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CYTOKINE GENE VARIATIONS ASSOCIATED WITH TRAIT AND STATE ANXIETY IN ONCOLOGY PATIENTS AND THEIR FAMILY CAREGIVERS

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Abstract

Purpose—Anxiety is common among cancer patients and their family caregivers (FCs) and is associated with poorer outcomes. Recently, associations between inflammation and anxiety were identified. However, the relationship between variations in cytokine genes and anxiety warrants investigation. Therefore, phenotypic and genotypic characteristics associated with trait and state anxiety were evaluated in a sample of 167 oncology patients with breast, prostate, lung, or brain cancer and 85 of their FCs.

Methods—Using multiple regression analyses, the associations between participants' demographic and clinical characteristics, as well as variations in cytokine genes and trait and state anxiety were evaluated.

Results—In the bivariate analyses, a number of phenotypic characteristics were associated with both trait and state anxiety (e.g., age, functional status). However, some associations were specific only to trait anxiety (e.g., number of comorbid conditions) or state anxiety (e.g., participation with a FC). Variations in three cytokine genes (i.e., interleukin (IL) 1 beta, IL1 receptor 2 (IL1R2), nuclear factor kappa beta 2 (NFKB2)) were associated with trait anxiety and variations in two genes (i.e., IL1R2, tumor necrosis factor alpha (TNFA)) were associated with state anxiety.

Conclusions—These findings suggest that both trait and state anxiety need to be assessed in oncology patients and their FCs. Furthermore, variations in cytokine genes may contribute to higher levels of anxiety in oncology patients and their FCs.

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Keywords

anxiety; radiation therapy; cytokines; single nucleotide polymorphisms; cancer; family caregiver; trait anxiety; state anxiety

INTRODUCTION

While anxiety is a common psychological symptom in oncology patients and their family caregivers (FC), compared to depression, it is studied less frequently. When evaluated, clinically significant anxiety occurs in 7% to 30% of oncology patients [1–5] and 20% to 40% of their FCs [1,2]. Most of these studies evaluated patients and FCs at the time of diagnosis or at the initiation of new treatments. In patients, higher levels of state anxiety were associated with increased levels of dyspnea [6], fatigue [7], nausea and pain [8,9], and decreased emotional, social, and cognitive function [10,11]. Moreover, increased anxiety was associated with decreased treatment adherence [12], longer hospital stays [13], and poorer quality of life (QOL) [10]. In a review of the symptom experience of FCs [14], higher anxiety scores were associated with higher levels of anger, depression, sleep disturbance, and fatigue, as well as poorer QOL.

A valid and reliable measure of anxiety is the Speilberger State-Trait Anxiety Inventory (STAI-T, STAI-S) [15]. Trait anxiety is defined as an individual's predisposition to anxiety determined by his/her personality and estimates how a person generally feels [15]. Trait anxiety is considered by some to be a proxy for neuroticism [16]. State anxiety is defined as an individual's transitory emotional response to a stressful situation [15]. While these two dimensions of anxiety are highly correlated [17], evidence suggests that they are distinct dimensions of anxiety [17].

Most studies of oncology patients and their FCs have evaluated state anxiety [18]. However, in the studies that evaluated trait anxiety in oncology patients [19–25], significant associations were found between higher levels of trait anxiety and depression [19,20], psychological distress [21], and pain [22], as well as decrements in health status [23], body image and sexual function [20], and QOL [24]. In addition, patients with higher trait anxiety expressed more negative emotions after diagnosis (e.g., concerns about cancer) as well as more negative perspectives on the future [21].

While the phenotypic characteristics that place oncology patients and their FCs at higher risk for clinically meaningful levels of trait and state anxiety require additional investigation, recent meta-analyses suggest that genetic factors may be involved in the development of anxiety disorders [26–31]. In addition, building on studies that suggest a role for inflammatory mediators in depressive disorders, a need exists to evaluate the role of cytokines in the pathogenesis of anxiety disorders [32,33]. Results of animal studies provide preliminary support for an association between cytokines and anxiety [34–37]. Furthermore, in a study of healthy volunteers who received endotoxin [38], higher anxiety scores were associated with increased levels of circulating pro-inflammatory cytokines.

