinical characteristics among the three latent classes: Significant differences among the three latent classes were found for several characteristics including age, gender, KPS score, number of comorbid conditions, distribution of patients versus FCs, and cancer diagnoses (patients

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only) (Table 2). Participants in the "All high" class were significantly younger (p=.005) and had a lower KPS score (p<.0001) than participants in the "Low depression and low pain" class. The average number of comorbid conditions of participants in the "All high" class was significantly higher than for the other two classes. Compared to the "Low depression and low pain" class, the "All high" classes had a higher percentage of female participants (p<.05). Participants in the "All high" class were more likely to be patients rather than FCs as compared to the other two classes (p=.01). Finally, the "All high" class was composed primarily of patients with breast cancer compared to the "Low depression and low pain" class that was composed primarily of patients with prostate cancer (p=.001). No significant differences were found among the latent classes in education level, ethnicity, employment status, cohabitation, or marital status.

Differences in mean symptom scores among the three latent classes: As summarized in Table 4, the mean CES-D score for the "All high" class was significantly higher than for the "Low depression and low pain" group (p<.0001). The mean CES-D scores for both the "All high" and the "High depression and low pain" classes were above the clinically meaningful cutoff score (≥16) for depressive symptoms. The mean GSDS score for the "All high" class was significantly higher than for either the "High depression and low pain" or "Low depression and low pain" classes (p<.0001). The "All high" class had clinically meaningful levels of sleep disturbance (≥43). The mean LFS score for the "All high" class was significantly higher than for the "Low depression and low pain" class (p<.0001). The mean worst pain score for the "All high" class was

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significantly higher than for either the "High depression and low pain" or "Low depression and low pain" classes (p<.0001).

Candidate gene analyses of the three LCPA classes: As summarized in Table 1, the minor allele frequency (MAF) was significantly different among the latent classes for four SNPs: IL1R1 rs2228139, IL4 rs2243248, IL8 rs2227306, and IL8 rs2227543. The minor allele of IL1R1 rs2228139 was more common in participants in the "High depression and low pain" class than in either of the other two classes (p=.013). However, post-hoc contrasts for IL1R1 rs2228139 were unable to distinguish the class(es) driving the differences between classes.

As shown in Figure 1, the minor allele of IL4 rs2243248 was more common in participants in the "All high" class than in the other two classes (p=.007). Post-hoc contrasts of IL4 rs2243248 revealed that differences among the three classes in terms of carriers of the minor allele was driven by the "All high" class as compared with the "Low depression and low pain" class (p=.008). The minor allele of IL8 rs2227306 was more common in participants in the "High depression and low pain" class than in the other two classes (p=.027). As shown in Figure 2, the minor allele of IL8 rs2227543 was more common in participants in the "High depression and low pain" class than in the "Low depression and low pain" class (p=.04). Post-hoc contrasts of IL8 rs2227306 and IL8 rs2227543 suggested that differences among the three classes in terms of rare allele homozygotes was driven by the "High depression and low pain" class as compared with the "Low depression and low pain" class (p=.020 and .026,

respectively). No significant differences were found among the latent classes for any of the haplotypes analyzed.

Regression analyses of IL-4 and IL-8 genotypes and symptom experience classification: The associations that remained significant in the multiple regression analyses were IL4 rs2243248 ("All high" class versus "Low depression and low pain" and "High depression and low pain" classes; Figure 1), IL8 rs2227306 ("Low depression and low pain" class versus "High depression and low pain" class; Figure 2), and IL8 rs2227543 ("Low depression and low pain" class versus "High depression and low pain" class). In the models fit for IL1R1 rs2228139, genotype was no longer significant after adjusting for potential covariates.

In order to better estimate the magnitude (i.e., odds ratio, OR) and precision (95% confidence interval, CI) of genotype on LCPA group membership, multiple variable logistic regression models were fit in a pairwise fashion between the three LCPA groups (i.e., the "Low depression and low pain" class compared to the "High depression and low pain" class, the "Low depression and low pain" class compared to the "All high" class, and the "High depression and low pain" class compared to the "All high" class). In addition to genotype, the phenotypic variables evaluated in the model were of gender, ethnicity (i.e., white vs non-white), patient/FC status, functional status (i.e., KPS score), and number of comorbid conditions

