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

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Abstract

Because multiple symptoms associated with “sickness behavior” have a negative impact on functional status and quality of life, increased information on 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 pain, fatigue, sleep disturbance, and depression; if these classes differed on demographic and clinical characteristics; and if variations in pro- and anti- inflammatory cytokine genes were associated with latent class membership.

Self-report measures of pain, fatigue, sleep disturbance, and depression were completed by 168 oncology outpatients and 85 family caregivers (FCs). Using latent class profile analysis (LCPA), three relatively distinct classes were identified: those who reported low depression and low pain (83%), 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 younger age, being White, being a patient (versus a FC), having a lower functional status score, and having a higher number of comorbid conditions.

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

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Keywords

fatigue; pain; sleep disturbance; depression; cancer; genetics; cytokines; symptom clusters; single nucleotide polymorphism; sickness behavior

INTRODUCTION

Oncology patients and their family caregivers (FC) report experiencing, with the same frequency and severity, pain, fatigue, sleep disturbance, and depression.[1–5] While these symptoms can occur singly, they often co-occur as a cluster[6–12] and have significant deleterious effects on an individual's functional status and quality of life (QOL).[3,9,13–17] In addition, several studies have identified distinct subgroups of individuals based on their experiences with these four symptoms.[14,17–20] Across these studies, a consistent finding was a subgroup of individuals who reported high levels of pain, fatigue, sleep disturbance, and depression. These individuals may represent a high risk group with a distinct phenotype.

Recent reviews suggest that inter-individual variability in symptom experiences may result from an individual's genetically determined ability to respond to physical and psychological stressors through changes in pro- and anti-inflammatory cytokines.[10,21,22] In fact, in studies that induced "sickness behavior" through the administration of inflammatory agents,[23–29] individuals reported the co-occurrence of lethargy, anorexia, depression, anxiety, sleepiness, and hyperalgesia. For oncology patients and their FCs, both physical and psychosocial stressors may modulate the release of pro-inflammatory cytokines.[30] Therefore, the sickness behavior model provides a framework for evaluation of four common symptoms (i.e., pain, fatigue, sleep disturbance, and depression) in oncology patients and their FCs.[7]

The associations between common symptoms and specific pro-inflammatory cytokines were the focus of a number of studies.[31] For example, in animal models, pro-inflammatory cytokines and chemokines are associated with pain hypersensitivity.[32,33] In humans, individuals with a painful neuropathy had twofold higher mRNA levels for the pro-inflammatory cytokines, interleukin (IL)-2 and tumor necrosis factor (TNF)-α and twofold lower mRNA levels for the anti-inflammatory IL-10 than individuals with a painless neuropathy.[34] 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.[35]

In addition, in patients with a variety of cancer diagnoses, increased levels of fatigue severity were associated with increased levels of IL6 and IL1 receptor antagonists,[36] as well as higher levels of tumor growth factor (TGF-α).[37] More recently, associations between single nucleotide polymorphisms (SNPs) in the promoter regions of IL6 and TNF-α and increased levels of fatigue and sleep disturbance were shown in a sample of oncology patients and their FCs.[38,39] Additionally, a SNP in the promoter region of IL1β was found to be associated with persistent fatigue in breast cancer survivors.[40]

In terms of depressive symptoms, several studies and a meta-analysis found significant elevations in circulating levels of pro-inflammatory cytokines, particularly IL6 and TNF-α, in patients with major depression.[41–47] Furthermore, depressive behaviors and mood alterations including sadness, inability to feel, depressed mood, and suicidal ideation were observed in patients who received repeated injections of recombinant cytokines, for the treatment of autoimmune diseases, viral infections, and cancer.[48,49]

While knowledge of the mechanisms through which pro-inflammatory cytokines contribute to inter-individual variability in single symptoms continues to grow, less is known about the association between cytokines and multiple symptoms or symptom clusters.[10,11] In addition, little is known about the role of anti-inflammatory cytokines in this phenomenon. While Reyes-Gibby et al [50] advanced a hypothesis-driven, pathway-based approach that assessed the contributions of both pro- and anti-inflammatory cytokine genes to cancer symptoms, no studies have evaluated this hypothesis using a comprehensive panel of pro- and anti-inflammatory cytokine genes 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. 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 (≥18 years 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

A demographic questionnaire obtained information on age, gender, marital status, living arrangements, education, ethnicity, employment status, and the presence of a number of comorbid conditions.

Pain was evaluated using a modified version of the Brief Pain Inventory.[51] Participants who responded yes to the question of having 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) numeric rating scales (NRS).[52]

Fatigue was evaluated using the Lee Fatigue Scale that consists of 13 items designed to assess physical fatigue.[53] 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 [53,54] and with patients with cancer and HIV.[20,55–57] A cutoff score of ≥5.6 indicates clinically significant levels of evening fatigue.[3] 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.

