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

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Abstract
Pain, fatigue, sleep disturbance, and depression are common and frequently co-occurring symptoms reported by oncology patients. Recent studies have revealed that these symptoms are related to each other and can form a symptom cluster (Barsevick, 2007; Kim, Barsevick, Beck, & Dudley, 2012). This symptom cluster is associated with significant decrements in oncology patients’ functional status and quality of life (Miaskowski et al., 2006; Pud et al., 2008). Evidence suggests that the model of cytokine-induced sickness behavior may be one mechanism to explain the occurrence of this symptom cluster in oncology patients (Gilbertson-White, Aouizerat, & Miaskowski, 2011).

Sickness behavior refers to the physiologic changes and associated behaviors that develop in individuals with an infection (Dantzer, 2001; Dantzer & Kelley, 2007) or through the administration of agents that trigger an inflammatory response (Eisenberger et al., 2010; Maier & Watkins, 2003). Sickness behavior occurs as a result of the release of proinflammatory cytokines from the central nervous system. A growing body of evidence suggests that some of the most common symptoms reported by oncology patients are associated with changes in the levels of pro- and anti-inflammatory cytokines.
(Miaskowski & Aouizerat, 2012; Seruga, Zhang, Bernstein, & Tannock, 2008).

For example, in recent studies of oncology patients, sleep disturbance and increased fatigue were associated with changes in a number of serum interleukins (ILs; Collado-Hidalgo, Bower, Ganz, Irwin, & Cole, 2008; Liu et al., 2012; Saligan & Kim, 2012; Schubert, Hong, Natarajan, Mills, & Dimsdale, 2007). Recent work from our research team identified associations between a polymorphism in tumor necrosis factor alpha (TNFα) and increased levels of fatigue and sleep disturbance in oncology patients and their family caregivers (Aouizerat et al., 2009). In addition, studies have shown that higher levels of IL6 and TNFα (Alesci et al., 2005; Dowlati et al., 2010; Sukoff Rizzo et al., 2012) and polymorphisms in IL1 receptor 2 (IL1R2), IL10, and TNFA (Dunn et al., 2013) are associated with depressive symptoms. Finally, in another recent study, elevated plasma levels of proinflammatory cytokines, including IL1-β, IL6, and TNFα, were associated with the development and maintenance of neuropathic pain (Wang, Lehky, Brell, & Dorsey, 2012). Collectively, these findings suggest that variations in both pro- and anti-inflammatory cytokine genes may influence the symptom experience of oncology patients.

In a recent study of oncology patients and their family caregivers, we used latent class analysis (LCA) to identify subgroups of participants with distinct symptom profiles and evaluated for associations between polymorphisms in a number of cytokine genes and high levels of self-reported pain, fatigue, sleep disturbance, and depression. We found that individuals who were homozygous for the rare allele rs2243248 in IL4 were significantly more likely to be classified into the all high symptom group (Illi et al., 2012). However, our heterogeneous and relatively small sample limited our ability to identify additional genetic associations. In this study, we sought to replicate this finding in a larger, different, and more homogeneous sample of patients with breast cancer.

While surgery can trigger the release of cytokines, several studies found that fatigue, pain, sleep disturbance, and depression can occur prior to and may trigger higher levels of cancer-related symptoms after breast cancer surgery (De Vries, Van der Steeg, & Roukema, 2009; Wright et al., 2009). For example, in one study, patients with chronic preoperative pain were 3 times more likely to report postoperative pain compared to patients without preoperative pain (Sheridan et al., 2012). In addition, patients who developed chronic pain after breast cancer surgery reported higher preoperative levels of depression and anxiety (Miaskowski, Cooper, Paul, et al., 2012).

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

**Method**

**Patients and Settings**

More detailed information on this study is published elsewhere (Miaskowski et al., 2013). In brief, patients were recruited from breast care centers located in a comprehensive cancer center, two public hospitals, and four community practices. A patient was eligible to participate if she was an adult woman (≥18 years) who would undergo breast cancer surgery on one breast; was able to read, write, and understand English; agreed to participate; and gave written informed consent. Patients were excluded if they were having breast cancer surgery on both breasts and/or had distant metastasis at the time of diagnosis. A total of 516 patients were approached to participate, 410 were enrolled in the study (response rate 79.5%), and 398 completed the study questionnaires.

**Instruments**

The demographic questionnaire obtained information on age, education, ethnicity, marital status, employment status, living situation, and financial status. The Karnofsky Performance Status (KPS) Scale was used to evaluate patient’s functional status (Karnofsky, 1977). The Self-Administered Comorbidity Questionnaire (SCQ) was used to evaluate comorbidity with 13 common medical conditions. The SCQ has well-established validity and reliability and has been used in studies of patients with a variety of chronic conditions (Sangha, Stucki, Liang, Fossel, & Katz, 2003).

