# Polymorphisms in Tumor Necrosis Factor-α Are Associated With Higher Anxiety Levels in Women After Breast Cancer Surgery

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## Abstract

**The present study identified 2 subgroups of patients with higher and lower levels of anxiety during the 6 months after breast cancer surgery. The women in the higher anxiety class were younger and reported a lower functional status. In addition, 2 polymorphisms in the tumor necrosis factor-α gene were associated with membership in the higher anxiety class.**

**Introduction:** Before and after breast cancer surgery, women have reported varying anxiety levels. Recent evidence has suggested that anxiety has a genetic basis and is associated with inflammation. The purposes of the present study were to identify the subgroups of women with distinct anxiety trajectories; to evaluate for differences in the phenotypic characteristics between these subgroups; and to evaluate for associations between polymorphisms in cytokine genes and subgroup membership. **Patients and Methods:** Patients with breast cancer (n = 398) were recruited before surgery and followed up for 6 months. The patients completed the Spielberger State Anxiety Inventory and provided a blood sample for genomic analyses. Growth mixture modeling was used to identify the subgroups of patients with distinct anxiety trajectories. **Results:** Two distinct anxiety subgroups were identified. The women in the higher anxiety subgroup were younger and had a lower functional status score. Two single nucleotide polymorphisms in tumor necrosis factor-α (rs1799964, rs3093662) were associated with the higher anxiety subgroup. **Conclusion:** The results of the present exploratory study suggest that polymorphisms in cytokine genes could partially explain the interindividual variability in anxiety. The determination of phenotypic and molecular markers associated with greater levels of anxiety can assist clinicians to identify high-risk patients and initiate appropriate interventions.

*Clinical Breast Cancer,* Vol. 16, No. 1, 63-71 © 2016 Elsevier Inc. All rights reserved.
**Keywords:** Cytokine genes, Depression, Growth mixture modeling, Psychological distress, Symptom trajectories

## Introduction

Moderate to high levels of psychological distress have been reported by most women before breast cancer surgery that gradually decreases during the first 12 months after surgery.[1-12] Across a number of studies, a younger age[3,10,13] and child care responsibilities[3] were associated with greater levels of psychological distress. Of the clinical characteristics, although no differences in psychological distress were found between women who had undergone breast conserving surgery and those who had undergone mastectomy,[4,9,10] the receipt of adjuvant treatment was associated with greater levels of distress.[3,14] However, one of the major limitations of these studies was that "general measures" of psychological distress were used rather than a specific measure of anxiety (eg, Spielberger State-Trait Anxiety Inventory [STAI][15]). Therefore, additional research is warranted to determine which demographic and clinical characteristics are specifically associated with greater levels of anxiety that persist after surgery.

Although the phenotypic characteristics that place patients with breast cancer at greater risk of more severe and persistent anxiety require additional investigation, the results from recent meta-analyses suggest that anxiety disorders have a genetic

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Submitted: Aug 11, 2014; Revised: Dec 9, 2014; Accepted: Dec 16, 2014; Epub: Dec 24, 2015

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1526-8209/$ - see frontmatter 
http://dx.doi.org/10.1016/j.clbc.2014.12.001

basis.[16-21] In addition, an emerging body of evidence suggests that inflammatory mechanisms may contribute to the development of anxiety disorders (for review, see Sokolowska and Hovatta[22] and Miller et al[23]). These investigators' reviews suggest that acute physical or psychological stressors modulate cytokine expression in the central nervous system. These changes in cytokine expression are adaptive, temporary, and controlled. However, when the stress becomes chronic, which is the case with a chronic illness such as cancer, the resultant chronic inflammatory responses contribute to the development of maladaptive behavioral symptoms (eg, anxiety, depression) and neuropsychiatric disorders.

Because of the associations between stress and its associated inflammatory responses and greater levels of symptoms,[24,25] our research team investigated the role of cytokine gene polymorphisms and an increased risk for depression,[26] pain,[27,28] sleep disturbances,[29] and attentional fatigue[30] in patients before and after surgery for breast cancer. In these studies, we used growth mixture modeling (GMM) to identify subgroups of patients with distinct symptom trajectories. In the present study, we extended this approach to an evaluation of state anxiety in women who were enrolled before breast cancer surgery and followed up for 6 months. The purposes of the present study, in a sample of 398 women who had undergone surgery for breast cancer, were to identify the subgroups (ie, latent classes) of women with distinct anxiety trajectories; to evaluate for differences in phenotypic characteristics between the latent classes; and to evaluate for associations between polymorphisms in genes for pro- and anti-inflammatory cytokines, their receptors, and their transcriptional regulators and latent class membership.

## Patients and Methods

### *Patients and Settings*

The present analysis was a part of a larger, longitudinal study that evaluated neuropathic pain and lymphedema in women who had undergone breast cancer surgery. The study methods have been previously described in detail.[27,31-33] In brief, the patients were recruited from breast care centers located at a comprehensive cancer center, 2 public hospitals, and 4 community practices.

Patients were eligible to participate if they were adult women (age $\geq$ 18 years) scheduled to undergo breast cancer surgery on 1 breast; who were able to read, write, and understand English; and who had agreed to participate and given written informed consent. Patients were excluded if they were scheduled to undergo bilateral breast cancer surgery or had distant metastases at the diagnosis. A total of 516 patients were approached, 410 were enrolled (response rate, 79.5%), and 398 completed the enrollment assessment. The most common reasons for refusal were too busy, overwhelmed with the cancer diagnosis, and insufficient time available to complete the enrollment assessment before surgery.

### *Instruments*

The demographic questionnaire obtained information on age, marital status, education, ethnicity, employment status, and living situation. Patients rated their functional status using the Karnofsky performance status (KPS) scale that ranged from 30 (I feel severely disabled and need to be hospitalized) to 100 (I feel normal; I have no complaints or symptoms).[34] The self-administered comorbidity questionnaire (SCQ) was used to evaluate comorbidity.[35] Patients were asked to indicate whether they had 1 of 13 common medical conditions; whether they had received treatment for it (proxy for disease severity); and whether it had limited their activities (indication of functional limitations). For each condition, a patient could receive a maximum of 3 points, with a maximum total score of 39. The SCQ has well-established validity and reliability.[36,37]

The Spielberger State-Trait Anxiety Inventories (STAI-T, STAI-S) consist of 20 items, each rated from 1 to 4. The scores for each scale are summed and range from 20 to 80. A higher score indicates greater anxiety. The STAI-T measures an individual's predisposition to anxiety determined by the person's personality and estimates how a person generally feels. The STAI-S measures an individual's transitory emotional response to a stressful situation. It evaluates the emotional responses of worry, nervousness, tension, and feelings of apprehension related to how a person feels "right now" in a stressful situation. A cutoff score of $\geq$ 31.8 and $\geq$ 32.2 indicates a high level of trait and state anxiety, respectively.[15] The STAI-S and STAI-T inventories have well-established validity and reliability.[38,39] In the present study, Cronbach's α for the STAI-T and STAI-S were 0.88 and 0.95, respectively.

The Center for Epidemiological Studies Depression Scale (CES-D) consists of 20 items selected to represent the major symptoms in the clinical syndrome of depression. The scores range from 0 to 60, with scores of $\geq$ 16 indicating the need for individuals to seek clinical evaluation for major depression. The CES-D has well-established validity and reliability.[40-42] In the present study, Cronbach's α for the CES-D was 0.90.

### *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 present study. During the patient's preoperative visit, a clinician explained the study, determined the patient's willingness to participate, and introduced the patient to the research nurse. The research nurse met with the women, determined their eligibility, and obtained written informed consent before surgery. After obtaining consent, the patients completed the enrollment questionnaires an average of 4 days before their surgery. The patients completed the STAI-S at enrollment and monthly for 6 months (ie, 7 assessments). The patients' medical records were reviewed for disease and treatment information.