In keeping with the findings in the literature that stress and inflammation are associated with higher levels of common symptoms, our research team has investigated the role of cytokine gene polymorphisms and increased risk for pain [39,40], depression [39,41], fatigue [39], and sleep disturbance [39,42] in oncology patients and their FCs. Based on these findings and the initial evidence that supports a role for inflammatory mediators in stress and anxiety [35,43], the purposes of this study, in the same sample of patients and FCs, who were evaluated prior to the initiation of the patient's radiation therapy (RT), were: to evaluate for differences in trait and state anxiety between patients and FCs; to evaluate the relationships between select demographic and clinical characteristics and levels of trait and state anxiety; and to investigate the associations between pro- and anti-inflammatory cytokine genes and levels of trait and state anxiety.

METHODS

Participants and Settings

This study is part of a larger, longitudinal study that evaluated multiple physical and psychological symptoms in patients who underwent primary or adjuvant RT and their FCs. A detailed description of the methods is published elsewhere [25,39,40,42]. In brief, participants were enrolled from two RT departments located in a Comprehensive Cancer Center and a community-based oncology program. Patients were eligible to participate if they were ≥18 years of age; were able to read, write, and understand English; had a self-reported Karnofsky Performance Status (KPS) score of ≥60; and were scheduled to receive primary or adjuvant RT. Patients were excluded if they had metastatic disease, more than one cancer diagnosis, or a diagnosed sleep disorder. FCs were eligible to participate if they were an adult (≥18 years of age); were able to read, write, and understand English; gave written informed consent; had a KPS score of ≥60; were living with the patient; and did not have a diagnosed sleep disorder.

Instruments

A demographic questionnaire obtained information on age, gender, marital status, education, ethnicity, employment status, and the presence of a number of co-morbid conditions. Patients' medical records were reviewed for disease and treatment information.

The STAI-T and STAI-S consist of 20 items each that are rated from 1 to 4. The scores for each scale are summed and can range from 20 to 80. A higher score indicates greater anxiety. The STAI-T measures an individual's predisposition to anxiety determined by his/her 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. In individuals with chronic medical conditions, cutoff scores of ≥31.8 and ≥32.2 indicate high levels of trait and state anxiety, respectively. The STAI-S and STAI-T inventories have well-established criterion and construct validity and internal consistency reliability coefficients [15,44,45]. In the current study, Cronbach's alphas for the STAI-T and STAI-S were .92 and .95 for patients and .89 and .93 for FCs, respectively.

The Center for Epidemiological Studies-Depression scale (CESD) consists of 20 items selected to represent the major symptoms in the clinical syndrome of depression. Scores can range from 0 to 60, with scores of ≥16 indicating the need for individuals to seek clinical evaluation for major depression. The CES-D has well established concurrent and construct validity [46,47]. In the current study, the Cronbach's alpha for the CES-D was .88 for patients and .84 for FCs.

Study Procedures

The study was approved by the Committee on Human Research at the University of California, San Francisco and at the second site. Approximately one week prior to the start of RT (i.e., simulation visit when the measurements for RT are made), patients were invited to participate in the study. If the FC was present, a research nurse explained the study protocol to both the patient and FC, determined eligibility, and obtained written informed consent. FCs who were not present were contacted by phone to determine their interest in participation. These FCs completed the enrollment procedures at home.

Phenotypic Data Analysis

Data were analyzed using the Statistical Package for the Social Sciences (SPSS) Version 21. Data collected at the enrollment visit were used in these analyses. Descriptive statistics and frequency distributions were generated on the sample characteristics and anxiety scores. Independent sample t-tests and Chi-square analyses were done to evaluate for differences in demographic and clinical characteristics between patients and FCs. Independent sample t-tests were used to evaluate for differences in anxiety scores between patients who participated with and without FCs. Bivariate analyses were performed to describe the relationships between trait and state anxiety scores and a number of demographic and clinical characteristics. In these bivariate analyses, correlations were used to analyze continuous variables and t-tests and analyses of variance (ANOVAs) were used for categorical variables.