Patients were asked to rate the intensity of their average and worst pain, using a 0 (no pain) to 10 (worst imaginable pain) numeric rating scale (NRS). The NRS is a valid and reliable measure of pain intensity (Jensen, 2003).

The Center for Epidemiologic Studies–Depression (CES-D) Scale was used to evaluate depressive symptoms. CES-D scores can range from 0 to 60, with scores of ≥16 indicating the need for individuals to seek clinical evaluation for major depression (Radloff, 1977). Cronbach’s α for the CES-D was .90.

The General Sleep Disturbance Scale (GSDS) was used to evaluate sleep disturbance. The GSDS total score can range from 0 (no disturbance) to 147 (extreme sleep disturbance). A GDS total score of ≥43 indicates a significant level of sleep disturbance (Fletcher et al., 2008; Miaskowski, et al., 2006). Cronbach’s α for the GDS total score was .86.

The Lee Fatigue Scale was used to evaluate physical fatigue and energy (Lee, Hicks, & Nino-Murcia, 1991). Total fatigue and energy scores are calculated as the mean of the 13 fatigue items and the 5 energy items, respectively, with higher scores indicating greater fatigue severity and higher levels of energy. A cutoff score of ≥4.4 indicates high levels of fatigue. A cutoff score of ≤4.8 indicates low levels of energy (Dhruva et al., 2010). Cronbach’s αs for fatigue and energy were .96 and .93, respectively.
Table 1. Fit Indices for Two-Through Four-Latent Class Solutions.

<table>
<thead>
<tr>
<th>Model</th>
<th>LL</th>
<th>AIC</th>
<th>BIC</th>
<th>BLRT</th>
<th>Entropy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Two class</td>
<td>-2,487.97</td>
<td>5,017.95</td>
<td>5,101.40</td>
<td>69.83</td>
<td>.84</td>
</tr>
<tr>
<td>Three class</td>
<td>-2,469.03</td>
<td>4,992.06</td>
<td>5,099.35</td>
<td>37.89</td>
<td>.75</td>
</tr>
<tr>
<td>Four class</td>
<td>-2,455.45</td>
<td>4,976.89</td>
<td>5,108.03</td>
<td>27.17</td>
<td>.81</td>
</tr>
</tbody>
</table>

Note. AIC = Akaike information criterion; BIC = Bayesian information criterion; BLRT = bootstrapped likelihood ratio test for the K versus K – 1 model; LL = log likelihood.

*p < .05. †p < .0001.

Study Procedures

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

Methods of Analysis for Phenotypic Data

Descriptive statistics and frequency distributions were generated for the sample characteristics and symptom data. All calculations used actual values. No adjustments were 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.

LCA was used to identify subgroups of patients with similar experiences with the symptom cluster of pain, fatigue, sleep disturbance, and depression (i.e., latent classes; Vermunt & Magdison, 2002). The final number of latent classes was identified by evaluating the Bayesian information criterion (BIC), the parametric bootstrapped likelihood ratio test (BLRT), and entropy. With this analysis, the model that fits the data best has the lowest BIC and/or BLRT, which indicates that the estimated model is a better fit than the model with one fewer class (Nylund, Asparouhov, & Muthen, 2007). In addition, better-fitting models should produce higher entropy values (Celeux & Soromenho, 1996). 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., 2007).

Latent class models often use categorical variables (Lanza, Flaherty, & Collins, 2003). 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, one of the continuous variables in this study, namely “worst pain,” which was reported on a 0 to 10 NRS, had a large number of 0s because a number of the patients did not report pain prior to surgery. We accommodated this large number of 0s 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; L. K. Muthen & Muthen, 1998–2010b).

The LCPA was performed using Mplus™ Version 6 (L. K. Muthen & Muthen, 1998–2010a). Estimation was carried out with robust maximum likelihood and the expectation-maximization algorithm (B. Muthen & Shedden, 1999). Due to the inclusion of a categorical variable (i.e., the binary variable for the occurrence of pain vs. no pain), Gauss–Hermite adaptive numeric integration with 20 integration points was employed. Subsequent analyses of differences among the identified classes were carried out with the Statistical Package for the Social Sciences (SPSS, 2012) Version 19 for Windows™.

Methods of Analysis for Genomic Data

Gene selection. Cytokines and their receptors are classes of polypeptides that mediate inflammatory processes (Verri et al., 2006). Proinflammatory cytokines promote systemic inflammation and include interferon gamma 1 (IFNG1), IFNG receptor 1 (IFNGR1), IL1R1, IL2, IL8, IL17A, nuclear factor kappa beta 1 (NFKB1), NFKB2, and TNFz. Anti-inflammatory cytokines suppress the activity of proinflammatory cytokines and include IL1R2, IL4, IL10, and IL13. Of note, IFNG1, IL1B, and IL6 perform pro- and anti-inflammatory functions (Seruga et al., 2008; Verri et al., 2006). The specific single-nucleotide polymorphisms (SNPs) for each of the relevant genes are listed in Supplementary Table 1.