### *Genomic Analyses*

*Gene Selection.* Cytokines, their receptors, and their transcriptional regulators are classes of polypeptides that mediate inflammatory processes. Cytokine dysregulation has been associated with anxiety (for review, see Sokolowska and Hovatta[22] and Miller et al[23]). These polypeptides are divided into pro- and anti-inflammatory cytokines. Pro-inflammatory mediators promote systemic inflammation and include interferon-γ (IFNG), IFNG receptor 1 (IFNGR1), interleukin-1 receptor 1 (IL1R1), interleukin (IL) 2, IL8, IL17A, and tumor necrosis factor-α (TNFA). Anti-inflammatory mediators suppress the activity of pro-inflammatory cytokines and include IL1R2, IL4, IL10, and IL13. Of note, IFNGR1, IL1B, and IL6 possess pro- and anti-inflammatory

functions. Nuclear factor κβ-1 (NFKB1) and NFKB2 are transcriptional regulators of these cytokine genes.[43]

*Blood Collection and Genotyping.* Of the 398 patients who completed the baseline assessment, 310 provided a blood sample from which DNA could be isolated from the peripheral blood mononuclear cells (PBMCs). Genomic DNA was extracted from PBMCs using the PUREGene DNA Isolation System (Invitrogen, Carlsbad, CA). DNA was quantified using a Nanodrop spectrophotometer (model no. ND-1000; Thermo Scientific, ThermoFisher Scientific, Waltham, MA) and normalized to a concentration of 50 ng/μL. Genotyping was performed without awareness of the patients' clinical status, and positive and negative controls were included. The samples were genotyped using the Golden Gate genotyping platform (Illumina, San Diego, CA) and processed according to the standard protocol using Genome Studio (Illumina). Two reviewers who were unaware of the patient characteristics visually inspected the signal intensity profiles and resulting genotype calls for each single nucleotide polymorphism (SNP).

*SNP Selection.* A combination of tagging SNPs and literature-driven SNPs were selected for analysis. The tagging SNPs were required to be common (ie, a minor allele frequency $\geq$ 0.05) in public databases. SNPs with call rates of $<$ 95% or Hardy-Weinberg $P$ values of $<$ .001 were excluded. As listed in Supplemental Table 1 (in the online version), a total of 82 SNPs among the 15 candidate genes passed all the quality control filters and were included in the genetic association analyses. The potential functional roles of the SNPs associated with state anxiety were examined using PUPASuite, version 2.0.[44]

### Statistical Analyses for Phenotypic Data

The data were analyzed using the Statistical Package for Social Sciences,[45] version 20 (IBM Corp, Armonk, NY), and STATA,[46] version 13 (StataCorp, College Station, TX).[46] Descriptive statistics and frequency distributions were generated for sample characteristics. Independent sample $t$ tests, Mann-Whitney $U$ tests, and $\chi^2$ analyses were used to evaluate for differences in the demographic and clinical characteristics between the 2 latent classes. All calculations used actual values. Adjustments were not made for missing data. Therefore, the cohort for each analysis was dependent on the largest set of available data between the 2 groups.

Unconditional GMM with robust maximum likelihood estimation was performed to identify latent classes with distinct anxiety trajectories using M*plus*, version 5.21 (available at: www.statmodel.com). These methods have been previously described in detail.[33] In brief, a single growth curve that represented the "average" change trajectory was estimated for the whole sample. Next, the number of latent growth classes that best fit the data was identified using the guidelines recommended in published studies.[47-49]

### Statistical Analyses for Genetic Data

The allele and genotype frequencies were determined by gene counting. Hardy-Weinberg equilibrium was assessed using the $\chi^2$ or Fisher exact test. Measures of linkage disequilibrium (LD; ie, D′ and $r^2$) were computed from the patients' genotypes using Haploview, version 4.2 (Broadview Institute, Cambridge, MA). The LD-based haplotype block definition was based on the D′ confidence interval.

For the SNPs that were members of the same haploblock, haplotype analyses were conducted to localize the association signal within each gene and to determine whether the haplotypes improved the strength of the association with the phenotype. Haplotypes were constructed using the program PHASE, version 2.1.[50] To improve the stability of haplotype inference, the haplotype construction procedure was repeated 5 times using different seed numbers with each cycle. Only those haplotypes that were inferred with probability estimates of $\geq$ .85, across the 5 iterations, were retained for the downstream analyses. Only the inferred haplotypes that occurred with a frequency estimate of $\geq$ 15% were included in the association analyses, assuming a dosage model (ie, analogous to the additive model).

Ancestry informative markers (AIMs) were used to minimize confounding due to population stratification.[51-53] The homogeneity in ancestry among the patients was verified by principal component analysis[54] using HelixTree (GoldenHelix, Bozeman, MT). In brief, the number of principal components (PCs) was sought that distinguished the major racial or ethnic groups in the sample by visual inspection of the scatter plots of the orthogonal PCs (ie, PC1 vs. PC2, PC2 vs. PC3). This procedure was repeated until no discernable clustering of patients by their self-reported race/ethnicity was possible (data not shown). The first 3 PCs were selected to adjust for potential confounding due to population substructure (ie, race/ethnicity) by including them in all logistic regression models. Finally, 106 AIMs were included in the analysis.

For the association tests, 3 genetic models were assessed for each SNP: additive, dominant, and recessive. Barring trivial improvements (ie, change $<$ 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, controlling for significant covariates and genomic estimates of and self-reported race/ethnicity, were used to evaluate the association between genotype and anxiety class. Only those genetic associations identified as significant from the bivariate analyses were evaluated in the multivariate analyses. A backward stepwise approach was used to create a parsimonious model. Except for the genomic estimates of and self-reported race/ethnicity, only the predictors with $P < .05$ were retained in the final model. The genetic model fit and both unadjusted and covariate-adjusted odds ratios were estimated using STATA, version 13 (StataCorp, College Station, TX).[46]

Just as was performed in our previous studies,[55-57] and in accordance with the recommendations in published studies,[58,59] with 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, the significant SNPs identified in the bivariate analyses were evaluated further using logistic regression analyses that controlled for differences in phenotypic characteristics, potential confounding due to population stratification, and variations in other SNPs/haplotypes within the same gene. Only those SNPs that remained significant were included in the final presentation of the results. Therefore, the significant independent associations reported

were unlikely to have resulted from chance. Unadjusted (bivariate) associations are reported for all the SNPs that passed the quality control criteria in Supplemental Table 1 (available in the online version), to allow for subsequent comparisons and meta-analyses.

## Results

### *GMM Analysis*

Two distinct latent classes of anxiety trajectories were identified using GMM (Figure 1). A 2-class model was selected because its Bayesian information criterion was smaller than that of the 1-class model. In addition, a 3-class solution could not be reliably fit (Table 1).

As listed in Table 2, most patients were classified into the higher anxiety group (63.1%). These patients had state anxiety scores that were high at enrollment (47.6 ± 12.4) and that gradually decreased during the 6 months of the study. The patients in the lower anxiety group (36.9%) had state anxiety scores that were lower at enrollment (31.8 ± 8.8) and that gradually decreased over time.

### *Differences in Demographic and Clinical Characteristics*

As summarized in Table 3, the patients in the higher anxiety class were significantly younger and had a lower KPS score and a higher SCQ score. In addition, a greater percentage of patients in the higher anxiety class had a Hispanic/mixed ethnic background (compared with whites), had received neoadjuvant chemotherapy (CTX), and had received adjuvant CTX during the first 6 months after breast cancer surgery. The patients in the higher anxiety class had higher trait anxiety scores and higher CES-D scores at enrollment.

### *Candidate Gene Analyses of Both GMM Classes*

As summarized in Supplemental Table 1 (available in the online version), the minor allele frequency was significantly different between the 2 latent classes for 6 SNPs and 2 haplotypes: IL1R1 rs3917332, IL6 rs2069840, IL6 HapA5, IL13 rs1295686, IL13 HapA1, NFKB2 rs1056890, TNFA rs1799964, and TNFA rs3093662.