Sleep disturbance was evaluated using the General Sleep Disturbance Scale (GSDS) that 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) 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.[3] The GSDS has well-established validity and reliability in shift workers, pregnant women, and patients with cancer and HIV.[56,58–60] In the current study, the Cronbach's alphas for the GSDS total score for patients and FCs were 0.84 and 0.79, respectively.

Depressive symptoms were assessed using the Center for Epidemiological Studies-Depression (CES-D) scale that 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 ≥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.[61–63] In the current study, the Cronbach's alphas for the CES-D for patients and FCs were 0.88 and 0.84, respectively.

Study Procedures

This study was approved by the Committee on Human Research at the University of California, San Francisco and 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 study questionnaires. 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. Data from these enrollment questionnaires were used in the subsequent analyses. Both patients and FCs had blood drawn for genomic analyses. In addition, patients' 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 or multiple testing.[64] 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,[65,66] was used to identify participants with similar experiences with the symptom cluster of pain, fatigue, sleep disturbance, and depression (i.e., latent classes). 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 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.[70] In addition, better-fitting models should produce higher entropy values.[71] 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.[70]

Latent class models often use categorical variables.[72,73] However, 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 participants 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).[74,75]

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. Due to the small number of respondents within clusters (only one for singletons and two for dyads); because no differences were found between the models with and without the inclusion of the dyadic term; and because dyads constituted only 65% of the sample, the results of the LCPA are reported without dyadic status.

The LCPA was performed using Mplus™ 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 numerical integration with 20 integration points was employed. Subsequent analyses of differences among the identified classes were carried out with SPSS Version 18 for Windows™.[77]

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".[22,78] These polypeptides are divided into pro- and anti-inflammatory cytokines. Pro-inflammatory cytokines promote systemic inflammation and include: interferon (IFN) gamma 1 receptor (IFNGR1), IL1R1, IL2, IL8, IL17A, nuclear factor kappa beta (NFKB1), NFKB2, and TNF-α Anti-inflammatory cytokines suppress the activity of pro-inflammatory cytokines and include: IL1R2, IL4, IL10, and IL13. Of note, INFG1, IL1β, and IL6 possess pro- and anti-inflammatory functions.[31]

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 and clinical characteristics between participants 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—Because no studies of the association between a specific symptom cluster and candidate genes has evaluated a comprehensive panel of pro-and anti-inflammatory cytokine genes, 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, a total of 104 SNPs among the 15 candidate genes (IFNG1: 6 SNPs, IFNGR1: 1SNP; IL1β: 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; NFKB1: 15 SNPs; NFKB2: 4 SNPs; TNF-α: 10 SNPs) passed all quality control filters and were included in the genetic association analyses. Potential functional roles of SNPs associated with specific symptoms were examined using PUPASuite 2.0,[79] a comprehensive search engine that tests a series of functional effects (i.e., nonsynonymous 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 4.2. LD-based haplotype block definition was based on the D' confidence interval method.[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 ≥ 0.85 across the five iterations were retained for downstream analyses. Only inferred haplotypes that occurred with a frequency estimate of ≥15% were included in the association analyses, assuming a dosage model (i.e., analogous to the additive model).

For association tests, three genetic models were assessed for each SNP: additive, dominant, and recessive. Barring trivial improvements (delta <10%), the genetic model that best fit the data, by maximizing the significance of the p-value, was selected for each SNP. Logistic

regression analysis, that controlled for significant covariates as well as race/ethnicity, was used to evaluate the association between genotype and latent class group membership. Only those genetic associations identified as significant in the post hoc contrasts from the univariate analyses were evaluated in the multivariate analyses. A backwards stepwise approach was used to create the most parsimonious model. Except for race/ethnicity, only predictors with a p-value of <0.05 were retained in the final model. Genetic model fit and both unadjusted and covariate-adjusted odds ratios were estimated using the STATA software package, version 9 (STATA Corp).

Ancestry informative markers (AIMs) can be used as a tool to minimize confounding due to population stratification in case-control association studies.[82–84] Homogeneity in ancestry among participants was verified by principal component analysis (PCA),[85] using HelixTree (GoldenHelix, Bozeman, MT). Briefly, the number of principal components (PCs) was sought which distinguished the major racial/ethnic groups in the sample by visual inspection of scatter plots of orthogonal PCs (i.e., PC 1 versus PC2, PC2 versus PC3). This procedure was repeated until no discernable clustering of participants by their self-reported race/ethnicity was possible (data not shown). The first three PCs were selected to adjust for potential confounding due to population substructure (i.e., race/ethnicity) by including them in all logistic regression models (described in the preceding paragraph). One hundred and six ancestry informative markers were included in the analysis.