Blood collection and genotyping. Of the 398 patients who completed the baseline assessment, 302 provided a blood sample. Genomic deoxyribonucleic acid (DNA) was extracted from archived buffy coats using the PUREGene DNA Isolation System (Invitrogen, Carlsbad, CA). Study personnel performing genotyping were blinded to LCPA status, and positive and negative controls were included. Samples were genotyped using the GoldenGate genotyping platform (Illumina, San Diego, CA) and processed using GenomeStudio (Illumina, San Diego, CA).

SNP selection. A combination of tagging SNPs and literature-driven SNPs was selected for analysis. We required that the tagging SNPs were common (i.e., estimated to have a minor allele frequency ≥.05) in public databases. 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 Supplementary Table 1, a total of 82 SNPs among the 15 candidate genes passed all quality control filters and were included in the genetic association analyses. Potential functional roles of SNPs associated with the symptoms of pain, fatigue, sleep disturbance, and depression were examined using PUPASuite 2.0 (Conde et al., 2006).
**Statistical Analyses**

Allele and genotype frequencies were determined by gene counting. Hardy–Weinberg equilibrium was assessed by the $\chi^2$ or Fisher exact tests. Measures of linkage disequilibrium ([LD] i.e., $D'$ and $r^2$) were computed from the patients’ genotypes with Haplovie 4.2. LD-based haplotype block definition was based on $D'$ confidence interval (CI; Gabriel et al., 2002).

Haplotypes were constructed using the program PHASE Version 2.1 (Stephens, Smith, & Donnelly, 2001). Only haplotypes that were inferred with probability estimates of $\geq .85$ across five iterations were retained for downstream analyses. Haplotypes were evaluated assuming a dosage model (i.e., analogous to the additive model).

Ancestry informative markers (AIMs) were used to minimize confounding due to population stratification (Halder, Shriver, Thomas, Fernandez, & Frudakis, 2008; Hoggart et al., 2003; Tian, Gregersen, & Seldin, 2008). Homogeneity in ancestry among patients was verified by principal component (PC) analysis (Price et al., 2006) using Helix Tree (Golden Helix, Bozeman, MT; data not shown). We included 106 AIMs in the analysis. The first three PCs were selected to adjust for potential confounding due to population substructure (i.e., race/ethnicity) by including the three covariates in all regression models.

For association tests, the following three genetic models were assessed for each SNP: additive, dominant, and recessive. Barring trivial improvements (i.e., $\delta < 10\%$), the genetic model that best fit the data, by maximizing the significance of the $p$ value, was selected for each SNP. Logistic regression analyses, which controlled for significant covariates as well as genomic estimates of and self-reported race/ethnicity, were used to evaluate the relationship between genotype and LCPA group membership. A backward stepwise approach was used to create the most parsimonious model. Genetic model fit and both unadjusted and covariate-adjusted odds ratios (ORs) were estimated using STATA Version 9 (StataCorp, 2005).

As was done in our previous studies (Illi et al., 2012; McCann et al., 2012; Miaskowski, Cooper, Dhruva, et al., 2012), based on recommendations in the literature (Hattersley & McCarthy, 2005; Rothman, 1990), the implementation of rigorous quality controls for genomic data, the nonindependence of SNPs/haplotypes in LD, and the exploratory nature of the analyses, adjustments were not made for multiple testing. In addition, significant SNPs identified in the bivariate analyses were evaluated further using regression analyses that controlled for differences in phenotypic characteristics, potential confounding due to population stratification, and variation in other SNPs/haplotypes within the same gene. Only those SNPs that remained significant were included in the final presentation of the results. Therefore, the significant independent associations reported are unlikely to be due solely to chance. Unadjusted (bivariate) associations are reported for all SNPs passing quality control criteria in Supplementary Table 1 to allow for subsequent comparisons and meta-analyses.

**Results**

**LCA**

Using LCPA, we identified three distinct classes of patients 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 1. The three-class solution was selected because its BIC was lower than the BIC for both the two- and four-class solutions. As summarized in Table 2, the largest percentage of patients (61.0%) was classified in the all low class and had mean scores for all symptoms that were below the clinically meaningful cutoff scores. A second group, which comprised 7.1% of the patients, was classified as the all high class. All four of the symptom scores were above the clinically meaningful cutoff scores for members of this class. The third class, comprising 31.6% of the sample, was classified as the low pain and high fatigue class.