As shown in Table 5A, in the models fit for IL4 rs2243248, only the model that compared the "Low depression and low pain" class with the "All high" class

and the model that compared the "High depression and low pain" class with the "All high" class included a genotype predictor (rs2243248). In the model that compared the "Low depression and low pain" class with the "All high" class, IL4 rs2243248 genotype, KPS score, number of comorbid conditions, gender, and patient/FC status were the only predictors retained in the final model (p<.0001). The overall model explained 29.2% of the variance in LCPA group membership. Controlling for gender, patient/FC status, KPS score, and number of comorbid conditions, carrying a minor allele (i.e., AC + CC) was associated with over a four-fold increase in the odds of belonging to the "All high" class (OR: 4.51, 95% CI: 1.579, 12.866, p=.005). In the model that compared the "High depression and low pain" class with the "All high" class, IL4 rs2243248 genotype, number of comorbid conditions, and patient/FC status were the only predictors retained in the final model (p=.0002). The overall model explained 40.3% of the variance in LCPA group membership (Table 5). Controlling for the number of comorbid conditions and patient/FC status, carrying a minor allele (i.e., AC + CC) was associated with over a twenty-one-fold increase in the odds of belonging to the "All high" class (OR: 21.14, 95% CI: 1.647, 271.254, p=.019).

As shown in Table 5B, in the models fit for IL8 r rs2227306, only the model that compared the "Low depression and low pain" class with the "High depression and low pain" class included a genotype predictor. In the model that compared the "Low depression and low pain" class with the "High depression and low pain" class, IL8 rs2227306 genotype and ethnicity (white versus non-white) were the only predictors retained in the final model (p=.002). The overall

model explained 13.5% of the variance in LCPA group membership (Table 5). Controlling for ethnicity, carrying a minor allele (i.e., CT + TT) was associated with over a nine-fold increase in the odds of belonging to the "High depression and low pain" class (OR: 9.78, 95% CI: 2.184, 43.810, p=.003). Of note, no evidence of confounding of the genotype association by ethnicity was observed, as evidenced by the lack of a significant association between ethnicity and genotype, nor evidence of an interaction between genotype and ethnicity for odds of being in the "High depression and low pain" class. Given the near-complete linkage disequilibrium (LD) between rs2227306 and rs2227543 (only two individuals differed in their genotypes for these two SNPs), only the results of the model for rs2227306 were described, as model results were virtually identical.

DISCUSSION

This study is the first to use LCPA to characterize a sample of oncology patients and their FCs using a cluster of symptoms associated with "sickness behavior" and to identify an association between these latent classes and one pro- (i.e., IL8) and one anti- (i.e., IL4) inflammatory cytokine. The identification of distinct subgroups of individuals with different symptom experiences is consistent with our previous reports (Dodd et al., 2010; Dodd et al., 2011; Miaskowski et al., 2006; Pud et al., 2008), as well as those of others (Gwede et al., 2008). Consistent with our a priori hypothesis, differences in the severity of this "sickness behavior" symptom cluster was partially explained by genetic variation in two cytokine genes.

In this sample, the LCPA identified three relatively distinct classes of participants: those who reported low depression and low pain (83.0%); those who reported high depression and low pain (4.7%); and those who reported high levels of all four symptoms (12.3%). While previous studies from our research team identified four distinct latent classes using the same symptom cluster (Dodd et al., 2010; Miaskowski et al., 2006; Pud et al., 2008), one consistent finding across all four studies is that the "All high" class constituted between 10% and 15% (mean 13.0%) of the samples. This finding suggests that a subset of individuals may share common biologic mechanisms that influence their experience with the multiple symptoms associated with sickness behavior. Identification of the mechanism associated with a biological response may lead to the development of targeted interventions for this high-risk group.

It should be noted that LCPA is an exploratory data analysis procedure that facilitates the emergence of distinct latent classes based on similarities in their ratings of pain, fatigue, sleep disturbance, and depression. Other studies have shown that group membership, as well as the number of classes identified within the same sample, can change over time (Dodd et al., 2010; Dodd et al., 2011). Differences in the number of latent classes as well as the symptom characteristics of the various latent classes may be related to differences in sample characteristics (e.g., age, gender, number of chronic conditions); the inclusion or exclusion of FCs; as well as other unidentified phenotypic and environmental characteristics. Unfortunately, blood samples were not available from patients in our previous studies (Dodd et al., 2010; Miaskowski et al., 2006;

Pud et al., 2008), so verification of the associations between cytokine genes and latent class membership await future investigations by our research team and others.