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. The majority of the FCs (91%) were the patients' spouses. Approximately 38% of the patients had breast cancer, 49% had prostate cancer, 7% had brain cancer, and 6% had lung cancer. No significant differences were found between patients and FCs in age (60.9 (± 11.6) years versus 62.5 (± 10.5) years), KPS score (91.1 (± 11.9) versus 93.7 (± 10.6)), and number of comorbidities (4.8 (± 2.6) versus 4.2 (± 2.9)). In addition, no significant differences were found between patients and FCs in their ratings of worst pain (2.0 (± 3.2) versus 1.5 (± 3.1)), fatigue (4.2 (± 2.0) versus 4.5 (± 2.0)), sleep disturbance (38.9 (± 19.6) versus 38.7 (± 16.7)), and depression (9.1 (± 8.7) versus 8.3 (± 7.2)).

Results of LCPA

Using LCPA, three distinct latent classes of individuals 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 3. As summarized in Table 4, the largest percentage of participants (83%) 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, patient/FC status, KPS score, number of comorbid conditions, and cancer diagnoses (patients only) (Table 2). Participants in the “All high” class were significantly younger (p=.011) 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 (p=.04). Compared to the “Low depression and low pain” class, the “All high” class had a higher percentage of female participants (p<.05). Compared to the other two classes, participants in the “All high” class were more likely to be patients than FCs (p<.05). 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, living arrangements, or marital status.

Differences in mean symptom scores among the three latent classes

As summarized in Table 4, the mean CES-D score was significantly higher for the “All high” class compared to the “Low depression and low pain” class (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 was significantly higher for the “All high” class compared to the other two classes (both, p<.0001). The “All high” class had clinically meaningful levels of sleep disturbance (≥43). The mean LFS score was significantly higher for the “All high” class compared to the “Low depression and low pain” class (p<.0001). The mean worst pain score was significantly higher for the “All high” class compared to the other two classes (both, p=.001).

Candidate gene analyses of the three LCPA classes

As summarized in Table 1, the minor allele frequency was significantly different among the latent classes for four SNPs: IL1R1 rs2228139, IL4 rs2243248, IL8 rs2227306, and IL8 rs2227543. For IL1R1 rs2228139, a recessive model fit the data best (p=.013). However, post-hoc contrasts of IL1R1 rs2228139 were unable to determine which classes drove the differences among the latent classes.

For IL4 rs2243248, a dominant model fit the data best (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). For IL8 rs2227306 (p=.027) and IL8 rs2227543 (p=.04), a recessive model fit the data best. 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 IL4 and IL8 genotypes and symptom experience classification

In order to better estimate the magnitude (i.e., odds ratio, OR) and precision (95% confidence interval, CI) of genotype on LCPA group membership, multivariate logistic regression models were fit in a pairwise fashion between the LCPA groups that were identified through post hoc contrasts in the univariate analyses. In addition to genotype, the phenotypic variables evaluated in the model were age, gender, ethnicity (i.e., White, Asian/

Pacific Islander, Black, Hispanic/Mixed ethnic background/Other), patient/caregiver status, functional status (i.e., KPS score), and number of comorbid conditions.

The only genetic association that remained significant in the multivariate logistic regression analyses was for IL4 rs2243248 that compared the "Low depression and low pain" class with the "All high" class (Figure 1 and Table 5). In this model, IL4 rs2243248 genotype, functional status, number of comorbid conditions, age, ethnicity (White versus Black), and patient/FC status were the predictors retained in the final model ($p<.0001$). The overall model explained 34.1% of the variance in LCPA group membership. Controlling for KPS score, number of comorbid conditions, age, ethnicity, and patient/FC status, carrying a minor allele (i.e., TG + GG) was associated with over a six-fold increase in the odds of belonging to the "All high" class (OR: 6.02, 95% CI: 1.874, 19.366, $p=.003$).

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 anti-inflammatory cytokine (i.e., IL4). The identification of distinct subgroups of individuals with different symptom experiences is consistent with previous reports.[14,17–20] However, only one cytokine gene was associated with differences in the severity of this "sickness behavior" symptom cluster.

In this sample, three relatively distinct classes of participants were identified, namely those who reported low depression and low pain (83%), those who reported high depression and low pain (4.7%), and those who reported high levels of all four symptoms (12.3%). While our previous studies identified four distinct latent classes using the same symptom cluster, [18–20] a consistent finding across all four studies is that the "All high" class constituted between 10% and 15% (mean 13.0%) of the sample. This finding suggests that a subset of individuals share some common biological mechanisms that influence their experience with the multiple symptoms associated with sickness behavior. Identification of these mechanisms could lead to the development of targeted interventions for this high-risk group.

Because LCPA is an exploratory analytic procedure that facilitates the emergence of distinct latent classes based on similarities in some dependent variables (in this study – differences in participants' ratings of pain, fatigue, sleep disturbance, and depression), group membership can change based on sample characteristics as well as timing of the symptom assessments. Therefore, differences across studies[14,17–20] in the number of latent classes, as well as in the symptom characteristics of the various latent classes, may be related to differences in demographic and clinical characteristics of the samples; differences in inclusion and exclusion criteria, as well as differences in some unidentified phenotypic and environmental characteristics. Because blood samples were not obtained from participants in our previous studies,[18–20] the genetic association identified in this study awaits verification in future studies.