**Differences in Demographic and Clinical Characteristics Among the Three Latent Classes**

As shown in Table 3, we found significant differences among the three latent classes in age, years of education, ethnicity, living arrangements, annual income, KPS score, estrogen and progesterone receptor status, and receipt of neoadjuvant chemotherapy.

**Differences in Demographic and Clinical Characteristics Between the all low and the all high Latent Classes**

Because the subsequent genomic analyses were done using an extreme phenotype approach (Li, Lewinger, Gauderman, Murcay, & Conti, 2011), we have summarized differences in demographic and clinical characteristics between the all low and the all high latent classes in Table 3. Compared to the all low class, patients in the all high class were significantly younger, had fewer years of education, and more likely to be non-White, have a lower annual household income, and live alone. In addition, compared to the all low class, patients in the all high class had a lower functional status score, a higher comorbidity score, and a more advanced stage of disease at the time of diagnosis.

**Candidate Gene Analyses for the Two Latent Classes**

As summarized in Supplementary Table 1, the genotype frequency was significantly different between the two latent classes for nine SNPs and two haplotypes spanning seven genes (i.e., IL1R1, IL6, IL13, IL17A, NFKB1, NFKB2, and TNFA). One haplotype (HapA2, $p = .010$) was identified in IL1R1. For the two SNPs identified in IL6 (rs1554606, and rs2069845), a recessive model (both $p = .044$) fits the data best. Two SNPs (rs1295686 and rs20541) and one haplotype (HapA1, $p < .0001$) were identified in IL13. For these two SNPs, a dominant model fits the data best ($p < .0001$ and $p = .041$, respectively). For the SNP in IL17A (rs2275913), a dominant model fits the
### Table 2. Symptom Severity Scores at Enrollment by Latent Class

<table>
<thead>
<tr>
<th>Symptom score</th>
<th>All low (1)</th>
<th>Low pain and high fatigue (2)</th>
<th>All high (3)</th>
<th>Omnibus test p-value; Sidak post hoc contrasts</th>
</tr>
</thead>
<tbody>
<tr>
<td>Global Sleep Disturbance Scale</td>
<td>n = 241 (61.3%)</td>
<td>n = 124 (31.6%)</td>
<td>n = 28 (7.1%)</td>
<td>p &lt; .00005; 1 &lt; 2 and 3</td>
</tr>
<tr>
<td>Center for Epidemiological Studies–Depression Scale</td>
<td>40.1 (18.4)</td>
<td>59.8 (19.9)</td>
<td>67.6 (19.7)</td>
<td></td>
</tr>
<tr>
<td>Worst Pain Severity (0–10)$^{b}$</td>
<td>2.4 (1.1)</td>
<td>2.9 (1.2)</td>
<td>7.6 (1.6)</td>
<td>p &lt; .00005; 1 and 2 &lt; 3</td>
</tr>
<tr>
<td>Lee Fatigue Scale</td>
<td>1.5 (1.2)</td>
<td>5.8 (1.3)</td>
<td>4.6 (1.7)</td>
<td>p &lt; .00005; 2 &gt; 3 &gt; 1</td>
</tr>
</tbody>
</table>

Note. Values reported for symptom scores are mean (SD). Predicted classes based on the most likely latent class membership. Means and tests are only for patients who reported pain: Class 1 (n = 20), Class 2 (n = 31), and Class 3 (n = 48).