In this cohort, carrying the minor allele for IL4 rs2243248 was associated with membership in the "All high" class along with lower KPS score, higher number of comorbid conditions, being a patient (versus a FC), and being female. Carrying the minor allele for IL8 rs2227306 was associated with membership in the "High depression and low pain" class when compared to the "Low depression and low pain" class. For both candidate genes, the results of the multiple regression analysis revealed that the genetic associations were not confounded by any of the demographic or clinical characteristics evaluated. These findings are particularly interesting because IL4 and IL8 were not evaluated or not identified as gene candidates in prior research on symptoms. Previous studies, including those published by this research team, found genetic associations with between IL1B and IL6 and severity of fatigue (Collado-Hidalgo et al., 2008), as well as associations between SNPs in IL6 and TNFA and severity of fatigue and sleep disturbance (Aouizerat et al., 2009; Miaskowski et al., 2010). The discrepancy in study findings may be related to differences in symptom phenotypes (i.e. single symptoms versus a symptom cluster). Additional research is needed to evaluate associations between single symptoms and symptom clusters and pro- and anti- inflammatory cytokine genes.

The anti-inflammatory cytokine IL4 blocks the action of a number of pro-inflammatory cytokines (i.e., IL1-β, IL6, IL8, and TNF-α) (Hamblin, 1993). The IL4 SNP identified in this study (rs2243248) is known to occur in an evolutionarily conserved region. However, no functional effect of the SNP was identified (i.e., a non-synonymous change, a localization to a transcription factor binding site, a predicted alteration in splicing, or a localization to known or predicted microRNA binding sites). Even so, while the functional significance of the SNP in rs2243248 is not completely understood, findings from this study suggest that carrying the minor allele may result in alterations in the regulation of several pro-inflammatory cytokines. This dysregulation in IL4 function places these individuals in a high-risk group for experiencing multiple symptoms related to "sickness behavior".

The neuromodulatory effects of IL4 were associated with changes in sickness behavior observed in an animal model. For example, one study found that cytokine-induced sickness behavior in rats was inhibited when IL4 was administered 12 hours prior to lipopolysaccharide (LPS) but was potentiated when IL4 was co-administered with LPS, suggesting that the regulation of sickness behavior by IL4 can be either inhibitory or stimulatory (Parnet et al., 2002). Interestingly, LPS–induced sickness behavior was found to be much more profound in IL4 (-/-) mice, which suggests a more protective role for IL4 (Lyons et al., 2009). Furthermore, Sherry and colleagues (2010) observed decreased sickness behavior in wild type mice fed a soluble fiber diet which induced the up regulation of IL4. The protective effect of the soluble fiber diet was reduced in IL-4 (-/-) mice. The literature that evaluated the association between IL4 and

sickness behavior in humans is limited. However, one study found that an eight-week meditation program increased production of IL4 and decreased production of interferon (IFN)-γ and IL10 in individuals with early stage prostate or breast cancer (Carlson et al., 2003). These changes in serum cytokines were associated with reduced symptoms of stress (including depression), increased sleep quality, and increased QOL.

Conversely, in this study a polymorphism in IL8 was associated with high levels of depression but not high levels of pain or sleep disturbance. Again, no known functional effects for the IL8 SNPs evaluated in this study (rs2227306 and rs2227543) were identified. Nonetheless, our findings suggest that IL8 may influence the development of depressive symptoms more than the other symptoms associated with sickness behavior. In fact, previous studies found an association between the over-expression of IL8 and mild to moderate levels of depressive symptoms in both healthy and acutely ill individuals (Marsland et al., 2007). In addition, compared to healthy controls, significantly higher plasma levels of IL8 were identified in individuals diagnosed with bipolar disorder, measured during both manic and depressive phases (O'Brien et al., 2006). However, a meta-analysis that evaluated serum cytokine levels in individuals meeting the Diagnostic and Statistical Manuel (DSM) criteria for major depression did not support these findings (Dowlati et al., 2010). Indeed, more recent studies suggest that changes in inflammatory markers in individuals with depressive symptoms are more complicated than previously thought (Dowlati et al., 2010; Lehto et al., 2010; Song et al., 2007). For example, Lento and

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colleagues found that in individuals with IL8 levels below the median (i.e., <2.86 pg/ml) the likelihood of depression was 2.4 higher after adjustment for age, gender, BMI, smoking and alcohol consumption, somatic illness, and medications (Lehto et al., 2010). Song et al (2007) evaluated serum levels of IL8 in Chinese earthquake victims both with and without post traumatic stress disorder (PTSD) and depressive symptoms and found decreased levels of IL8 in the subgroup with PTSD when compared to those who remained asymptomatic. Additional research is warranted to determine the effects of IL8 or depressed symptoms.

Several study limitations need to be acknowledged. The majority of participants were middle-aged, Caucasian, well educated, and married/ partnered, which limits the generalizability of the self-reported symptom severity scores to other groups with similar demographic characteristics. The major reasons for enrollment refusal were being too overwhelmed with treatment or too busy which may have led to either underestimation or overestimation of symptom severity in study participants. The small sample sizes for the "All high" and the "High depression and low pain" classes may have prevented the identification of difference among the LCPA classes in other SNPs because of a low minor allele frequency. For example, findings for several SNPs in IL1R1, IL2, IL10, IL17A, and TNFA approached statistical significance and warrant investigation in future studies with larger sample sizes.