In this cohort, carrying the minor allele for IL4 rs2243248 was associated with membership in the "All high" class along with younger age, being White, being a patient (versus a FC), having a lower functional status, and having a higher number of comorbid conditions. Similar associations between higher symptom severity scores and various demographic and clinical characteristics were reported in previous studies. [14,17–20] However, it is important to note that the genetic association was not confounded by any of these demographic or clinical characteristics. These findings are particularly interesting because IL4 was either not evaluated or identified as a candidate gene in previous research on symptoms. Previous studies found genetic associations between IL1β and IL6 and severity

of fatigue,[40] as well as associations between IL6 and TNF-α and severity of fatigue and sleep disturbance.[38,39] The discrepancy in study findings may be related to differences in symptom phenotypes (i.e. single symptoms versus a symptom cluster). Given the fact that a number of reviews suggested that alterations in pro-inflammatory cytokines contributed to the symptoms associated with sickness behavior,[10,21] additional research is warranted 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. [86] The IL4 SNP identified in this study (rs2243248) is known to occur in an evolutionarily conserved region. While the functional effects of this SNP are not known, 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”.

In fact, the neuromodulatory effects of IL4 have been evaluated in animal models of sickness behavior. For example, in one study, 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. This finding suggests that the regulation of sickness behavior by IL4 can be either inhibitory or stimulatory.[87] Interestingly, LPS–induced sickness behavior was more profound in IL4 (−/−) mice, which suggests a more protective role for IL4.[88]. Furthermore, Sherry and colleagues observed decreased sickness behavior in wild type mice fed a soluble fiber diet which induced the up regulation of IL4.[89] The protective effect of the soluble fiber diet was reduced in IL-4 (−/−) mice. While research on the association between IL4 and sickness behavior in humans is limited, 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.[90] These changes in serum cytokines were associated with reduced symptoms of stress (including depression), increased sleep quality, and increased QOL.

Previous work with this sample of patients and FCs identified associations between TNF-α (rs1800629) and sleep disturbance and morning fatigue[38] and between IL6 (rs4719714) and sleep disturbance, evening fatigue, and morning fatigue.[39] The lack of an association between these SNPs and the subgroups of participants found in this study may be explained by a number of factors. First, the symptom phenotype that was evaluated in this study (i.e., symptom cluster of pain, fatigue, sleep disturbance and depression) compared to previous studies (i.e., single symptoms) are distinctly different and may be associated with different cytokine genes. This hypothesis is supported by the fact that the p-values of the additive models for TNF-α rs1800629 (p=.422) and IL6 rs 4719714 (p=.419) in this study did not approach statistical significance (Table 1). An equally plausible hypothesis is that additional research, with larger samples might identify additional candidate genes. In addition, rather than polymorphisms in various cytokine genes being directly responsible for the symptoms associated with sickness behavior and elevations in serum levels of cytokines, polymorphisms in other gene pathways may be involved in activation or inhibition of cytokine genes. This hypothesis warrants investigation in future studies.

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 these findings to individuals 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 symptoms in the

individuals included in this study. In addition, the exact etiologies for and duration of each of the individual symptoms within the symptom cluster were not evaluated. While most studies of sickness behavior reported symptoms associated with an acute stimulus (e.g., administration of lipopolysaccharide),[22,91,92] it is possible that a symptom cluster that occurs because of one or more chronic conditions is associated with genetic variations in pro- and anti-inflammatory cytokines. Future studies need to evaluate the relationships between cytokine genes and individual symptoms as well as symptom clusters.

Due to the small sample sizes for the “All high” and the “High depression and low pain” classes, it is plausible that some genetic associations were not identified because of low minor allele frequency. For example, findings for several SNPs in IL1R1, IL2, IL8, IL10, IL17A, and TNF-α 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.[14] Finally, future studies may need to evaluate levels of pro- and anti-inflammatory cytokines in order to refine our understanding of the associations between genotype and self-reported symptom experiences.

In summary, the recognition of a distinct phenotype that may represent sickness behavior reveals new evidence for the role of IL4 in the modulation of this symptom cluster in oncology patients and their FCs. Using new statistical approaches like LCPA to identify distinct phenotypes may provide new information about the biologic mechanisms that underline this symptom experience. Indeed, this study uncovered a role for an anti-inflammatory cytokine in the modulation of symptom experience that was not described previously and warrants confirmation in future studies.

Highlights

Distinct groups had high levels of pain, fatigue, sleep disturbance and depression.

Approximately 12% of patients reported high levels of all four symptoms.

New role for IL4 in the modulation of a sickness behavior symptom was identified.