### Table 3. Demographic and Clinical Characteristics by Latent Class

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>All low (1)</th>
<th>Low pain and high fatigue (2)</th>
<th>All high (3)</th>
<th>Comparison Among 1, 2, and 3</th>
<th>Comparison Between 1 and 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>n = 241 (61%)</td>
<td>n = 124 (31.6%)</td>
<td>n = 28 (7.1%)</td>
<td>t = 2.60, p = .01</td>
<td></td>
</tr>
<tr>
<td>Education (years)</td>
<td>15.7 (2.7)</td>
<td>16.0 (2.6)</td>
<td>14.0 (2.6)</td>
<td>F(2, 390) = 9.72, p &lt; .0001; 1 &gt; 2 and 3</td>
<td></td>
</tr>
<tr>
<td>Number biopsies in past year</td>
<td>1.5 (0.7)</td>
<td>1.5 (0.9)</td>
<td>1.7 (1.1)</td>
<td>t = 3.18, p = .002</td>
<td></td>
</tr>
<tr>
<td>Karnofsky Performance Status score</td>
<td>95.6 (8.2)</td>
<td>90.4 (11.8)</td>
<td>84.4 (12.5)</td>
<td>F(2, 284) = 23.0, p &lt; .0001; 1 &gt; 2 &gt; 3</td>
<td></td>
</tr>
<tr>
<td>Self-administered Comorbidity Questionnaire score</td>
<td>4.1 (2.7)</td>
<td>4.5 (3.1)</td>
<td>5.1 (2.7)</td>
<td>t = -2.00, p = .047</td>
<td></td>
</tr>
<tr>
<td>Body mass index (kg/m$^2$)</td>
<td>26.5 (6.1)</td>
<td>27.5 (6.2)</td>
<td>26.9 (6.7)</td>
<td>n.s.</td>
<td></td>
</tr>
<tr>
<td>Lives alone</td>
<td>22.9 (55)</td>
<td>23.0 (28)</td>
<td>46.2 (12)</td>
<td>$\chi^2 = 7.08, p = .029$</td>
<td></td>
</tr>
<tr>
<td>Married/partnered</td>
<td>42.3 (102)</td>
<td>38.2 (47)</td>
<td>60.0 (15)</td>
<td>n.s.</td>
<td></td>
</tr>
<tr>
<td>Ethnicity</td>
<td>n.s.</td>
<td>n.s.</td>
<td>n.s.</td>
<td>n.s.</td>
<td></td>
</tr>
<tr>
<td>White</td>
<td>65.4 (157)</td>
<td>69.9 (86)</td>
<td>32.1 (9)</td>
<td>$\chi^2 = 24.3, p &lt; .0001$</td>
<td></td>
</tr>
<tr>
<td>Black</td>
<td>8.8 (21)</td>
<td>7.3 (9)</td>
<td>28.6 (8)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Asian/Pacific Islander</td>
<td>13.3 (32)</td>
<td>8.1 (10)</td>
<td>28.6 (8)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hispanic/Mixed</td>
<td>12.5 (30)</td>
<td>14.6 (18)</td>
<td>10.7 (3)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Income</td>
<td>n.s.</td>
<td>n.s.</td>
<td>n.s.</td>
<td>n.s.</td>
<td></td>
</tr>
<tr>
<td>&lt;US$30,000</td>
<td>17.7 (35)</td>
<td>21.9 (23)</td>
<td>57.1 (12)</td>
<td>$\chi^2 = 19.5, p = .001$</td>
<td></td>
</tr>
<tr>
<td>US$30,000–$99,000</td>
<td>43.9 (87)</td>
<td>35.2 (37)</td>
<td>28.6 (6)</td>
<td>$\chi^2 = 17.55, p = .001$</td>
<td></td>
</tr>
<tr>
<td>$\geq$US$100,000</td>
<td>38.4 (76)</td>
<td>42.9 (45)</td>
<td>14.3 (3)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stage of disease</td>
<td>n.s.</td>
<td>MW = .018</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stage 0</td>
<td>19.9 (48)</td>
<td>16.9 (21)</td>
<td>14.3 (4)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stage I</td>
<td>38.2 (92)</td>
<td>40.3 (50)</td>
<td>21.4 (6)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stages IIa and IIb</td>
<td>35.3 (85)</td>
<td>33.9 (42)</td>
<td>42.9 (12)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stages IIIa, IIIb, IIIC, and IV</td>
<td>6.6 (16)</td>
<td>8.9 (11)</td>
<td>21.4 (6)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Estrogen receptor positive</td>
<td>82.1 (197)</td>
<td>69.4 (86)</td>
<td>67.9 (19)</td>
<td>$\chi^2 = 8.9, p = .012$</td>
<td></td>
</tr>
<tr>
<td>Progesterone receptor positive</td>
<td>75.4 (181)</td>
<td>61.3 (76)</td>
<td>64.3 (18)</td>
<td>$\chi^2 = 8.3, p = .016$</td>
<td></td>
</tr>
<tr>
<td>Gone through menopause</td>
<td>65.8 (158)</td>
<td>60.2 (118)</td>
<td>64.0 (16)</td>
<td>n.s.</td>
<td></td>
</tr>
<tr>
<td>Received neoadjuvant therapy</td>
<td>16.3 (39)</td>
<td>27.4 (34)</td>
<td>21.4 (6)</td>
<td>$\chi^2 = 6.4, p = .041$</td>
<td></td>
</tr>
<tr>
<td>Received hormone-replacement therapy prior to cancer diagnosis</td>
<td>17.5 (42)</td>
<td>17.1 (21)</td>
<td>10.7 (3)</td>
<td>n.s.</td>
<td></td>
</tr>
</tbody>
</table>

Note. FE = Fisher’s exact test; HER2 = human epidermal growth factor receptor 2; MW = Mann–Whitney U; n.s. = not significant; SD = standard deviation.
data best ($p = .048$). For the two SNPs identified in *NFKB1* (rs4648110, rs4648141), an additive model fits the data best ($p = .009$ and $p = .031$, respectively). For the one SNP identified in *NFKB2* (rs1056890), a dominant model fits the data best ($p = .026$). For the one SNP identified in *TNFA* (rs1800610), a dominant model fits the data best ($p = .039$).