It is plausible that other genetic associations with symptom clusters will emerge if the same analyses are conducted at several points over the trajectory of the patient's treatment as latent class membership can change over time

(Dodd et al., 2011). Studies that evaluate serum levels of IL4 and IL8 may contribute to more definitive conclusions regarding changes in IL4 and IL8 gene expression and inter individual differences in symptom experiences.

In summary, the recognition of a distinct phenotype that may represent sickness behavior reveals new evidence for the role of IL4 and IL8 in the modulation of this symptom cluster in oncology patients and their FCs. Using newer statistical approaches like LCPA to identify distinct phenotypes may provide important information about the biologic mechanisms that underlie inter-individual differences in the symptom experience. Indeed, findings from this study suggest a role for anti-inflammatory cytokines in the modulation of symptom experience that was not described previously and warrants confirmation in future studies.

ACKNOWLEDGEMENTS

Table 1 Summary of Single Nucleotide Polymorphisms Analyzed for Pro- and Anti-inflammatory Cytokine Genes

Gene	SNP	Position	Chr	MAF	Alleles	Chi square	p-value	Model
IL1B	rs1071676	106042060	2	0.198	G>C	5.48	.242	A
IL1B	rs1143643	106042929	2	0.331	G>A	4.97	.290	A
IL1B	rs1143642	106043180	2	0.095	C>T	2.40	.663	A
IL1B	rs1143634	106045017	2	0.196	C>T	5.37	.252	A
IL1B	rs1143633	106045094	2	0.345	G>A	3.33	.505	A
IL1B	rs1143630	106046282	2	0.103	C>A	0.67	.955	A
IL1B	rs3917356	106046990	2	0.432	G>A	5.64	.228	A
IL1B	rs1143629	106048145	2	0.353	A>G	5.81	.214	A
IL1B	rs1143627	106049014	2	0.390	A>G	3.12	.538	A
IL1B	rs16944	106049494	2	0.380	G>A	4.15	.386	A
IL1B	rs1143623	106050452	2	0.248	C>G	4.69	.321	A
IL1B	rs13032029	106055022	2	0.428	C>T	5.41	.248	A
IL1R1	rs949963	96533648	2	0.213	G>A	2.28	.685	A
IL1R1	rs2228139	96545511	2	0.066	C>G	8.75	.013	R
IL1R1	rs3917320	96556738	2	0.068	A>C	2.45	.294	A
IL1R1	rs2110726	96558145	2	0.333	C>T	5.06	.281	A
IL1R1	rs3917332	96560387	2	0.124	A>T	1.22	.875	A
IL1R2	rs4141134	96370336	2	0.401	A>G	3.09	.543	A
IL1R2	rs11674595	96374804	2	0.233	A>G	2.10	.718	A
IL1R2	rs7570441	96380807	2	0.393	G>A	2.74	.602	A
IL2	rs1479923	119096993	4	0.302	C>T	1.64	.802	A
IL2	rs2069776	119098582	4	0.244	A>G	3.03	.552	A
IL2	rs2069772	119099739	4	0.238	A>G	6.75	.150	A
IL2	rs2069777	119103043	4	0.054	C>T	3.94	.139	A
IL2	rs2069763	119104088	4	0.287	A>C	3.29	.511	A
IL4	**rs2243248**	**127200946**	**5**	**0.101**	**A>C**	**9.85**	**.007 (3>1&2)**	**D**
IL4	rs2243250	127201455	5	0.260	C>T	1.62	.806	A
IL4	rs2070874	127202011	5	0.219	C>T	2.58	.631	A
IL4	rs2227284	127205027	5	0.399	C>A	8.09	.088	A
IL4	rs2227282	127205481	5	0.401	C>G	8.01	.091	A
IL4	rs2243263	127205601	5	0.124	C>G	1.20	.549	A
IL4	rs2243266	127206091	5	0.203	G>A	2.85	.583	A
IL4	rs2243267	127206188	5	0.205	G>C	2.86	.581	A
IL4	rs2243274	127207134	5	0.262	G>A	0.50	.974	A
IL6	rs4719714	22643793	7	0.196	A>T	3.91	.419	A
IL6	rs2069827	22648536	7	0.071	G>T	1.35	.853	A
IL6	rs1800796	22649326	7	0.095	C>G	5.55	.235	A
IL6	rs1800795	22649725	7	0.355	C>G	5.34	.254	A
IL6	rs2069835	22650951	7	0.066	A>G	0.98	.613	A
IL6	rs2066992	22651329	7	0.091	G>T	6.76	.149	A