**Figure 1** Observed and Estimated State Anxiety Inventory Trajectories for Patients in Each of the Latent Classes and the Mean State Anxiety Scores for the Total Sample

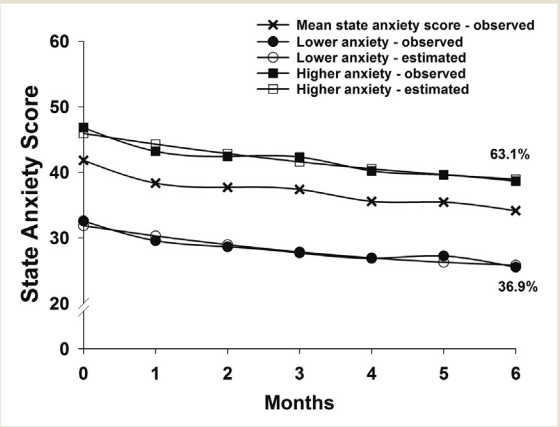



**Table 1** Fit Indexes for the Spielberger State Anxiety Scale GMM Class Solutions for 398 Patients With Breast Cancer

| GMM | LL | AIC | BIC | Entropy | BLRT | VLMR |
|---|---|---|---|---|---|---|
| 1-Class[a] | −9174.21 | 18380.42 | 18444.20 | NA | NA | NA |
| 2-Class[b] | −9136.32 | 18314.64 | 18398.36 | .63 | 75.78[c] | 75.78[d] |
| 3-Class[e] | | | | | | |

Abbreviations: AIC = Akaike information criterion; BIC = Bayesian information criterion; BLRT = parametric bootstrapped likelihood ratio test for K-1 (H0) versus K classes; GMM = growth mixture model; LL = log likelihood; VLMR = Vuong-Lo-Mendell-Rubin likelihood ratio test for K-1 (H0) versus K classes.
[a]Latent growth curve with linear and quadratic components: $\chi^2 = 40.19$, df = 19, $P = .01$, comparative fit index = 0.976, root mean square error of approximation = 0.053.
[b]A 2-class model was selected; the BIC was smaller than for the 1-class model, and the BLRT indicated that the 2-class solution fit the data better than did the 1-class solution.
[c]$P < .00005$.
[d]$P < .01$.
[e]A 3-class solution could not be reliably fit without fixing multiple (n = 29) parameter estimates at 0, owing to singularity in the information matrix and nonpositive definite covariance matrices of the parameter estimates for 2 classes; even with the parameters fixed at 0, the smallest class was only 3% of the total, which is not a reliable size for a latent class.

### *Regression Analyses for IL1R1, IL6, IL13, NFKB2, and TNFA Genotypes and Lower Versus Higher Anxiety Classes*

To better estimate the magnitude (ie, odds ratio) and precision (95% confidence interval) of the genotype on the odds of belonging to the higher versus lower anxiety class, multivariate logistic regression models were fit. In these regression analyses, which

**Table 2** Parameter Estimates for Spielberger State Anxiety Scale GMM Latent Classes From 7 Assessments of 398 Patients With Breast Cancer

| Variable | Lower Anxiety Class[a] (n = 147) | Higher Anxiety Class[a] (n = 251) |
|---|---|---|
| Parameter estimates | | |
| Intercept | 31.85 ± 1.54[b] | 45.93 ± 0.89[b] |
| Linear slope | −1.68 ± 0.62[c] | −1.72 ± 0.57[c] |
| Quadratic slope | 0.11 ± 0.08 | 0.09 ± 0.08 |
| Variances | | |
| Intercept | 10.52 ± 3.49[c] | 77.68 ± 13.24[b] |
| Linear slope | 0[d] | 29.71 ± 5.79[b] |
| Quadratic slope | 0[d] | 0.66 ± 0.14[b] |
| I with S | 0[d] | −26.55 ± 8.03[b] |
| I with Q | 0[d] | 3.27 ± 1.15[c] |
| S with Q | 0[d] | −4.20 ± 0.85[b] |

Data presented as mean ± standard error.
Abbreviations: GMM = growth mixture model; I = intercept; Q = quadratic slope; S = linear slope.
[a]Predicted class size according to most likely class membership.
[b]$P < .001$.
[c]$P < .01$.
[d]Random intercepts model only; random slopes were fixed at 0 to assist in estimation.

**Table 3** Differences in Demographic and Clinical Characteristics Between Lower (n = 147) and Higher (n = 251) Latent Classes

| Characteristic | Lower Anxiety Class (n = 147; 36.9%) | Higher Anxiety Class (n = 251; 63.1%) | Statistic and *P* Value |
|---|---|---|---|
| Age (years) | 57.5 ± 11.5 | 53.4 ± 11.3 | t = 3.46, *P* = .001 |
| Education (years) | 15.9 ± 2.6 | 15.6 ± 2.7 | NS |
| KPS score | 95.1 ± 9.5 | 92.1 ± 10.6 | t = 2.80, *P* = .005 |
| SCQ score | 3.9 ± 2.4 | 4.5 ± 3.0 | t = −2.07, *P* = .039 |
| Trait anxiety score at enrollment | 29.9 ± 5.6 | 38.5 ± 9.1 | t = −11.3, *P* < .0001 |
| State anxiety score at enrollment | 31.8 ± 8.8 | 47.6 ± 12.4 | t = −14.6, *P* < .0001 |
| CES-D scale score at enrollment | 7.6 ± 6.2 | 17.3 ± 9.7 | t = −11.9, *P* < .0001 |
| Breast biopsies in previous year (n) | 1.4 ± 0.6 | 1.6 ± 0.9 | NS |
| Positive lymph nodes (n) | 0.7 ± 1.7 | 1.0 ± 2.5 | NS |
| Lymph nodes removed (n) | 4.8 ± 5.7 | 6.3 ± 7.2 | NS |
| Ethnicity | | | $\chi^2$ = 17.801, *P* < .0001; *P* = .008[a] |
| White | 76.7 (112) | 57.2 (143) | |
| Black | 7.5 (11) | 11.6 (29) | |
| Asian/Pacific Islander | 10.3 (15) | 14.0 (35) | |
| Hispanic/mixed ethnic background/other | 5.5 (8) | 17.2 (43) | |
| Married/partnered (% yes) | 36.7 (54) | 44.9 (111) | NS |
| Work for pay (% yes) | 50.3 (74) | 46.4 (115) | NS |
| Lives alone (% yes) | 21.8 (32) | 25.6 (63) | NS |
| Postmenopausal (% yes) | 67.1 (98) | 62.2 (150) | NS |
| Disease stage | | | NS |
| 0 | 20.4 (30) | 17.1 (43) | |
| I | 43.5 (64) | 34.7 (87) | |
| IIA and IIB | 31.3 (46) | 37.8 (95) | |
| IIIA, IIIB, IIIC, IV | 4.8 (7) | 10.4 (26) | |
| Surgical treatment | | | NS |
| Breast conservation | 80.3 (118) | 79.7 (200) | |
| Mastectomy | 19.7 (29) | 20.3 (51) | |
| Sentinel node biopsy (% yes) | 85.7 (126) | 80.5 (202) | NS |
| Axillary lymph node dissection (% yes) | 32.2 (47) | 40.6 (102) | NS |
| Breast reconstruction at surgery (% yes) | 22.6 (33) | 21.1 (53) | NS |
| Neoadjuvant chemotherapy (% yes) | 13.0 (19) | 23.9 (60) | FE, *P* = .009 |
| Radiotherapy during first 6 mo (% yes) | 57.8 (85) | 55.4 (139) | NS |
| Chemotherapy during first 6 mo (% yes) | 25.9 (38) | 37.8 (95) | FE, *P* = .016 |

Data presented as mean ± standard deviation or % (n).
Abbreviations: CES-D = Center for Epidemiological Studies depression scale; FE = Fisher's exact test; KPS = Karnofsky performance status; NS = not significant; SCQ = self-administered comorbidity questionnaire.
[a]White versus Hispanic/mixed/other.

included genomic estimates of and self-reported race/ethnicity, the only phenotypic characteristic that remained significant in the multivariate model was age (in 5-year increments).

The only genetic associations that remained significant in the multivariate logistic regression analyses were for TNFA rs1799964 and TNFA rs3093662 (Table 4, Figure 2). In the regression analysis for TNFA rs1799964, controlling for age and rs3093662, carrying 2 of the rare C allele (ie, TT+TC vs. CC) was associated with an 88% reduction in the odds of belonging to the higher anxiety class. In the same analysis, controlling for age and rs1799964, carrying 1 or 2 doses of the rare G allele (ie, AA vs. AG+GG) in TNFA rs3093662 was associated with a 4.04 increase in the odds of belonging to the higher anxiety class.