**Regression Analyses of IL1R1, IL6, IL13, IL17A, NFkB1, NFkB2, and TNFA Genotypes and Haplotypes and Latent Class Membership**

In order to better estimate the magnitude (i.e., OR) and precision (95% CI) of the association between genotype and latent class membership, multivariate logistic regression models were used. In addition to genotype, the phenotypic variables included in the regression models were genomic estimates of race/ethnicity (i.e., White, Black, Asian, Hispanic/mixed ethnic background/other), age, KPS score, and living arrangements.

The only genetic associations that remained significant in the multivariate regression analyses were for *IL6* rs2069845, *IL13* rs1295686, and *TNFA* rs1800610 (Table 4). In the regression analysis for *IL6* rs2069845, carrying two doses of the rare G allele (i.e., AA + AG vs. GG) was associated with a 14-fold increase in the odds of being in the all high latent class. Of note, *IL6* rs1554606 and *IL6* rs2069845 were completely colinear (i.e., mutual surrogates). Therefore, only one (i.e., rs2069845) of the two SNPs was selected to represent these two SNPs.

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

**Discussion**

To the best of our knowledge, this study is the first to identify distinct subgroups of women prior to breast cancer surgery based on their experience with the symptom cluster of pain, fatigue, sleep disturbance, and depression. The LCPA identified the following three relatively distinct latent classes: fatigue, sleep disturbance, and depression. The LCPA identified the following three relatively distinct latent classes: fatigue, sleep disturbance, and depression. The LCPA identified the following three relatively distinct latent classes: fatigue, sleep disturbance, and depression.

In terms of *TNFA*, patients who were homozygous for the rare allele in *rs1800610* were more likely to be in all high class. This SNP is located in the intronic region of the *IL6* gene, it resides in region that is a likely binding site for two ribosomal binding proteins (RBPs; University of California Santa Cruz [UCSC] Genome Bioinformatics website at genome.ucsc.edu). In addition, recent findings suggest that it plays a role in the modulation of inflammatory responses. In one study (Sousa et al., 2012), carriers of the rare allele were at higher risk for the occurrence of leprosy Type 2 reactions. These Type 2 reactions presented as disseminated painful erythematous tender nodules, with or without neuritis, usually followed by systemic symptoms (e.g., fever and malaise) and were associated with elevated levels of TNFα. In another study (Tabassum et al., 2012), the minor allele was associated with an increased risk of childhood obesity. Interestingly, a systemic inflammatory response appears to occur in children with obesity that is related to elevated levels of inflammatory cytokines being produced by excessive amounts of adipose tissue (Schwarzenberg & Sinaiko, 2006). In a study of patients with myocardial infarction (Ljungman et al., 2009), the rare allele was associated with increased circulating levels of IL6. Finally, findings from our research group and others suggest that additional SNPs in IL6 are associated with the symptoms of fatigue (Miaskowski et al., 2010), sleep disturbance (Miaskowski, Cooper, Dhruva et al., 2012; Miaskowski et al., 2010), and depression (Dowlati et al., 2010; Sukoff Rizzo et al., 2012). Taken together, these findings suggest that variations in the *IL6* gene contribute to inflammatory responses and symptoms associated with sickness behavior.
In this study, an SNP in one anti-inflammatory cytokine gene was associated with membership in the all high class. Patients who were heterozygous or homozygous for the rare allele in \textit{IL13} rs1295686 were 29 times more likely to be classified in the all high class compared to those without the rare allele. It is not readily apparent why this SNP was not associated with membership in the all high class in our previous study (Illi et al., 2012). However, in the present sample of women with breast cancer, we found that each dose of the rare A allele in \textit{IL13} rs1295686 was associated with a 1.6-fold increase in the odds of reporting pain in the breast prior to surgery (McCann et al., 2012).

While rs1295686 is located in intron 3 of the \textit{IL13} gene, it resides in a region of the gene that is a binding site for the NFKB transcription factor (Hirota et al., 2012; Moffatt et al., 2010) and occurs within a region of \textit{IL13} that undergoes DNA methylation. Preclinical studies suggest that \textit{IL13} has anti-inflammatory effects in mice. This effect may be mediated through inhibition of the release of TNF\(\alpha\) (Vale et al., 2003) and IL1\(\beta\) (Karam, Al-Kouba, Bazzi, Smith, & Leung, 2011). In addition, in a preclinical study by Karam, Al-Kouba, Bazzi, Smith, and Leung (2011), mice that were infected with a low dose of Leishmania (which induces a sustained hyperalgesia) and received concomitant administration of \textit{IL13} (which reduced the hyperalgesic response) produced increased levels of IL6. Karam et al. concluded that the anti-hyperalgesia effect of \textit{IL13} was independent of its effect on IL6 release. Given the associations between latent class membership and variations in IL6 and \textit{IL13} found in the current study, additional research with larger samples is warranted to examine the relationships between these two molecular markers and the severity of pain, fatigue, sleep disturbance, and depression, not only as symptoms but also as individual symptoms.