IL6	rs2069840	22651652	7	0.308	C>G	0.87	.929	A
IL6	rs1554606	22651787	7	0.405	A>C	3.54	.472	A
IL6	rs2069845	22653229	7	0.405	A>G	3.54	.472	A
IL6	rs2069849	22654236	7	0.039	C>T	8.46	.076	A
IL6	rs2069861	22654734	7	0.083	C>T	2.77	.251	A
IL6	rs35610689	22656903	7	0.242	A>G	3.56	.469	A
IL8	rs4073	70417508	4	0.498	A>T	3.36	.500	A
IL8	**rs2227306**	**70418539**	**4**	**0.366**	**C>T**	**7.20**	**.027 (2>1&3)**	**R**
IL8	**rs2227543**	**70419394**	**4**	**0.374**	**C>T**	**6.44**	**.040 (2>1&3)**	**R**
IL10	rs3024505	177638230	1	0.138	C>T	6.00	.199	A
IL10	rs3024498	177639855	1	0.236	A>G	2.36	.670	A
IL10	rs3024496	177640190	1	0.459	A>G	1.06	.901	A
IL10	rs1878672	177642039	1	0.452	C>G	1.12	.891	A
IL10	rs3024492	177642438	1	0.207	A>T	4.44	.350	A
IL10	rs1518111	177642971	1	0.267	G>A	3.24	.519	A
IL10	rs1518110	177643187	1	0.267	G>T	3.24	.519	A
IL10	rs3024491	177643372	1	0.448	A>C	0.69	.952	A
IL13	rs1881457	127184713	5	0.192	A>C	1.84	.766	A
IL13	rs1800925	127185113	5	0.227	C>T	2.31	.678	A
IL13	rs2069743	127185579	5	0.021	A>G	0.58	.750	A
IL13	rs1295686	127188147	5	0.252	G>A	5.56	.235	A
IL13	rs20541	127188268	5	0.174	C>T	3.53	.473	A
IL17A	rs4711998	51881422	6	0.293	G>A	3.20	.526	A
IL17A	rs8193036	51881562	6	0.255	A>G	2.93	.569	A
IL17A	rs3819024	51881855	6	0.374	A>G	5.92	.205	A
IL17A	rs2275913	51882102	6	0.345	G>A	5.12	.275	A
IL17A	rs3804513	51884266	6	0.027	A>T	1.28	.865	A
IL17A	rs7747909	51885318	6	0.225	G>A	3.69	.450	A
TNFA	rs2857602	31533378	6	0.360	A>G	1.60	.809	A
TNFA	rs1800683	31540071	6	0.388	G>A	1.81	.772	A
TNFA	rs2239704	31540141	6	0.370	G>T	1.73	.785	A
TNFA	rs2229094	31540556	6	0.256	A>G	5.96	.202	A
TNFA	rs1041981	31540784	6	0.388	C>A	1.81	.772	A
TNFA	rs1799964	31542308	6	0.202	A>G	5.99	.200	A
TNFA	rs1800750	31542963	6	0.019	G>A	0.62	.961	A
TNFA	rs1800629	31543031	6	0.157	G>A	3.89	.422	A
TNFA	rs1800610	31543827	6	0.105	C>T	3.64	.458	A
TNFA	rs3093662	31544189	6	0.072	A>G	2.77	.598	A

A = additive model, Chr = chromosome, D = dominant model, IL = interleukin, MAF = minor allele frequency, R = recessive model, SNP= single nucleotide polymorphism, TNFA = tumor necrosis factor alpha

Table 2: Demographic and clinical characteristics of total sample and differences in characteristics among the latent classes