## Discussion

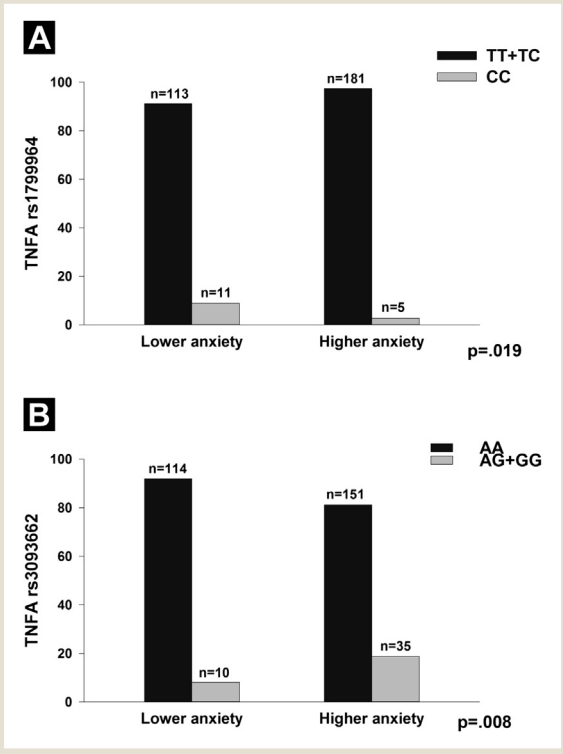
The present study is the first to use GMM to identify subgroups of women with distinct trajectories of anxiety from before through 6 months after breast cancer surgery. Before surgery, the women in the lower anxiety class reported both state and trait anxiety scores that approached the clinically meaningful cutoff score.[15] In contrast, in the higher anxiety class, both trait and state anxiety scores were greater than the cutoff scores. In the higher anxiety class, the state anxiety score of 47.6 was greater than the scores reported by patients

**Table 4** Multiple Logistic Regression Analyses for State Anxiety and Candidate Gene Markers

| Predictor | Odds Ratio | Standard Error | 95% CI | Z | *P* Value |
|---|---|---|---|---|---|
| TNFA rs1799964 | 0.12 | 0.084 | 0.030-0.471 | −3.03 | .002 |
| TNFA rs3093662 | 4.04 | 1.789 | 1.694-9.623 | 3.15 | .002 |
| Age | 0.83 | 0.047 | 0.745-0.930 | −3.26 | .001 |

Multiple logistic regression analysis of GMM latent classes for state anxiety scores (0 = lower, 1 = higher). For each model, the first 3 principle components identified from analysis of ancestry informative markers and self-reported race/ethnicity were retained in all models to adjust for potential confounding due to race or ethnicity (data not shown). The predictors evaluated in each model included genotype (TNFA rs1799964 genotype: TT+TC vs. CC; and TNFA rs3093662 genotype: AA vs. AG+GG), age (in 5-year increments), and self-reported race/ethnicity (white [reference group], Asian/Pacific Islander, black, Hispanic/mixed/other). Overall model fit: $\chi^2 = 42.81$, $P < .0001$.
Abbreviations: CI = confidence interval; GMM = growth mixture model; TNFA = tumor necrosis factor-α.

**Figure 2** (A) Differences Between the Latent Classes in the Percentages of Patients Who Were Homozygous or Heterozygous for the Common Allele (TT+TC) or Homozygous for the Rare Allele (CC) for rs1799964 in Tumor Necrosis Factor-α (TNFA). Values Were Plotted as Unadjusted Proportions With the Corresponding *P* Value. (B) Differences Between the Latent Classes in the Percentages of Patients Who Were Homozygous for the Common Allele (AA) or Heterozygous or Homozygous for the Rare Allele (AG+GG) for rs3093662 in TNFA. Values Were Plotted as Unadjusted Proportions With Corresponding *P* Values




with coronary artery disease[60] (ie, 39.0), chronic renal failure[61] (ie, 28.7), or chronic obstructive pulmonary disease[62] (ie, 34.9) but somewhat lower than the scores reported by patients with generalized anxiety disorder[63] (ie, 52.2).

The differences in the preoperative trait and state anxiety scores between the lower and higher anxiety classes were not only statistically significant but clinically meaningful for both scores (ie, d = 1.0 and d = 1.2, respectively, where d represents the difference between the 2 groups in standard deviation units).[64,65] Although the results of the GMM analysis demonstrated that in both anxiety classes, the severity of state anxiety decreased during the 6 months of the study, the reductions from before through 6 months after surgery were relatively modest (ie, 25.5 for the lower and 38.7 for the higher anxiety classes at 6 months). This finding suggests that for > 60% of the sample, relatively high levels of anxiety persisted after breast cancer surgery.

Age and ethnicity were the 2 demographic characteristics associated with the higher anxiety class. For each 5-year increase in age, the state anxiety scores decreased by approximately 1 point. Consistent with previous reports,[3,10,13] younger patients reported higher anxiety scores. In addition, compared with whites, the patients who self-reported their ethnicity as Hispanic or of mixed ethnic background were more likely to be included in the higher anxiety group. This finding is consistent with a recent meta-analysis that found that Hispanic oncology patients in the United States reported greater levels of psychological distress and poorer quality of life outcomes.[66]

A number of clinical and symptom characteristics were associated with the higher anxiety class. Consistent with previous findings,[67-69] patients in the higher anxiety class reported significantly lower KPS scores. In addition, and consistent with a previous report of oncology patients and their family caregivers,[69] patients with a more severe comorbidity profile were classified in the higher anxiety class. Although receipt of neoadjuvant CTX has not been identified as a risk factor for greater levels of anxiety, receipt of adjuvant CTX after breast cancer surgery was associated with greater levels of anxiety.[3,14] Finally, patients in the higher anxiety group reported depressive symptom scores that were greater than the clinically meaningful cutoff for the CES-D. In the 3 studies that evaluated the co-occurrence of anxiety and depressive symptoms (CAD) in patients with breast cancer,[70-72] the prevalence rates for CAD ranged from 10% to 28%, depending on the timing of the assessment. Taken together, clinicians can use these demographic, clinical, and symptom characteristics to identify patients who are at increased risk for clinically meaningful levels of anxiety before and after breast cancer surgery.

In the present study, 2 SNPs in TNFA (ie, rs1799964 and rs3093662) were associated with the higher anxiety class. Patients who were homozygous for the rare C allele in TNFA rs1799964 had an 88% decrease in the odds of belonging to the higher anxiety class. Although TNFA rs1799964 is 1 of the 8 SNPs used to infer HapA, rs3093662 is not included in this haplotype. Therefore, the finding that each SNP was independently associated with membership in the higher anxiety class is in agreement with the null association between the TNFA HapA and latent class membership. Although no studies were identified that evaluated an association

between this SNP and anxiety, several published findings are worth noting. In 1 study of patients with lung cancer,[73] this same SNP, which is located in the promoter region of the TNFA gene, was associated with decreased pain. In addition, in a community-based cohort study,[74] those who were homozygous for the rare C allele had a 17% decrease in the odds of cancer-related mortality. Finally, in the same sample of patients with breast cancer,[26] being homozygous for the rare C allele was associated with an 87% decrease in the odds of belonging to the subsyndromal depressive symptoms' class.

In contrast, patients who were heterozygous or homozygous for the rare G allele at rs3093662 were 4.0 times more likely to be in the higher anxiety class. Although this SNP lies in the intronic region of the TNFA gene, it was associated with inflammation and poorer outcomes in patients who had undergone renal transplantation.[75] In addition, in our previous study of patients who had undergone radiation therapy and their family caregivers,[76] the participants who were heterozygous or homozygous for the rare G allele were 3.8 times more likely to be classified in the higher morning fatigue class.

A growing body of evidence suggests that TNFA exerts a broad range of biologic functions within the peripheral and central nervous systems.[77-80] The findings from the present study suggest that polymorphisms in TNFA are associated with anxiety and other common symptoms in oncology patients. Although functional studies are needed to confirm these associations, our findings have been supported by a recent study that found that the administration of TNFA antagonists was associated with a decrease in the occurrence of generalized anxiety disorder in patients with rheumatoid arthritis.[81]

The present study had a number of study limitations. Although our sample size was adequate, larger, independent samples are needed to confirm these preliminary findings and identify additional latent classes, significant phenotypic predictors, and significant genetic associations. Also, although a valid and reliable self-report measure was used to evaluate state anxiety, future studies should incorporate a clinical evaluation of pre-existing and concurrent psychiatric conditions. In addition, no information was available on whether these patients were taking anti-anxiety medications. Finally, the generalizability of the study findings is limited to only female patients with breast cancer.

## Conclusion

Despite these limitations, these findings provide evidence to support distinct anxiety phenotypes in patients with breast cancer before and after surgery. In addition, in this sample, the higher risk phenotype was associated with higher levels of depressive symptoms before surgery. It is important that these higher risk patients be identified early to be able to provide pre-emptive and ongoing treatment.