In terms of clinical studies, associations between \textit{IL13} rs1295686 and immunoglobulin E (IgE) dysfunction were evaluated in the Framingham Heart Study (Granada et al., 2012) and in a study that evaluated total IgE concentrations in cord blood samples (Hong et al., 2010). In both of these studies, the rare allele rs1295686 in \textit{IL13} was associated with decreases in IgE concentrations. While we found no additional clinical studies on associations between this SNP and other symptoms, the preclinical and clinical findings suggest that additional research is warranted on the role of the \textit{IL13} gene, in conjunction with other pro- and anti-inflammatory cytokine genes, in the symptom experience of oncology patients.

In our previous study of oncology patients and their family caregivers (Illi et al., 2012), we found an association between another anti-inflammatory cytokine gene (i.e., IL4 rs2243248) and membership in the all high symptom class. Participants who were heterozygous or homozygous for the rare allele were 6 times more likely to be classified in the all high class than those without the rare allele. In the current study, we found no association between this SNP and the all high class. The reasons why this SNP was not associated with latent class membership in the current study may be related to differences in demographic (e.g., gender) and clinical (e.g., cancer

### Table 4. Multiple Logistic Regression Analyses for IL6, IL13, and TNFA Genotypes Comparing the Latent Classes All Low Versus All High.

<table>
<thead>
<tr>
<th>Predictor</th>
<th>OR</th>
<th>SE</th>
<th>[95% CI]</th>
<th>Z</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>IL6 genotype</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>0.72</td>
<td>0.131</td>
<td>[0.505, 1.030]</td>
<td>−1.80</td>
<td>.072</td>
</tr>
<tr>
<td>KPS score</td>
<td>0.36</td>
<td>0.120</td>
<td>[0.186, 0.689]</td>
<td>−3.07</td>
<td>.002</td>
</tr>
<tr>
<td>Lives alone</td>
<td>9.12</td>
<td>7.567</td>
<td>[1.793, 46.375]</td>
<td>2.68</td>
<td>.008</td>
</tr>
<tr>
<td><strong>Overall model fit:</strong> (\chi^2 )</td>
<td>36.05</td>
<td>p = .0001, (R^2 ) = .3768</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>IL13 genotype</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>0.68</td>
<td>0.148</td>
<td>[0.444, 1.040]</td>
<td>−1.78</td>
<td>.075</td>
</tr>
<tr>
<td>KPS score</td>
<td>0.44</td>
<td>0.135</td>
<td>[0.242, 0.805]</td>
<td>−2.67</td>
<td>.008</td>
</tr>
<tr>
<td>Lives alone</td>
<td>10.89</td>
<td>9.960</td>
<td>[1.815, 65.376]</td>
<td>2.61</td>
<td>.009</td>
</tr>
<tr>
<td><strong>Overall model fit:</strong> (\chi^2 )</td>
<td>40.32</td>
<td>p &lt; .0001, (R^2 ) = .4215</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>TNFA genotype</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>0.71</td>
<td>0.130</td>
<td>[0.500, 1.026]</td>
<td>−1.84</td>
<td>.065</td>
</tr>
<tr>
<td>KPS score</td>
<td>0.45</td>
<td>0.133</td>
<td>[0.253, 0.806]</td>
<td>−2.69</td>
<td>.007</td>
</tr>
<tr>
<td>Lives alone</td>
<td>10.77</td>
<td>8.864</td>
<td>[2.145, 54.050]</td>
<td>2.89</td>
<td>.004</td>
</tr>
<tr>
<td><strong>Overall model fit:</strong> (\chi^2 )</td>
<td>33.50</td>
<td>p = .0002, (R^2 ) = .3502</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Note. n = 196. OR = odds ratio; SE = standard error; CI = confidence interval; KPS = Karnofsky Performance Status; IL = interleukin; TNFA = tumor necrosis factor alpha. For each model, the three first principal components identified from the analysis of ancestry informative markers as well as self-report race/ethnicity (i.e., White, Black, Asian/Pacific Islander, Hispanic/mixed ethnic background/other) were retained in all models to adjust for potential confounding due to race or ethnicity (data not shown). Predictors evaluated in each model included genotype (IL6 rs2069845: AA + AG vs. GG, IL13 rs1295686: GG vs. GA = AA; TNFA rs1800610: CC vs. CT + TT), age (in 5-year increments), KPS score (in 10-point increments), and whether the patient lives alone (No/Yes).