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ABBREVIATIONS

AIMs	Ancestry informative markers
BIC	Bayesian Information Criterion
BLRT	Bootstrapped Likelihood Ratio Test
CES-D	Center for Epidemiological Studies- Depression Scale
CI	confidence interval
DNA	deoxyribonucleic acid

EM	expectation-maximization
FC	family caregiver
GSDS	General Sleep Disturbance Scale
IFN	interferon
IL	interleukin
KPS	Karnofsky Performance status
LCA	Latent class analysis
LCPA	Latent class profile analysis
LFS	Lee Fatigue Scale
MLR	robust maximum likelihood
PCA	Principal component analysis
NFKB	nuclear factor kappa beta
NRS	numeric rating scale
OR	odds ratio
QOL	quality of life
RT	radiation therapy
SNP	single nucleotide polymorphism
TGF	tumor growth factor
TNF	tumor necrosis factor
VLMR	Vuong-Lo-Mendell-Rubin likelihood ratio test

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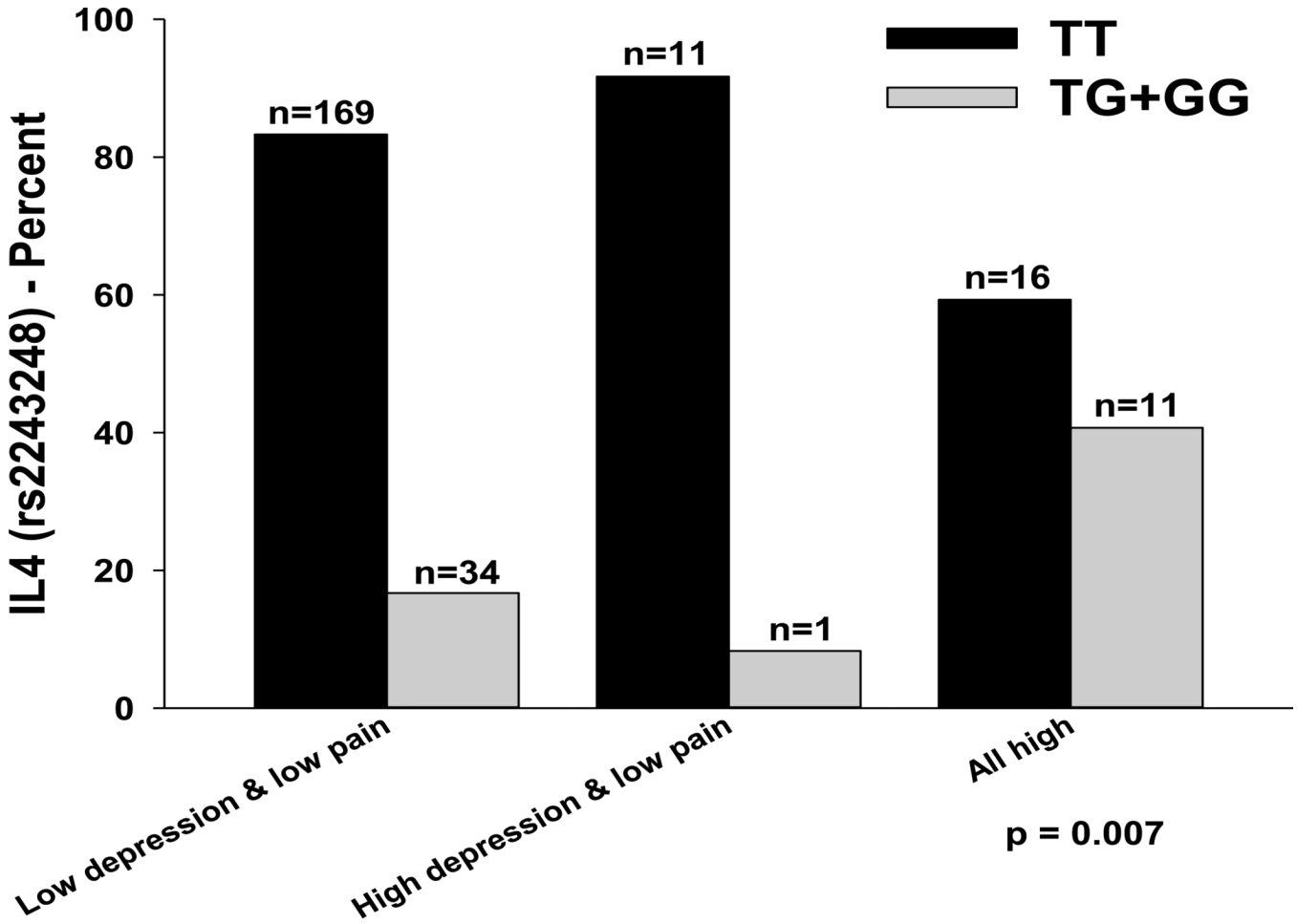

Figure 1.
Differences among the latent classes in the percentages of participants who were homozygous for the common allele (TT) or heterozygous or homozygous for the minor allele (TG + GG) for rs223248 in IL4.