### *Clinical Practice Points*

- Moderate to high levels of psychological distress are reported by most women after breast cancer surgery.
- Although the exact prevalence of anxiety is unknown in women after breast cancer surgery, recent evidence suggests that inflammatory mechanisms might contribute to the development of anxiety disorders.
- Using GMM, 2 subgroups of women with distinct trajectories of anxiety (ie, higher and lower anxiety classes) were identified.
- Younger patients, those with Hispanic or mixed ethnic background, and those with lower functional status scores were more likely to be in the higher anxiety class.
- Two single nucleotide polymorphisms in TNFA (ie, rs1799964 and rs3093662) were associated with membership in the higher anxiety class.
- Clinicians can use the phenotypic predictors associated with higher anxiety to identify women who warrant additional evaluation before breast cancer surgery.

## Acknowledgments

The present study was funded by grants from the National Cancer Institute (NCI; CA107091 and CA118658). Dr. Christine Miaskowski is an American Cancer Society Clinical Research Professor and is supported by a K05 award from the NCI (CA168960). This project is supported by National Institutes of Health (NIH)/ National Center for Research Resources University of California, San Francisco, Clinical and Translational Science Institute (grant UL1 RR024131). Its contents are solely the responsibility of the authors and do not necessarily represent the official views of the NIH.

## Disclosure

The authors have stated that they have no conflicts of interest.

## Supplemental Data

Supplemental tables accompanying this article can be found in the online version at http://dx.doi.org/10.1016/j.clbc.2014.12.001.

## References

1. Barez M, Blasco T, Fernandez-Castro J, Viladrich C. Perceived control and psychological distress in women with breast cancer: a longitudinal study. *J Behav Med* 2009; 32:187-96.
2. Burgess C, Cornelius V, Love S, Graham J, Richards M, Ramirez A. Depression and anxiety in women with early breast cancer: five year observational cohort study. *BMJ* 2005; 330:702.
3. Dean C. Psychiatric morbidity following mastectomy: preoperative predictors and types of illness. *J Psychosom Res* 1987; 31:385-92.
4. Fallowfield LJ, Hall A, Maguire GP, Baum M. Psychological outcomes of different treatment policies in women with early breast cancer outside a clinical trial. *BMJ* 1990; 301:575-80.
5. Gallagher J, Parle M, Cairns D. Appraisal and psychological distress six months after diagnosis of breast cancer. *Br J Health Psychol* 2002; 7:365-76.
6. Goldberg JA, Scott RN, Davidson PM, et al. Psychological morbidity in the first year after breast surgery. *Eur J Surg Oncol* 1992; 18:327-31.
7. Henselmans I, Helgeson VS, Seltman H, de Vries J, Sanderman R, Ranchor AV. Identification and prediction of distress trajectories in the first year after a breast cancer diagnosis. *Health Psychol* 2010; 29:160-8.
8. Maunsell E, Brisson J, Deschenes L. Psychological distress after initial treatment for breast cancer: a comparison of partial and total mastectomy. *J Clin Epidemiol* 1989; 42:765-71.
9. Millar K, Purushotham AD, McLatchie E, George WD, Murray GD. A 1-year prospective study of individual variation in distress, and illness perceptions, after treatment for breast cancer. *J Psychosom Res* 2005; 58:335-42.
10. Parker PA, Youssef A, Walker S, et al. Short-term and long-term psychosocial adjustment and quality of life in women undergoing different surgical procedures for breast cancer. *Ann Surg Oncol* 2007; 14:3078-89.
11. Ramirez AJ, Richards MA, Jarrett SR, Fentiman IS. Can mood disorder in women with breast cancer be identified preoperatively? *Br J Cancer* 1995; 72: 1509-12.