**Table 4. Multiple Logistic Regression Analyses for IL6, IL13, and TNFA Genotypes Comparing the Latent Classes All Low Versus All High.**

**Predictor** | **OR** | **SE** | **[95% CI]** | **Z** | **p Value**
--- | --- | --- | --- | --- | ---
**IL6 genotype** | | | | | |
Age | 0.72 | 0.131 | [0.505, 1.030] | −1.80 | .072 |
KPS score | 0.36 | 0.120 | [0.186, 0.689] | −3.07 | .002 |
Lives alone | 9.12 | 7.567 | [1.793, 46.375] | 2.68 | .008 |
**Overall model fit:** \(\chi^2 \) = 36.05, p = .0001, \(R^2 \) = .3768
**IL13 genotype** | | | | | |
Age | 0.68 | 0.148 | [0.444, 1.040] | −1.78 | .075 |
KPS score | 0.44 | 0.135 | [0.242, 0.805] | −2.67 | .008 |
Lives alone | 10.89 | 9.960 | [1.815, 65.376] | 2.61 | .009 |
**Overall model fit:** \(\chi^2 \) = 40.32, p < .0001, \(R^2 \) = .4215
**TNFA genotype** | | | | | |
Age | 0.71 | 0.130 | [0.500, 1.026] | −1.84 | .065 |
KPS score | 0.45 | 0.133 | [0.253, 0.806] | −2.69 | .007 |
Lives alone | 10.77 | 8.864 | [2.145, 54.050] | 2.89 | .004 |
**Overall model fit:** \(\chi^2 \) = 33.50, p = .0002, \(R^2 \) = .3502

Note. n = 196. OR = odds ratio; SE = standard error; CI = confidence interval; KPS = Karnofsky Performance Status; IL = interleukin; TNFA = tumor necrosis factor alpha. For each model, the three first principal components identified from the analysis of ancestry informative markers as well as self-report race/ethnicity (i.e., White, Black, Asian/Pacific Islander, Hispanic/mixed ethnic background/other) were retained in all models to adjust for potential confounding due to race or ethnicity (data not shown). Predictors evaluated in each model included genotype (IL6 rs2069845: AA + AG vs. GG, IL13 rs1295686: GG vs. GA = AA; TNFA rs1800610: CC vs. CT + TT), age (in 5-year increments), KPS score (in 10-point increments), and whether the patient lives alone (No/Yes).
diagnoses) characteristics between the two samples, as well as differences in the symptom characteristics of the latent classes in the two studies. Additional research is needed with larger samples to explore the role of variations in **IL4** and common symptoms in oncology patients.

Several study limitations need to be acknowledged. First, most of the patients were Caucasian, middle aged, well educated, mildly overweight, married, and postmenopausal. The relative homogeneity of this sample of women limits the generalizability of the study findings. Second, the exact etiologies for the symptom cluster of pain, fatigue, sleep disturbance, and depression warrant additional investigation. In this study, we hypothesized that sickness behavior associated with inflammation was the mechanism for this symptom cluster. However, sickness behavior is not the only potential mechanism for these four symptoms. The specific interactions that occur among **IL6**, **IL13**, and **TNFα** and how they trigger these four symptoms remain to be determined. In addition, each cytokine may contribute in a differential manner to the development of these four symptoms. Therefore, additional research is warranted to evaluate each single gene’s role in the development of sickness behavior and to evaluate for interactions among multiple genes in order to understand the causes of symptom clusters. Third, patients in the current study reported a comorbidity score of between 4 and 5. Therefore, we cannot rule out the fact that some of the associations identified in this study might be attributable to other chronic conditions. Fourth, we did not measure serum cytokine levels in these patients. In addition, future studies need to evaluate whether the polymorphisms identified in this study affect protein production and/or binding of these cytokines to their receptors. Finally, it is possible that we did not identify all of the SNPs in the cytokine genes that are associated with the symptom cluster due to the small sample size in the all high class. A larger sample is needed to increase the power to detect differences in other cytokine genes in future studies.

In conclusion, findings from this study partially confirm our previous findings of subgroups of patients with distinct experiences with the symptom cluster of pain, fatigue, sleep disturbance, and depression (Miaskowski et al., 2006; Pud et al., 2008). In addition, our findings provide new evidence that genetic variations in **IL6**, **IL13**, and **TNFA** are associated with the symptom cluster of pain, fatigue, sleep disturbance, and depression. The association between pro- and anti-inflammatory cytokine genes and this symptom cluster provides additional support for the role of inflammatory mechanisms in the development of these symptoms.

**Authors’ Note**
The authors Bradley E. Aouizerat and Christine Miaskowski have dual senior authorship. The contents of this project are solely the responsibility of the authors and do not necessarily represent the official views of the NIH.

**Declaration of Conflicting Interests**
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**Supplemental Material**
The online supplemental tables are available at http://brn.sagepub.com/supplemental

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