Genomic Data Analysis

Blood collection and genotyping—Genomic deoxyribonucleic acid (DNA) was extracted from archived buffy coats using the PUREGene DNA Isolation System (Invitrogen, Carlsbad, CA). Of the 287 participants recruited, DNA was recovered from the archived buffy coats of 253 (i.e., 168 patients and 85 FCs).

DNA samples were quantitated with a Nanodrop Spectrophotometer (ND-1000) and normalized to a concentration of 50 ng/μL (diluted in 10 mM Tris/1 mM EDTA). Samples were genotyped using the GoldenGate genotyping platform (Illumina, San Diego, CA) and processed according to the standard protocol using GenomeStudio (Illumina, San Diego, CA). Signal intensity profiles and resulting genotype calls for each single nucleotide polymorphism (SNP) were visually inspected by two blinded reviewers.

Gene and SNP Selection—Genes for pro- and anti-inflammatory cytokines and cytokine receptors were selected for analysis (Supplementary Table 1). The pro-inflammatory cytokine genes included: interferon gamma 1 (IFNG1), IFNG receptor 1 (IFNGR1),

interleukin (IL) 1, IL1R1, IL2, IL8, IL17A, and tumor necrosis factor alpha (TNFA). Anti-inflammatory cytokines included: IL1R2, IL4, IL10, and IL13. Of note, IFNG1, IL1B, and IL6 possess pro- and anti-inflammatory functions. Two genes in the nuclear factor-kappa beta (NFKB) family of transcription factors (i.e., NFKB1, NFKB2) were evaluated [48].

A combination of tag-SNPs and literature driven SNPs were selected for analysis. Tagging SNPs were required to be common (defined as having a minor allele frequency ≥.05) in public databases (e.g., HapMap). In order to ensure robust genetic association analyses, quality control filtering of SNPs was performed. SNPs with call rates of <95% or Hardy-Weinberg p-values of <.001 were excluded.

A total of 92 SNPs among the 15 candidate genes (IFNG1: 5 SNPs, IFNGR1: 1 SNP; IL1B: 12 SNPs; IL1R1: 5 SNPs; IL1R2: 3 SNPs; IL2: 5 SNPs; IL4: 8 SNPs; IL6: 9 SNPs; IL8: 3 SNPs; IL10: 8 SNPs; IL13: 4 SNPs; IL17A: 5 SNPs; NFKB1: 11 SNPs; NFKB2: 4 SNPs; TNFA: 9 SNPs) passed all quality control filters and were included in the genetic association analyses (see Supplementary Table 1). Potential functional roles of SNPs were examined using PUPAS uite 2.0 [49].

Statistical Analyses for the Genetic Data

Allele and genotype frequencies were determined by gene counting. Hardy-Weinberg equilibrium was assessed by the Chi-square or Fisher Exact tests. Measures of linkage disequilibrium (i.e., D' and r^2) were computed from the participants' genotypes with Haploview 4.2. Linkage disequilibrium (LD)-based haplotype block definition was based on D' confidence interval [50].

For SNPs that were members of the same haploblock, haplotype analyses were conducted in order to localize the association signal within each gene and to determine if haplotypes improved the strength of the association with the phenotype. Haplotypes were constructed using the program PHASE version 2.1 [51]. In order to improve the stability of haplotype inference, the haplotype construction procedure was repeated five times using different seed numbers with each cycle. Only haplotypes that were inferred with probability estimates of ≥. 85, across the five iterations, were retained for downstream analyses. Haplotypes were evaluated assuming a dosage model (i.e., analogous to the additive model).

Ancestry informative markers (AIMS) were used to minimize confounding due to population stratification [52–54]. Homogeneity in ancestry among participants was verified by principal component analysis [55], using Helix Tree (Golden Helix, Bozeman, MT). Briefly, the number of principal components (PCs) was sought that distinguished the major racial/ethnic groups in the sample by visual inspection of scatter plots of orthogonal PCs (i.e., PC 1 versus PC2, PC2 versus PC3). This procedure was repeated until no discernible clustering of participants by their self-reported race/ethnicity was possible (data not shown). One hundred and six AIMs were included in the analysis. The first three PCs were selected to adjust for potential confounding due to population substructure (i.e., race/ethnicity) by including these three covariates in all regression models.