12. Vahdaninia M, Omidvari S, Montazeri A. What do predict anxiety and depression in breast cancer patients? A follow-up study. *Soc Psychiatry Psychiatr Epidemiol* 2010; 45:355-61.
13. Epping-Jordan JE, Compas BE, Osowiecki DM, et al. Psychological adjustment in breast cancer: processes of emotional distress. *Health Psychol* 1999; 18:315-26.
14. Kissane DW, Clarke DM, Ikin J, et al. Psychological morbidity and quality of life in Australian women with early-stage breast cancer: a cross-sectional survey. *Med J Aust* 1998; 169:192-6.
15. Spielberger CG, Gorsuch RL, Suchene R, Vagg PR, Jacobs GA. *Manual for the State-Anxiety (Form Y): Self-Evaluation Questionnaire*. Palo Alto, CA: Consulting Psychologists Press; 1983.
16. Luciano M, Huffman JE, Arias-Vasquez A, et al. Genome-wide association uncovers shared genetic effects among personality traits and mood states. *Am J Med Genet B Neuropsychiatr Genet* 2012; 159B:684-95.
17. Webb BT, Guo AY, Maher BS, et al. Meta-analyses of genome-wide linkage scans of anxiety-related phenotypes. *Eur J Hum Genet* 2012; 20:1078-84.
18. Domschke K, Deckert J. Genetics of anxiety disorders—status quo and quo vadis. *Curr Pharm Des* 2012; 18:5691-8.
19. McGrath LM, Weill S, Robinson EB, Macrae R, Smoller JW. Bringing a developmental perspective to anxiety genetics. *Dev Psychopathol* 2012; 24:1179-93.
20. Binder EB. The genetic basis of mood and anxiety disorders—changing paradigms. *Biol Mood Anxiety Disord* 2012; 2:17.
21. Sokolowska E, Hovatta I. Anxiety genetics—findings from cross-species genome-wide approaches. *Biol Mood Anxiety Disord* 2013; 3:9.
22. Miller AH, Haroon E, Raison CL, Felger JC. Cytokine targets in the brain: impact on neurotransmitters and neurocircuits. *Depress Anxiety* 2013; 30:297-306.
23. Hou R, Baldwin DS. A neuroimmunological perspective on anxiety disorders. *Hum Psychopharmacol* 2012; 27:6-14.
24. Dantzer R, Capuron L, Irwin MR, et al. Identification and treatment of symptoms associated with inflammation in medically ill patients. *Psychoneuroendocrinology* 2008; 33:18-29.
25. Dantzer R, Kelley KW. Twenty years of research on cytokine-induced sickness behavior. *Brain Behav Immun* 2007; 21:153-60.
26. Saad S, Dunn LB, Koetters T, et al. Cytokine gene variations associated with subsyndromal depressive symptoms in patients with breast cancer. *Eur J Oncol Nurs* 2014; 18:397-404.
27. McCann B, Miaskowski C, Koetters T, et al. Associations between pro- and anti-inflammatory cytokine genes and breast pain in women prior to breast cancer surgery. *J Pain* 2012; 13:425-37.
28. Stephens K, Cooper BA, West C, et al. Associations between cytokine gene variations and severe persistent breast pain in women following breast cancer surgery. *J Pain* 2014; 15:169-80.
29. Alfaro E, Dhruva A, Langford DJ, et al. Associations between cytokine gene variations and self-reported sleep disturbance in women following breast cancer surgery. *Eur J Oncol Nurs* 2014; 18:85-93.
30. Merriman JD, Aouizerat BE, Cataldo JK, et al. Association between an interleukin 1 receptor, type I promoter polymorphism and self-reported attentional function in women with breast cancer. *Cytokine* 2014; 65:192-201.
31. Miaskowski C, Cooper B, Paul SM, et al. Identification of patient subgroups and risk factors for persistent breast pain following breast cancer surgery. *J Pain* 2012; 13:1172-87.
32. Miaskowski C, Dodd M, Paul SM, et al. Lymphatic and angiogenic candidate genes predict the development of secondary lymphedema following breast cancer surgery. *PLoS One* 2013; 8:e60164.
33. Dunn LB, Cooper BA, Neuhaus J, et al. Identification of distinct depressive symptom trajectories in women following surgery for breast cancer. *Health Psychol* 2011; 30:683-92.
34. Karnofsky D, Abelmann WH, Craver LV, Burchenal JH. The use of nitrogen mustards in the palliative treatment of carcinoma. *Cancer* 1948; 1:634-56.
35. Sangha O, Stucki G, Liang MH, Fossel AH, Katz JN. The self-administered comorbidity questionnaire: a new method to assess comorbidity for clinical and health services research. *Arthritis Rheum* 2003; 49:156-63.
36. Brunner F, Bachmann LM, Weber U, et al. Complex regional pain syndrome 1—the Swiss cohort study. *BMC Musculoskelet Disord* 2008; 9:92.
37. Cieza A, Geyh S, Chatterji S, Kostanjsek N, Ustun BT, Stucki G. Identification of candidate categories of the International Classification of Functioning Disability and Health (ICF) for a Generic ICF Core Set based on regression modelling. *BMC Med Res Methodol* 2006; 6:36.
38. Bieling PJ, Antony MM, Swinson RP. The State-Trait Anxiety Inventory, trait version: structure and content re-examined. *Behav Res Ther* 1998; 36:777-88.
39. Kennedy BL, Schwab JJ, Morris RL, Beldia G. Assessment of state and trait anxiety in subjects with anxiety and depressive disorders. *Psychiatr Q* 2001; 72: 263-76.
40. Carpenter JS, Andrykowski MA, Wilson J, et al. Psychometrics for two short forms of the Center for Epidemiologic Studies-Depression scale. *Issues Ment Health Nurs* 1998; 19:481-94.
41. Radloff LS. The CES-D scale: a self-report depression scale for research in the general population. *Appl Psychol Measure* 1977; 1:385-401.
42. Sheehan TJ, Fifield J, Reisine S, Tennen H. The measurement structure of the Center for Epidemiologic Studies Depression scale. *J Pers Assess* 1995; 64: 507-21.
43. Seruga B, Zhang H, Bernstein LJ, Tannock IF. Cytokines and their relationship to the symptoms and outcome of cancer. *Nat Rev Cancer* 2008; 8:887-99.
44. Conde L, Vaquerizas JM, Dopazo H, et al. PupaSuite: finding functional single nucleotide polymorphisms for large-scale genotyping purposes. *Nucleic Acids Res* 2006; 34:W621-5.
45. SPSS. *IBM SPSS for Windows (version 21)*. Chicago: SPSS, Inc.; 2012.
46. StataCorp. *Stata Statistical Software: release 13*. College Station, TX: StataCorp; 2014.
47. Jung T, Wickrama KAS. An introduction to latent class growth analysis and growth mixture modeling. *Soc Person Psychol Compass* 2008; 2:302-17.
48. Nylund KL, Asparouhov T, Muthen BO. Deciding on the number of classes in latent class analysis and growth mixture modeling: a Monte Carlo simulation study. *Struct Equ Modeling* 2007; 14:535-69.
49. Tofighi D, Enders CK. *Identifying the Correct Number of Classes in Growth Mixture Models*. Charlotte, NC: Information Age Publishing; 2008.
50. Stephens M, Smith NJ, Donnelly P. A new statistical method for haplotype reconstruction from population data. *Am J Hum Genet* 2001; 68:978-89.
51. Halder I, Shriver M, Thomas M, Fernandez JR, Frudakis T. A panel of ancestry informative markers for estimating individual biogeographical ancestry and admixture from four continents: utility and applications. *Hum Mut* 2008; 29: 648-58.
52. Hoggart CJ, Parra EJ, Shriver MD, et al. Control of confounding of genetic associations in stratified populations. *Am J Hum Genet* 2003; 72:1492-504.
53. Tian C, Gregersen PK, Seldin MF. Accounting for ancestry: population substructure and genome-wide association studies. *Hum Mol Genet* 2008; 17: R143-50.
54. Price AL, Patterson NJ, Plenge RM, Weinblatt ME, Shadick NA, Reich D. Principal components analysis corrects for stratification in genome-wide association studies. *Nat Genet* 2006; 38:904-9.
55. Dunn LB, Aouizerat BE, Langford DJ, et al. Cytokine gene variation is associated with depressive symptom trajectories in oncology patients and family caregivers. *Eur J Oncol Nurs* 2013; 17:346-53.
56. Illi J, Miaskowski C, Cooper B, et al. Association between pro- and anti-inflammatory cytokine genes and a symptom cluster of pain, fatigue, sleep disturbance, and depression. *Cytokine* 2012; 58:437-47.
57. Miaskowski C, Cooper BA, Dhruva A, et al. Evidence of associations between cytokine genes and subjective reports of sleep disturbance in oncology patients and their family caregivers. *PLoS One* 2012; 7:e40560.
58. Rothman KJ. No adjustments are needed for multiple comparisons. *Epidemiology* 1990; 1:43-6.
59. Bakan D. The test of significance in psychological research. *Psychol Bull* 1966; 66: 423-37.
60. Bunevicius A, Staniute M, Brozaitiene J, Pop VJ, Neverauskas J, Bunevicius R. Screening for anxiety disorders in patients with coronary artery disease. *Health Qual Life Outcomes* 2013; 11:37.
61. Theofilou P. Depression and anxiety in patients with chronic renal failure: the effect of sociodemographic characteristics. *Int J Nephrol* 2011; 2011:514070.
62. Giardino ND, Curtis JL, Andrei AC, et al. Anxiety is associated with diminished exercise performance and quality of life in severe emphysema: a cross-sectional study. *Respir Res* 2010; 11:29.
63. Linden M, Zubraegel D, Baer T, Franke U, Schlattmann P. Efficacy of cognitive behaviour therapy in generalized anxiety disorders: results of a controlled clinical trial (Berlin CBT-GAD study). *Psychother Psychosom* 2005; 74:36-42.
64. Osoba D. Interpreting the meaningfulness of changes in health-related quality of life scores: lessons from studies in adults. *Int J Cancer Suppl* 1999; 12: 132-7.
65. Osoba D, Rodrigues G, Myles J, Zee B, Pater J. Interpreting the significance of changes in health-related quality-of-life scores. *J Clin Oncol* 1998; 16: 139-44.
66. Luckett T, Goldstein D, Butow PN, et al. Psychological morbidity and quality of life of ethnic minority patients with cancer: a systematic review and meta-analysis. *Lancet Oncol* 2011; 12:1240-8.
67. Valdes-Stauber J, Vietz E, Kilian R. The impact of clinical conditions and social factors on the psychological distress of cancer patients: an explorative study at a consultation and liaison service in a rural general hospital. *BMC Psychiatry* 2013; 13:226.
68. Reilly CM, Bruner DW, Mitchell SA, et al. A literature synthesis of symptom prevalence and severity in persons receiving active cancer treatment. *Support Care Cancer* 2013; 21:1525-50.
69. Miaskowski C, Cataldo JK, Baggott CR, et al. Cytokine gene variations associated with trait and state anxiety in oncology patients and their family caregivers. *Support Cancer Cancer* 2015; 23:953-65.
70. Brintzenhofe-Szoc KM, Levin TT, Li Y, Kissane DW, Zabora JR. Mixed anxiety/depression symptoms in a large cancer cohort: prevalence by cancer type. *Psychosomatics* 2009; 50:383-91.
71. Van Esch L, Roukema JA, Ernst MF, Nieuwenhuijzen GA, De Vries J. Combined anxiety and depressive symptoms before diagnosis of breast cancer. *J Affect Disord* 2012; 136:895-901.
72. So WK, Marsh G, Ling WM, et al. Anxiety, depression and quality of life among Chinese breast cancer patients during adjuvant therapy. *Eur J Oncol Nurs* 2010; 14:17-22.
73. Rausch SM, Gonzalez BD, Clark MM, et al. SNPs in PTGS2 and LTA predict pain and quality of life in long term lung cancer survivors. *Lung Cancer* 2012; 77:217-23.
74. Gallicchio L, Chang H, Christo DK, et al. Single nucleotide polymorphisms in inflammation- related genes and mortality in a community-based cohort in Washington County, Maryland. *Am J Epidemiol* 2008; 167:807-13.

75. Israni AK, Li N, Cizman BB, et al. Association of donor inflammation- and apoptosis-related genotypes and delayed allograft function after kidney transplantation. *Am J Kidney Dis* 2008; 52:331-9.
76. Dhruva A, Aouizerat BE, Cooper B, et al. Cytokine gene associations with self-report ratings of morning and evening fatigue in oncology patients and their family caregivers [e-pub ahead of print]. *Biol Res Nurs* 2015; 17: 175-94.
77. Eyre H, Baune BT. Neuroplastic changes in depression: a role for the immune system. *Psychoneuroendocrinology* 2012; 37:1397-416.
78. Falip M, Salas-Puig X, Cara C. Causes of CNS inflammation and potential targets for anticonvulsants. *CNS Drugs* 2013; 27:611-23.
79. Olmos G, Llado J. Tumor necrosis factor alpha: a link between neuroinflammation and excitotoxicity. *Mediators Inflamm* 2014; 2014:861231.
80. Santello M, Volterra A. TNFalpha in synaptic function: switching gears. *Trends Neurosci* 2012; 35:638-47.
81. Uguz F, Akman C, Kucuksarac S, Tufekci O. Anti-tumor necrosis factor-alpha therapy is associated with less frequent mood and anxiety disorders in patients with rheumatoid arthritis. *Psychiatry Clin Neurosci* 2009; 63:50-5.