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
IFNG1	rs2069728	66834051	12	.079	G>A	5.70	.222	A
IFNG1	rs2069727	66834490	12	.411	A>G	4.76	.313	A
IFNG1	rs2069718	66836429	12	.442	C>T	0.41	.982	A
IFNG1	rs1861493	66837463	12	.264	A>G	1.38	.848	A
IFNG1	rs1861494	66837676	12	.279	T>C	1.53	.821	A
IFNG1	rs2069709	66839970	12	.008	G>T	0.78	.677	A
IFNG1	HapA3	n/a	12			1.38	.848	
IFNG1	HapA5	n/a	12			4.76	.313	
IFNGR1	rs9376268	137574444	6	.246	G>A	1.70	.719	A
IL1B	rs1071676	106042060	2	.198	G>C	5.48	.242	A
IL1B	rs1143643	106042929	2	.331	G>A	4.97	.290	A
IL1B	rs1143642	106043180	2	.095	C>T	2.40	.663	A
IL1B	rs1143634	106045017	2	.196	C>T	5.37	.252	A
IL1B	rs1143633	106045094	2	.345	G>A	3.33	.505	A
IL1B	rs1143630	106046282	2	.103	C>A	0.67	.955	A
IL1B	rs3917356	106046990	2	.432	A>G	5.64	.228	A
IL1B	rs1143629	106048145	2	.353	T>C	5.81	.214	A
IL1B	rs1143627	106049014	2	.390	T>C	3.12	.538	A
IL1B	rs16944	106049494	2	.380	G>A	4.15	.386	A
IL1B	rs1143623	106050452	2	.248	G>C	4.69	.321	A
IL1B	rs13032029	106055022	2	.428	C>T	5.41	.248	A
IL1B	HapA1	n/a				1.49	.829	
IL1B	HapA3	n/a				2.16	.339	
IL1B	HapA4	n/a				5.03	.284	
IL1B	HapA5	n/a				5.38	.251	
IL1B	HapB1	n/a				3.16	.531	

Gene	SNP*	Position	Chr	MAF	Alleles	Chi square	p-value	Model
IL1B	HapB7	n/a				4.01	.405	
IL1B	HapB9	n/a				5.50	.240	
IL1B	HapB11	n/a				0.47	.976	
IL1R1	rs949963	96533648	2	.213	G>A	2.28	.685	A
IL1R1	**rs2228139**	**96545511**	**2**	**.066**	**C>G**	**8.75**	**.013**	**R**
IL1R1	rs3917320	96556738	2	.068	A>C	2.45	.294	A
IL1R1	rs2110726	96558145	2	.333	C>T	5.06	.281	A
IL1R1	rs3917332	96560387	2	.124	T>A	1.22	.875	A
IL1R2	rs4141134	96370336	2	.401	T>C	3.09	.543	A
IL1R2	rs11674595	96374804	2	.233	T>C	2.10	.718	A
IL1R2	rs7570441	96380807	2	.393	G>A	2.74	.602	A
IL1R2	HapA1	n/a				7.69	.104	
IL1R2	HapA2	n/a				7.31	.120	
IL1R2	HapA4	n/a				2.93	.569	
IL2	rs1479923	119096993	4	.302	C>T	1.64	.802	A
IL2	rs2069776	119098582	4	.244	T>C	3.03	.552	A
IL2	rs2069772	119099739	4	.238	A>G	6.75	.150	A
IL2	rs2069777	119103043	4	.054	C>T	3.94	.139	A
IL2	rs2069763	119104088	4	.287	T>G	3.29	.511	A
IL2	HapA1	n/a				8.23	.084	
IL2	HapA2	n/a				6.81	.146	
IL2	HapA3	n/a				2.93	.570	
IL2	HapA5	n/a				1.64	.802	
IL4	**rs2243248**	**127200946**	**5**	**.101**	**T>G**	**9.85**	**.0073>1**	**D**
IL4	rs2243250	127201455	5	.260	C>T	1.62	.806	A
IL4	rs2070874	127202011	5	.219	C>T	2.58	.631	A
IL4	rs2227284	127205027	5	.399	C>A	8.09	.088	A
IL4	rs2227282	127205481	5	.401	C>G	8.01	.091	A
IL4	rs2243263	127205601	5	.124	G>C	1.20	.549	A