For association tests, using Independent Student's t-tests or ANOVAs, 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.

Linear regression analysis that controlled for significant covariates, as well as genomic estimates of and self-reported race/ethnicity, was used to evaluate the associations between genotype and anxiety scores. Only those genetic associations identified as significant from the bivariate analyses were evaluated in the multivariate analyses. A backwards stepwise approach was used to create a parsimonious model. Except for self-reported and genomic estimates of race/ethnicity, only predictors with a p-value of <.05 were retained in the final model. Genetic model fit and both unadjusted and covariate-adjusted coefficients were estimated using STATA version 13.

As was done in our previous studies [39,40,42] based on recommendations in the literature [56,57], the implementation of rigorous quality controls for genomic data, the non-independence of SNPs/haplotypes in LD, and the exploratory nature of the analyses, adjustments were not made for multiple testing. In addition, significant SNPs identified in the bivariate analyses were evaluated further using regression analyses that controlled for differences in phenotypic characteristics, potential confounding due to population stratification, and variation in other SNPs/haplotypes within the same gene. Only those SNPs that remained significant were included in the final presentation of the results. Therefore, the significant independent associations reported are unlikely to be due solely to chance. Unadjusted (bivariate) associations are reported for all SNPs passing quality control criteria in Supplementary Table 1 to allow for subsequent comparisons and meta-analyses.

RESULTS

Participant Characteristics

A detailed description of the demographic and clinical characteristics of the participants is published elsewhere [42]. In brief, as shown in Table 1, the majority of participants were female, Caucasian, and well educated. Patients and FCs differed only on gender and marital status. Compared to the patients, a greater proportion of FCs was female (p<.0001) and married/partnered (p<.0001).

Relationships Between Demographic and Clinical Characteristics and State and Trait Anxiety Scores

As shown in Table 1, no significant differences were found in patients' and FCs' trait (p=.484) and state (p=.951) anxiety scores at enrollment. Trait anxiety did not differ significantly between patients who participated with (32.5, SD=10.1) or without a FC (35.5, SD=9.7, p=.06). However, mean state anxiety scores were higher for patients who participated without a FC (33.0, SD=11.7) compared to those of patients who were members of a dyad (29.4, SD=10.0, p=.04).

As shown in Table 2, for the entire sample, both trait and state anxiety scores were negatively correlated with age (both p ≤.001) and KPS score (both p<.001). Both trait and

state anxiety scores were positively correlated with the number of comorbid conditions (both p ≤.015). In addition, women reported higher trait and state anxiety scores (both p <.05). Higher levels of trait anxiety were associated with lower weight (p=.043) and caring for children at home (p=.036).

Associations Between Cytokine Gene Variations and Trait and State Anxiety

In the bivariate analyses, using Independent sample t-tests, five SNPs (i.e., IFNG1 rs2069727, IL1R2 rs4141134, IL17A rs7747909, NFKB2 rs7897947, TNFA rs1800629) and two haplotypes (i.e., IFNG1 Haplotype A5 (HapA5), IL1R2 HapA2) were associated with both trait and state anxiety. Six SNPs in IL1B (i.e., rs3917356, rs1143629, rs1143627, rs16944, rs1143623, rs13032029) were associated only with trait anxiety. Three SNPs (i.e., IL1R2 rs7570441, IL6 rs2069861, IL13 rs2069743) and two haplotypes (i.e., IL1R2 HapA4, IL6 HapA6) were associated only with state anxiety.

Regression Analyses for Trait Anxiety

After controlling for age, functional status, number of comorbidities, and genomic estimates of and self-reported race/ethnicity, the only genotypic predictors of trait anxiety that remained significant were: IL1B rs1143629 (Figure 1A); IL1R2 HapA2 (Figure 2); and NFKB2 rs7897947 (Figure 1B; Table 3).