Characteristics	Total sample N=253	Low Depression & Low Pain (1) N=210 83.0%	High Depression & Low Pain (2) N=12 4.7%	All High (3) N=31 12.3%	p-value post-hoc contrasts
	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)	
Age (years)	61.5 (11.3)	62.5 (11.3)	57.0 (11.1)	56.2 (9.3)	p=.005 3<1; p=.011
Education (years)	16.0 (3.0)	15.9(3.0)	15.4 (3.9)	16.4 (2.6)	p=.586
Karnofsky Performance Status score	92.0 (11.5)	93.7 (9.9)	86.4 (17.5)	81.4 (13.3)	p<.0001 3<1; p<.0001
Number of comorbid conditions	4.6 (2.7)	4.4 (2.6)	3.8 (2.0)	6.6 (2.9)	p=.004 3>1 and 2; p=.04
	%	%	%	%	
Gender					
Male	46.2	50.5	25.0	25.8	p=.012 1<3; p<.05
Female	53.8	49.5	75.0	74.2	
Ethnicity					
White	74.7	76.7	50.0	71.0	p=.104
Non-white	25.3	23.3	50.0	29.0	
Participant					
Patient	66.4	62.9	66.7	90.3	p=.01 3>1 and 2; p<.05
Family caregiver	33.6	37.1	33.3	9.7	
Lives alone					
Yes	32.1	29.5	50.0	39.3	p=.327
No	67.9	70.5	50.0	60.7	
Married/ partnered?					
Yes	69.3	71.4	58.3	58.6	p=.262
No	30.7	28.6	41.7	41.4	
Work for pay?					
Yes	46.4	48.3	41.7	34.5	p=.356
No	53.6	51.7	58.3	65.5	
Cancer Diagnosis					
Breast (N=64)	38.1	31.1	62.5	64.3	p=.001
Prostate (N=82)	48.8	57.6	25.0	14.3	
Brain (N=12)	7.1	6.8	12.5	7.1	
Lung (N=10)	6.0	4.5	0.0	14.3	

kg = kilogram; m = meters; SD = standard deviation

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Table 3. Latent Class Solutions and Fit Indices for Two-Through Four-Class Solutions

Model	LL	BIC	BIC_{SSAdj}	VLMR	Entropy
2 Class	-1683.05	3490.60	3420.84	96.67**	.90
3 Class	-1666.38	3496.88	3404.92	33.34*	.92
4 Class	-1651.93	3507.60	3393.44	28.90^{ns}	.86

* p < .05; ** p < .01; ns = not significant
LL = log-likelihood; BIC= Bayesian Information Criterion; BIC_{SSAdj} = sample-size adjusted BIC; VLMR= the Vuong-Lo-Mendel-Rubin likelihood ratio test for the K vs. K-1 model.

Table 4: Symptom severity scores for the total sample and differences in symptom severity scores among the latent classes

Symptom severity scores	Total sample N=253		Low Depression & Low Pain (1) N=210 83.0%	High Depression & Low Pain (2) N=12 4.7%	All High (3) N=31 12.3%	p-value post-hoc contrasts
	Mean (SD)		Mean (SD)	Mean (SD)	Mean (SD)	
Total CES-D score	8.8 (8.2)		5.9 (4.7)	20.6 (6.8)	24.5 (5.0)	p<.0001 3>1; p<.0001
Total GSDS score	38.9 (18.6)		35.3 (15.4)	27.5 (12.0)	68.1 (14.6)	p<.0001 3>1 and 2; p<.0001
LFS score (evening fatigue)	4.3 (2.0)		4.1 (2.0)	4.4 (2.6)	5.6 (1.3)	p<.0001 3>1; p<.0001
BPI worst pain score	1.9 (3.2)		1.5 (2.9)	0.8 (2.7)	4.7 (3.7)	p<.0001 3>1 and 2; p=.001

BPI = Brief Pain Inventory; CES-D=Center for Epidemiological Studies – Depression; GSDS= General Sleep Disturbance Scale; LFS= Lee Fatigue Scale; SD= standard deviation

Table 5. Multiple Logistic Regression Analyses for IL4 and IL8

Table 5A IL4 (rs2243248)

LCPA Class Comparison	Predictor	Odds Ratio	Standard Error	95% CI	Z	p-value
Class 1 vs. 2 (n=215)	Genotype	0.45	0.480	0.056, 3.617	-0.75	.454
	Overall model fit: $\chi^2 = 0.69$, p = .407 $R^2 = 0.0074$					
Class 1 vs. 3 (n=225)	Genotype	4.51	2.412	1.579, 12.866	2.81	.005
	KPS	0.93	0.016	0.903, 0.967	-3.91	<.001
	# of comorbidities	2.90	1.559	1.008, 8.316	1.97	.048
	Gender	0.30	0.166	0.102, 0.887	-2.18	.029
	Pt/ FC	0.15	0.106	0.037, 0.603	-2.67	.008
	Overall model fit: $\chi^2 = 46.96$, p < .0001 $R^2 = 0.2915$					
Class 2 vs. 3 (n=39)	Genotype	21.14	27.522	1.647, 271.254	2.34	.019
	# of comorbidities	39.81	51.718	3.121, 507.879	2.84	.005
	Pt/ FC	0.06	0.075	0.004, 0.790	-2.13	.033
	Overall model fit: $\chi^2 = 19.41$, p = .0002 $R^2 = 0.403$					

Table 5B IL8 (rs2227306)