Gene	SNP*	Position	Chr	MAF	Alleles	Chi square	p-value	Model
IL4	rs2243266	127206091	5	.203	G>A	2.85	.583	A
IL4	rs2243267	127206188	5	.205	G>C	2.86	.581	A
IL4	rs2243274	127207134	5	.262	G>A	0.50	.974	A
IL4	HapA1	n/a				5.78	.216	
IL4	HapA10	n/a				2.71	.608	
IL6	rs4719714	22643793	7	.196	A>T	3.91	.419	A
IL6	rs2069827	22648536	7	.071	G>T	1.35	.853	A
IL6	rs1800796	22649326	7	.095	G>C	5.55	.235	A
IL6	rs1800795	22649725	7	.355	C>G	5.34	.254	A
IL6	rs2069835	22650951	7	.066	T>C	0.98	.613	A
IL6	rs2066992	22651329	7	.091	G>T	6.76	.149	A
IL6	rs2069840	22651652	7	.308	C>G	0.87	.929	A
IL6	rs1554606	22651787	7	.405	T>G	3.54	.472	A
IL6	rs2069845	22653229	7	.405	G>A	3.54	.472	A
IL6	rs2069849	22654236	7	.039	C>T	8.46	.076	A
IL6	rs2069861	22654734	7	.083	C>T	2.77	.251	A
IL6	rs35610689	22656903	7	.242	A>G	3.56	.469	A
IL6	HapA4	n/a				0.85	.932	
IL6	HapA6	n/a				5.62	.229	
IL8	rs4073	70417508	4	.498	T>A	3.36	.500	A
IL8	**rs2227306**	**70418539**	**4**	**.366**	**C>T**	**7.20**	**.0272>1**	**R**
IL8	**rs2227543**	**70419394**	**4**	**.374**	**C>T**	**6.44**	**.0402>1**	**R**
IL8	HapA1	n/a				1.30	.861	
IL8	HapA3	n/a				8.93	.063	
IL8	HapA4	n/a				3.36	.500	
IL10	rs3024505	177638230	1	.138	C>T	6.00	.199	A
IL10	rs3024498	177639855	1	.236	A>G	2.36	.670	A
IL10	rs3024496	177640190	1	.459	T>C	1.06	.901	A
IL10	rs1878672	177642039	1	.452	G>C	1.12	.891	A

Gene	SNP*	Position	Chr	MAF	Alleles	Chi square	p-value	Model
IL10	rs3024492	177642438	1	.207	A>T	4.44	.350	A
IL10	rs1518111	177642971	1	.267	G>A	3.24	.519	A
IL10	rs1518110	177643187	1	.267	G>T	3.24	.519	A
IL10	rs3024491	177643372	1	.448	T>G	0.69	.952	A
IL10	HapA5	n/a				1.08	.898	
IL10	HapA6	n/a				3.51	.476	
IL10	HapA8	n/a				3.89	.421	
IL10	HapA9	n/a				5.95	.203	
IL13	rs1881457	127184713	5	.192	A>C	1.84	.766	A
IL13	rs1800925	127185113	5	.227	C>T	2.31	.678	A
IL13	rs2069743	127185579	5	.021	A>G	0.58	.750	A
IL13	rs1295686	127188147	5	.252	G>A	5.56	.235	A
IL13	rs20541	127188268	5	.174	C>T	3.53	.473	A
IL13	HapA1	n/a				4.63	.328	
IL13	HapA4	n/a				3.10	.542	
IL17A	rs4711998	51881422	6	.293	G>A	3.20	.526	A
IL17A	rs8193036	51881562	6	.255	T>C	2.93	.569	A
IL17A	rs3819024	51881855	6	.374	A>G	5.92	.205	A
IL17A	rs2275913	51882102	6	.345	G>A	5.12	.275	A
IL17A	rs3804513	51884266	6	.027	A>T	1.28	.865	A
IL17A	rs7747909	51885318	6	.225	G>A	3.69	.450	A
NFKB1	rs3774933	103645369	4	.444	T>C	1.29	.864	A
NFKB1	rs170731	103667933	4	.397	T>A	2.89	.576	A
NFKB1	rs17032779	103685279	4	.023	T>C	4.28	.118	A
NFKB1	rs230510	103695201	4	.366	T>A	5.21	.267	A
NFKB1	rs230494	103706005	4	.477	A>G	4.64	.326	A
NFKB1	rs4648016	103708706	4	.017	C>T	1.04	.594	A
NFKB1	rs4648018	103709236	4	.025	G>C	0.38	.827	A
NFKB1	rs3774956	103727564	4	.479	C>T	4.70	.319	A