**Supplemental Table 1** Summary of Cytokine Gene SNPs and Haplotypes Analyzed for Lower Versus Higher Anxiety Latent Classes

| Gene | SNP | Position | Chromosome | Haplotype[a] | MAF | Alleles | $\chi^2$ | *P* Value | Model |
|---|---|---|---|---|---|---|---|---|---|
| *IFNG1* | rs2069728 | 66834051 | 12 | HapA | .110 | G>A | 3.460 | .177 | A |
| *IFNG1* | rs2069727 | 66834490 | 12 | HapA | .384 | A>G | 1.928 | .381 | A |
| *IFNG1* | rs2069718 | 66836429 | 12 | HapA | .494 | C>T | 0.119 | .942 | A |
| *IFNG1* | rs1861493 | 66837463 | 12 | HapA | .266 | A>G | 0.545 | .761 | A |
| *IFNG1* | rs1861494 | 66837676 | 12 | HapA | .273 | T>C | 0.719 | .698 | A |
| *IFNG1* | rs2069709 | 66839970 | 12 | | .003 | G>T | NA | NA | NA |
| *IFNG1* | HapA3 | | | | | | 0.467 | .792 | |
| *IFNG1* | HapA5 | | | | | | 2.484 | .289 | |
| *IFNGR1* | rs9376268 | 137574444 | 6 | | .254 | G>A | 0.938 | .626 | A |
| *IL1B* | rs1071676 | 106042060 | 2 | HapA | .189 | G>C | 1.669 | .434 | A |
| *IL1B* | rs1143643 | 106042929 | 2 | HapA | .383 | G>A | 1.948 | .378 | A |
| *IL1B* | rs1143642 | 106043180 | 2 | HapA | .082 | C>T | 0.171 | .918 | A |
| *IL1B* | rs1143634 | 106045017 | 2 | HapA | .187 | C>T | 1.514 | .469 | A |
| *IL1B* | rs1143633 | 106045094 | 2 | HapA | .392 | G>A | 2.469 | .291 | A |
| *IL1B* | rs1143630 | 106046282 | 2 | HapB | .115 | C>A | 1.653 | .438 | A |
| *IL1B* | rs3917356 | 106046990 | 2 | HapB | .450 | G>A | 1.076 | .584 | A |
| *IL1B* | rs1143629 | 106048145 | 2 | HapB | .389 | T>C | 1.060 | .589 | A |
| *IL1B* | rs1143627 | 106049014 | 2 | HapB | .397 | T>C | 0.636 | .728 | A |
| *IL1B* | rs16944 | 106049494 | 2 | HapB | .386 | G>A | 0.394 | .821 | A |
| *IL1B* | rs1143623 | 106050452 | 2 | HapB | .277 | G>C | 0.072 | .965 | A |
| *IL1B* | rs13032029 | 106055022 | 2 | HapB | .448 | C>T | 0.784 | .676 | A |
| *IL1B* | HapA1 | | | | | | 0.811 | .667 | |
| *IL1B* | HapA4 | | | | | | 2.010 | .366 | |
| *IL1B* | HapA6 | | | | | | 1.549 | .461 | |
| *IL1B* | HapB1 | | | | | | 2.410 | .300 | |
| *IL1B* | HapB6 | | | | | | 1.015 | .602 | |
| *IL1B* | HapB8 | | | | | | 0.717 | .699 | |
| *IL1R1* | rs949963 | 96533648 | 2 | | .223 | G>A | 1.589 | .452 | A |
| *IL1R1* | rs2228139 | 96545511 | 2 | | .053 | C>G | 3.171 | .205 | A |
| *IL1R1* | rs3917320 | 96556738 | 2 | | .047 | A>C | NA | NA | NA |
| *IL1R1* | rs2110726 | 96558145 | 2 | HapA | .317 | C>T | 4.116 | .128 | A |
| *IL1R1* | rs3917332 | 96560387 | 2 | HapA | .187 | A>T | FE | .020 | D |
| *IL1R1* | HapA1 | | | | | | 3.231 | .199 | |
| *IL1R1* | HapA2 | | | | | | 3.604 | .165 | |
| *IL1R1* | HapA3 | | | | | | 5.971 | .051 | |
| *IL1R2* | rs4141134 | 96370336 | 2 | | .362 | T>C | 3.443 | .179 | A |
| *IL1R2* | rs11674595 | 96374804 | 2 | HapA | .258 | T>C | 0.681 | .711 | A |
| *IL1R2* | rs7570441 | 96380807 | 2 | HapA | .408 | G>A | 2.116 | .347 | A |
| *IL1R2* | HapA1 | | | | | | 2.301 | .316 | |
| *IL1R2* | HapA2 | | | | | | FE | .117 | |
| *IL1R2* | HapA4 | | | | | | 0.050 | .975 | |
| *IL2* | rs1479923 | 119096993 | 4 | | .308 | C>T | 2.763 | .251 | A |
| *IL2* | rs2069776 | 119098582 | 4 | | .184 | T>C | NA | NA | NA |
| *IL2* | rs2069772 | 119099739 | 4 | HapA | .241 | A>G | 3.192 | .203 | A |
| *IL2* | rs2069777 | 119103043 | 4 | | .047 | C>T | NA | NA | NA |
| *IL2* | rs2069763 | 119104088 | 4 | HapA | .277 | T>G | 2.574 | .276 | A |
| *IL2* | HapA1 | | | | | | 2.008 | .366 | |
| *IL2* | HapA2 | | | | | | 2.703 | .259 | |
| *IL2* | HapA3 | | | | | | 3.192 | .203 | |
| *IL4* | rs2243248 | 127200946 | 5 | | .086 | T>G | 3.239 | .198 | A |
| *IL4* | rs2243250 | 127201455 | 5 | HapA | .269 | C>T | NA | NA | NA |
| *IL4* | rs2070874 | 127202011 | 5 | HapA | .245 | C>T | NA | NA | NA |