LCPA Class Comparison	Predictor	Odds Ratio	Standard Error	95% CI	Z	p-value
Class 1 vs. 2 (n=215)	Genotype	9.78	7.483	2.184, 43.810	2.98	.003
	White	0.15	0.112	0.035, 0.647	-2.55	.011
	Overall model fit: $\chi^2 = 12.45$, p = .002 $R^2 = 0.1345$					
Class1 vs. 3 (n=225)	Genotype	0.97	0.636	0.269, 3.502	-0.04	.964
	KPS	0.93	0.016	0.898, 0.960	-4.36	<.001
	Gender	0.35	0.180	0.126, 0.961	-2.04	.042
	Pt/ FC	0.17	0.119	0.044, 0.669	-2.54	.011
	Overall model fit: $\chi^2 = 36.60$, p < .0001 $R^2 = 0.2272$					
Class 2 vs. 3 (n=39)	Genotype	0.19	0.188	0.028, 1.311	-1.68	.092
	# of comorbidities	17.17	20.408	1.674, 176.277	2.39	.017
	Pt/ FC	0.05	0.071	0.004, 0.722	-2.21	.027
	Overall model fit: $\chi^2 = 14.47$, p = .0023 $R^2 = 0.301$					

Abbreviations; LCPA = latent class profile analysis; KPS= karnofsky performance status; Pt = patient; FC = family caregiver