Gene	SNP*	Position	Chr	MAF	Alleles	Chi square	p-value	Model
NFKB1	rs10489114	103730426	4	.025	A>G	0.38	.827	A
NFKB1	rs4648068	103737343	4	.366	A>G	6.29	.179	A
NFKB1	rs4648095	103746914	4	.052	T>C	0.69	.709	A
NFKB1	rs4648110	103752867	4	.205	T>A	5.30	.258	A
NFKB1	rs4648135	103755716	4	.060	A>G	0.36	.835	A
NFKB1	rs4648141	103755947	4	.188	G>A	6.54	.162	A
NFKB1	rs1609798	103756488	4	.337	C>T	3.04	.551	A
NFKB1	HapA1	n/a				5.47	.242	
NFKB1	HapA9	n/a				2.58	.631	
NFKB2	rs12772374	104146901	10	.157	A>G	2.87	.580	A
NFKB2	rs7897947	104147701	10	.229	T>G	5.87	.209	A
NFKB2	rs11574849	104149686	10	.085	G>A	0.31	.989	A
NFKB2	rs1056890	104152760	10	.317	C>T	5.23	.265	A
TNFA	rs2857602	31533378	6	.360	T>C	1.60	.809	A
TNFA	rs1800683	31540071	6	.388	G>A	1.81	.772	A
TNFA	rs2239704	31540141	6	.370	G>T	1.73	.785	A
TNFA	rs2229094	31540556	6	.256	T>C	5.96	.202	A
TNFA	rs1041981	31540784	6	.388	C>A	1.81	.772	A
TNFA	rs1799964	31542308	6	.202	T>C	5.99	.200	A
TNFA	rs1800750	31542963	6	.019	G>A	0.62	.961	A
TNFA	rs1800629	31543031	6	.157	G>A	3.89	.422	A
TNFA	rs1800610	31543827	6	.105	C>T	3.64	.458	A
TNFA	rs3093662	31544189	6	.072	A>G	2.77	.598	A
TNFA	HapA1	n/a				3.91	.419	
TNFA	HapA5	n/a				4.69	.321	
TNFA	HapA8	n/a				1.75	.783	

A = additive model, Chr = chromosome, D = dominant model, Hap = haplotype, IFNG, interferon gamma, IL = interleukin, n/a = not applicable, MAF = minor allele frequency, R = recessive model, NFKB, nuclear factor kappa beta, SNP= single nucleotide polymorphism, TNFA = tumor necrosis factor alpha

*Haplotypes are listed as the last variables for each gene.

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 (KPS) 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 Female	46.2 53.8	50.5 49.5	25.0 75.0	25.8 74.2	p= .012 1<3; p<.05
Ethnicity White Asian/Pacific Islander Black Hispanic/Mixed/Other	74.6 6.3 13.5 5.6	76.6 5.3 13.4 4.8	50.0 16.7 25.0 8.3	71.0 9.7 9.7 9.7	p=.328
Participant Patient Family caregiver	66.4 33.6	62.9 37.1	66.7 33.3	90.3 9.7	p=.01 3>1 and 2; p<.05
Lives alone Yes No	32.1 67.9	29.5 70.5	50.0 50.0	39.3 60.7	p=.327
Married/ partnered? Yes No	69.3 30.7	71.4 28.6	58.3 41.7	58.6 41.4	p=.262
Work for pay? Yes No	46.4 53.6	48.3 51.7	41.7 58.3	34.5 65.5	p=.356

SD = standard deviation

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	28.09*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 scores at enrollment	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)	
CES-D scores	8.8 (8.2)	5.9 (4.7)	20.6 (6.8)	24.5 (5.0)	p<.0001 3>1; p<.0001
GSDS scores	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 scores for evening fatigue	4.3 (2.0)	4.1 (2.0)	4.4 (2.6)	5.6 (1.3)	p<.0001 3>1; p<.0001
Worst pain intensity scores	1.9 (3.2)	1.5 (2.9)	0.8 (2.7)	4.7 (3.7)	p<.0001 3>1 and 2; p=.001

CES-D = Center for Epidemiologic Studies-Depression Scale; GSDS = General Sleep Disturbance Scale; LFS = Lee Fatigue Scale; SD= standard deviation

Table 5

Multiple Logistic Regression Analyses for IL4 rs2243248

LCPA Class Comparison	Predictor	Odds Ratio	Standard Error	95% CI	Z	p-value
Class 1 versus Class 3 (n=224)	Genotype	6.02	3.589	1.873, 19.366	3.01	.003
	Functional status	0.48	0.092	0.330, 0.697	−3.85	<.001
	# of comorbidities	1.35	0.134	1.109, 1.638	3.00	.003
	Age	0.78	0.093	0.619, 0.986	−2.08	.037
	White versus Black	0.04	0.058	0.003, 0.647	−2.27	.023
	Patient/FC	0.21	0.160	0.045, 0.946	−2.03	.042
	Overall model fit: $\chi^2 = 54.80$, $p < .0001$ $R^2 = 0.3407$					

Pair-wise multiple logistic regression analysis of Latent Class Profile Analysis (LCPA) groups. Class 1: “Low depression and low pain”, Class 2: “High depression and low pain”, and Class 3: “All high”. For each model, the first three principle components identified from the analysis of ancestry informative markers were retained to adjust for potential confounding due to race or ethnicity (data not shown). For Table 5, predictors evaluated in the model included IL4 rs2243248 genotype (TT versus TG+GG), age (years), gender (female versus male), self-reported ethnicity (white, Asian/Pacific Islander, Black, Hispanic/Mixed race/Other), patient versus family caregiver (FC) status, functional status estimated using Karnofsly Performance Status (KPS) score, and number of comorbid conditions.