**Supplemental Table 1** Continued

| Gene | SNP | Position | Chromosome | Haplotype[a] | MAF | Alleles | $\chi^2$ | *P* Value | Model |
|---|---|---|---|---|---|---|---|---|---|
| *IL4* | rs2227284 | 127205027 | 5 | HapA | .387 | C>A | NA | NA | NA |
| *IL4* | rs2227282 | 127205481 | 5 | HapA | .390 | C>G | NA | NA | NA |
| *IL4* | rs2243263 | 127205601 | 5 | HapA | .124 | C>G | 0.181 | .913 | A |
| *IL4* | rs2243266 | 127206091 | 5 | HapA | .237 | G>A | NA | NA | NA |
| *IL4* | rs2243267 | 127206188 | 5 | HapA | .237 | G>C | NA | NA | NA |
| *IL4* | rs2243274 | 127207134 | 5 | HapA | .261 | G>A | NA | NA | NA |
| *IL4* | HapA1 | | | | | | 0.658 | .720 | |
| *IL4* | HapA3 | | | | | | 0.174 | .917 | |
| *IL4* | HapX1 | | | | | | 3.396 | .183 | |
| *IL6* | rs4719714 | 22643793 | 7 | | .255 | A>T | 4.170 | .124 | A |
| *IL6* | rs2069827 | 22648536 | 7 | | .069 | G>T | 0.803 | .669 | A |
| *IL6* | rs1800796 | 22649326 | 7 | | .134 | C>G | NA | NA | NA |
| *IL6* | rs1800795 | 22649725 | 7 | HapA | .285 | C>G | 2.818 | .244 | A |
| *IL6* | rs2069830 | 22650951 | 7 | HapA | .061 | T>C | NA | NA | NA |
| *IL6* | rs2066992 | 22651329 | 7 | | .049 | G>T | 4.361 | .113 | A |
| *IL6* | rs2069840 | 22651652 | 7 | HapA | .333 | C>G | 6.624 | .036 | A |
| *IL6* | rs1554606 | 22651787 | 7 | HapA | .319 | G>T | 1.085 | .581 | A |
| *IL6* | rs2069845 | 22653229 | 7 | HapA | .319 | A>G | 1.085 | .581 | A |
| *IL6* | rs2069849 | 22654236 | 7 | | .024 | C>T | NA | NA | NA |
| *IL6* | rs2069861 | 22654734 | 7 | HapA | .056 | C>T | 4.126 | .127 | A |
| *IL6* | rs35610689 | 22656903 | 7 | | .259 | A>G | 1.268 | .530 | A |
| *IL6* | HapA1 | | | | | | 3.165 | .205 | |
| *IL6* | HapA5 | | | | | | 8.421 | .015 | |
| *IL6* | HapA8 | | | | | | 2.595 | .273 | |
| *IL8* | rs4073 | 70417508 | 4 | HapA | .455 | T>A | 1.750 | .417 | A |
| *IL8* | rs2227306 | 70418539 | 4 | HapA | .366 | C>T | 2.107 | .349 | A |
| *IL8* | rs2227543 | 70419394 | 4 | HapA | .368 | C>T | 1.239 | .538 | A |
| *IL8* | HapA1 | | | | | | 1.750 | .417 | |
| *IL8* | HapA4 | | | | | | 1.536 | .464 | |
| *IL10* | rs3024505 | 177638230 | 1 | HapA | .129 | C>T | 0.309 | .857 | A |
| *IL10* | rs3024498 | 177639855 | 1 | HapA | .204 | A>G | 0.728 | .695 | A |
| *IL10* | rs3024496 | 177640190 | 1 | HapA | .421 | T>C | 0.105 | .949 | A |
| *IL10* | rs1878672 | 177642039 | 1 | HapA | .416 | G>C | 0.140 | .933 | A |
| *IL10* | rs3024492 | 177642438 | 1 | | .190 | T>A | NA | NA | NA |
| *IL10* | rs1518111 | 177642971 | 1 | HapA | .303 | G>A | 2.862 | .239 | A |
| *IL10* | rs1518110 | 177643187 | 1 | HapA | .301 | G>T | 2.973 | .226 | A |
| *IL10* | rs3024491 | 177643372 | 1 | HapA | .408 | G>T | 0.021 | .989 | A |
| *IL10* | HapA1 | | | | | | 2.703 | .259 | |
| *IL10* | HapA2 | | | | | | 3.079 | .214 | |
| *IL10* | HapA8 | | | | | | 0.620 | .734 | |
| *IL13* | rs1881457 | 127184713 | 5 | | .210 | A>C | 2.660 | .265 | A |
| *IL13* | rs1800925 | 127185113 | 5 | | .233 | C>T | 1.933 | .380 | A |
| *IL13* | rs2069743 | 127185579 | 5 | | .019 | A>G | NA | NA | NA |
| *IL13* | rs1295686 | 127188147 | 5 | HapA | .265 | G>A | FE | .027 | D |
| *IL13* | rs20541 | 127188268 | 5 | HapA | .212 | C>T | 1.799 | .407 | A |
| *IL13* | HapA1 | | | | | | 6.032 | .049 | |
| *IL13* | HapA4 | | | | | | 1.479 | .477 | |
| *IL17A* | rs4711998 | 51881422 | 6 | | .346 | G>A | 1.733 | .421 | A |
| *IL17A* | rs8193036 | 51881562 | 6 | | .327 | T>C | 0.661 | .719 | A |
| *IL17A* | rs3819024 | 51881855 | 6 | | .372 | A>G | 0.083 | .959 | A |
| *IL17A* | rs2275913 | 51882102 | 6 | | .361 | G>A | 2.138 | .343 | A |
| *IL17A* | rs3804513 | 51884266 | 6 | | .023 | A>T | NA | NA | NA |

**Supplemental Table 1** Continued

| Gene | SNP | Position | Chromosome | Haplotype[a] | MAF | Alleles | $\chi^2$ | *P* Value | Model |
|---|---|---|---|---|---|---|---|---|---|
| *IL17A* | rs7747909 | 51885318 | 6 | | .217 | G>A | 1.817 | .403 | A |
| *NFKB1* | rs3774933 | 103645369 | 4 | | .409 | T>C | 0.335 | .846 | A |
| *NFKB1* | rs170731 | 103667933 | 4 | HapA | .358 | A>T | 0.373 | .830 | A |
| *NFKB1* | rs17032779 | 103685279 | 4 | | .011 | T>C | NA | NA | NA |
| *NFKB1* | rs230510 | 103695201 | 4 | HapA | .410 | T>A | 2.362 | .307 | A |
| *NFKB1* | rs230494 | 103706005 | 4 | HapA | .434 | A>G | 0.332 | .847 | A |
| *NFKB1* | rs4648016 | 103708706 | 4 | | .010 | C>T | NA | NA | NA |
| *NFKB1* | rs4648018 | 103709236 | 4 | | .018 | G>C | NA | NA | NA |
| *NFKB1* | rs3774956 | 103727564 | 4 | HapA | .435 | C>T | 0.231 | .891 | A |
| *NFKB1* | rs10489114 | 103730426 | 4 | | .018 | A>G | NA | NA | NA |
| *NFKB1* | rs4648068 | 103737343 | 4 | | .363 | A>G | 0.448 | .799 | A |
| *NFKB1* | rs4648095 | 103746914 | 4 | | .052 | T>C | FE | 1.000 | A |
| *NFKB1* | rs4648110 | 103752867 | 4 | | .170 | T>A | 0.179 | .915 | A |
| *NFKB1* | rs4648135 | 103755716 | 4 | | .061 | A>G | FE | .860 | A |
| *NFKB1* | rs4648141 | 103755947 | 4 | | .180 | G>A | 1.269 | .530 | A |
| *NFKB1* | rs1609798 | 103756488 | 4 | | .337 | C>T | 0.532 | .766 | A |
| *NFKB1* | HapA1 | | | | | | 2.039 | .361 | |
| *NFKB1* | HapA9 | | | | | | 0.409 | .815 | |
| *NFKB2* | rs12772374 | 104146901 | 10 | | .168 | A>G | 0.011 | .995 | A |
| *NFKB2* | rs7897947 | 104147701 | 10 | | .221 | T>G | 0.631 | .730 | A |
| *NFKB2* | rs11574849 | 104149686 | 10 | | .070 | G>A | 2.091 | .351 | A |
| *NFKB2* | rs1056890 | 104152760 | 10 | | .305 | C>T | FE | .027 | D |
| *TNFA* | rs2857602 | 31533378 | 6 | HapA | .341 | T>C | 1.473 | .479 | A |
| *TNFA* | rs1800683 | 31540071 | 6 | HapA | .390 | G>A | 0.707 | .702 | A |
| *TNFA* | rs2239704 | 31540141 | 6 | HapA | .335 | G>T | 1.574 | .455 | A |
| *TNFA* | rs2229094 | 31540556 | 6 | HapA | .278 | T>C | 1.163 | .559 | A |
| *TNFA* | rs1041981 | 31540784 | 6 | HapA | .386 | C>A | 0.713 | .700 | A |
| *TNFA* | rs1799964 | 31542308 | 6 | HapA | .224 | T>C | FE | .019 | R |
| *TNFA* | rs1800750 | 31542963 | 6 | | .016 | G>A | NA | NA | NA |
| *TNFA* | rs1800629 | 31543031 | 6 | HapA | .149 | G>A | 2.240 | .326 | A |
| *TNFA* | rs1800610 | 31543827 | 6 | HapA | .100 | C>T | 0.083 | .959 | A |
| *TNFA* | rs3093662 | 31544189 | 6 | | .074 | A>G | FE | .008 | D |
| *TNFA* | HapA1 | | | | | | 4.818 | .090 | |
| *TNFA* | HapA5 | | | | | | 5.194 | .074 | |
| *TNFA* | HapA6 | | | | | | 2.166 | .339 | |

Abbreviations: A = additive model; D = dominant model; FE = Fisher's exact test; Hap = haplotype; IFNG = interferon-γ; IL = interleukin; MAF = minor allele frequency; NA = not assayed because SNP violated Hardy-Weinberg expectations ($P < .001$) or because MAF was < 0.05; NFKB = nuclear factor κβ; R = recessive model; SNP = single nucleotide polymorphism; TNFA = tumor necrosis factor-α.

[a]The SNPs used to infer the haplotypes for each gene are identified in the "Haplotype" column (eg, for IL13, HapA was inferred using rs1295686 and rs20541).