Table 5 caption: Pair-wise multiple logistic regression analysis of LPA groups. Class 1: "Low depression, low pain", Class 2: "High depression, low pain", and Class 3: "All high". For Table 5A, predictors evaluated in each model included IL4 rs2243248 genotype (AA versus AC+CC), age (years), gender (female versus male), ethnicity (white versus non-white), patient versus FC, functional status as estimated by KPS score, and number of comorbid conditions. An interaction term for ethnicity and genotype was examined. For Table 5B, predictors evaluated in each model included: IL8 rs2227306 genotype (CC versus CT+TT), age (years), gender (female versus male), ethnicity (white versus other), patient versus FC, functional status as estimated by KPS score, and number of comorbid conditions. Interaction terms for: ethnicity and genotype, and genotype x genotype were examined.
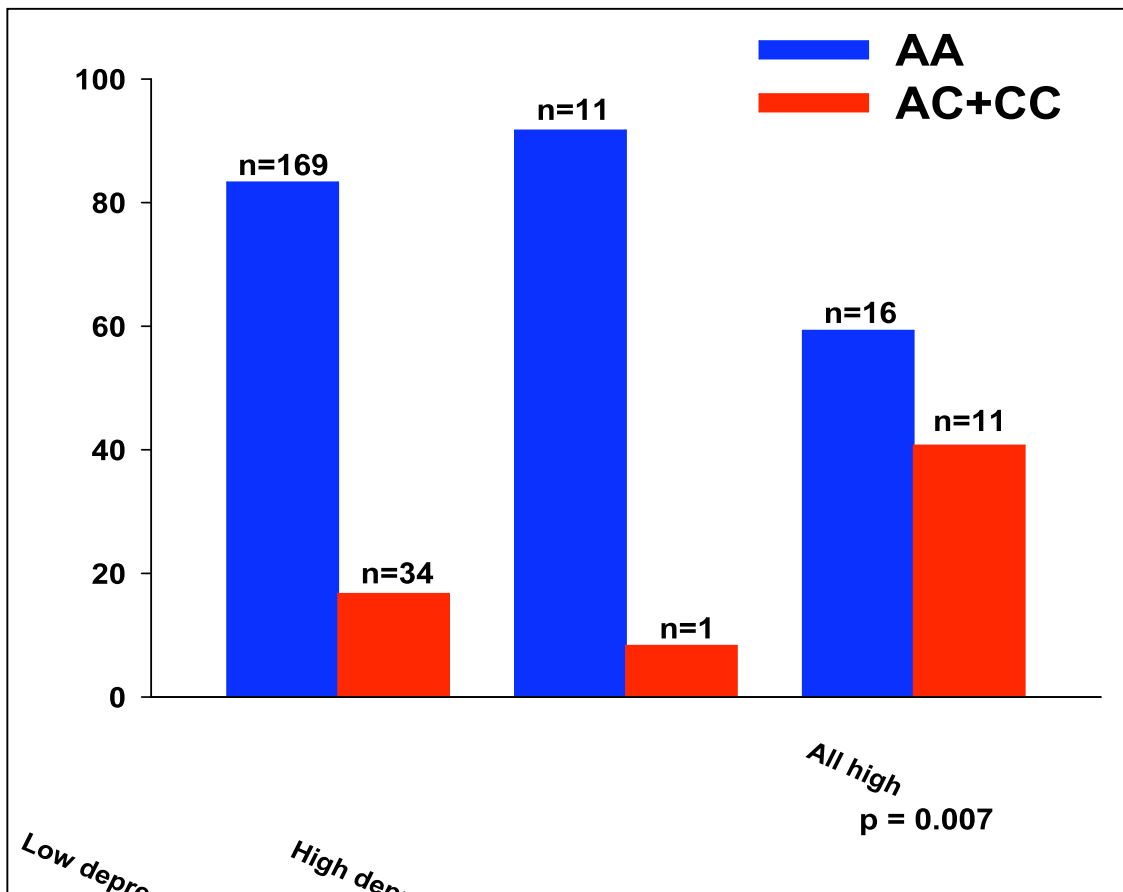
Figure 1 – Differences among the latent classes for those who were homozygous for the common allele (AA) or heterozygous or homozygous for the minor allele (AC+CC) for rs2243248 in IL4.
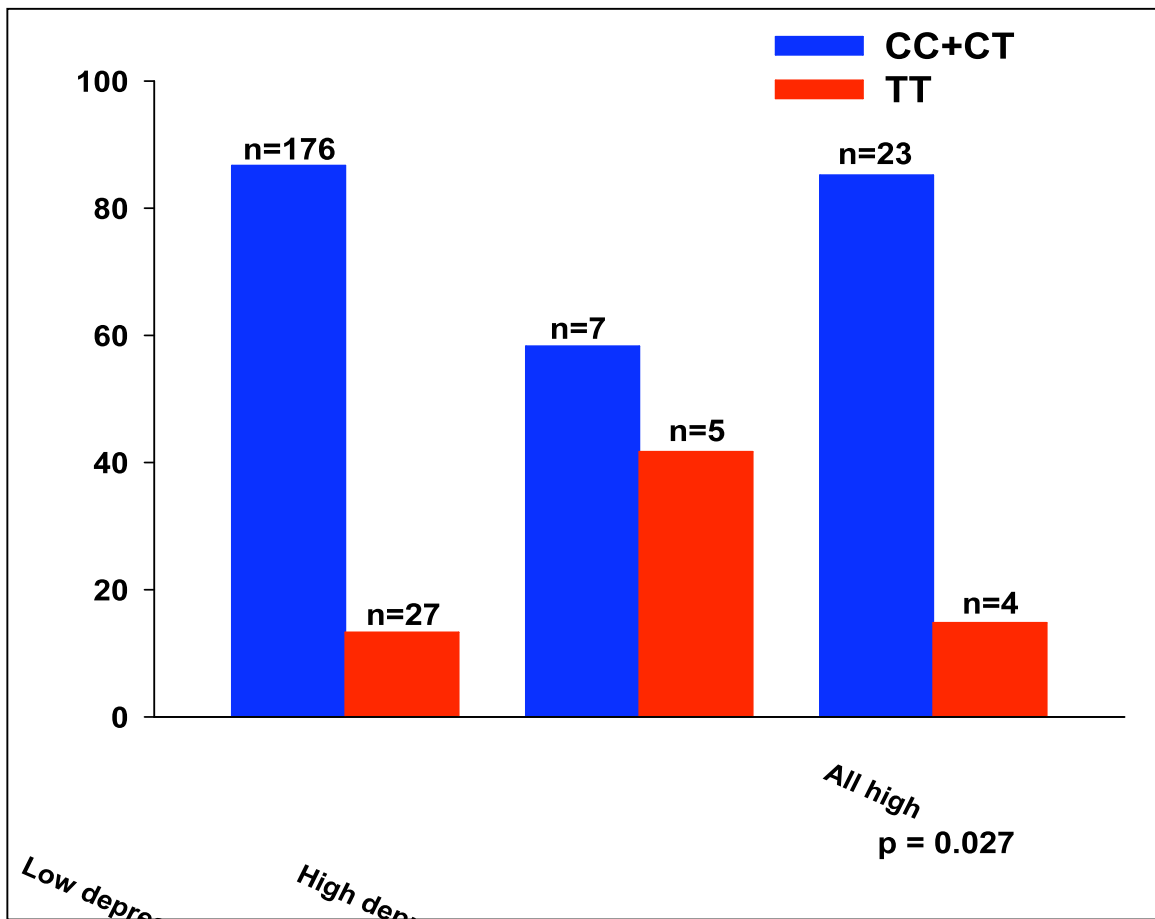
Figure 2 – Differences among the latent classes for those who were homozygous or heterozygous for the common allele (CC+CT) or homozygous for the minor allele (TT) for rs2227306 in IL8.

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