For IL1B, rs1143629, individuals who carried one or two doses of the rare “C” allele (i.e., TC+CC) had a mean trait anxiety score that was 2.98 points higher than common “T” allele carriers (95% confidence interval [CI]: .52, 5.44, p=.018). The overall model explained 19.2% of the variance in trait anxiety.

Each dose of the haplotype of IL1R2 HapA2 (composed of IL1R2 rs4141134 [rare “C” allele], rs11674595 [common “T” allele], and rs7570441 [common “G” allele]) was associated with a 2.74-point increase in trait anxiety score (95% CI: .31, 5.18, p = .027). The overall model explained 19.0% of the variance in trait anxiety.

For NFKB2 rs78979947, individuals who carried one or two doses of the rare “G” allele (TG+GG) had a mean trait anxiety score that was 2.70 points lower than common “T” allele carriers (95% CI: −5.10, −.30, p=.028). The overall model explained 19.0% of the variance in trait anxiety.

Regression Analyses for State Anxiety

After controlling for age, functional status, and genomic estimates of and self-reported race/ethnicity, the only genotypic predictors that remained significant for state anxiety were IL1R2 HapA2 (Figure 2) and TNFA rs1800629 (Figure 1C, Table 4).

Each dose of IL1R2 HapA2 haplotype was associated with a 3.01 point increase in state anxiety scores (95% CI: .29, 5.74, p=.030). The overall model explained 15.3% of the variance in state anxiety.

For TNFA rs1800629, individuals who carried of one or two doses of the rare “A” allele (GA+AA) had a mean state anxiety score that was 3.68 points lower than carriers of the

common “G” allele (95% CI: −6.56, −.80, p=.013). The overall model explained 15.9% of the variance in state anxiety.

DISCUSSION

This study is the first to evaluate the relationships between trait and state anxiety and cytokine gene variations in oncology patients and their FCs. Findings from this study suggest that trait and state anxiety are related both phenotypically and genotypically. However, consistent with two previous reports [17,58], unique phenotypic and genotypic predictors of trait and state anxiety were identified that suggest that these two symptoms are distinct.

Comparisons of Phenotypic Characteristics of Anxiety

In this sample, trait and state anxiety scores were highly correlated (r=.78, p=.001). While the bivariate analyses revealed common and distinct demographic and clinical characteristics associated with trait and state anxiety, in the multivariate analyses, only age and functional status were retained in the final regression models for both trait and state anxiety. For each 5-year increase in age, both trait and state anxiety scores decreased by approximately one point. Consistent with previous reports [59], younger patients reported higher trait and state anxiety scores. The mean scores for trait and state anxiety (i.e., 34.1 and 31.0, respectively) reported by study participants are comparable to two previous reports in oncology patients at the initiation of RT [60,61].

In addition, consistent with previous reports [62,63], participants who reported poorer functional status scores reported higher levels of trait and state anxiety scores. For each 10-point decrease in KPS scores (which equates with a clinically meaningful decrement in functional status), both trait and state anxiety scores increased by approximately 2 points.

In terms of trait anxiety, only number of comorbid conditions was a unique predictor. On average, each additional comorbid condition was associated with a half-point increase in mean trait anxiety scores (p<.05). In previous studies [24,64], higher levels of trait anxiety at the initiation of cancer treatment predicted decreases in health status and QOL measured one to five years later.

Some of the findings from the bivariate analyses warrant additional consideration. Both patients and FCs reported similar levels of trait and state anxiety, which suggests that both groups experience psychological distress at the initiation of a new cancer treatment. In addition, state anxiety, but not trait anxiety, was significantly higher in patients who did not participate with a FC. This finding is consistent with previous reports that suggest that social support can reduce psychological distress in oncology patients [65–68]. Taken together, these phenotypic findings suggest that clinicians need to perform assessments of patients’ and FCs’ levels of anxiety prior to the initiation of a new cancer treatment and utilize appropriate interventions to reduce distress.

Comparisons of Genotypic Characteristics of Anxiety

Only one genomic marker, an IL1R2 haplotype, composed of rs4141134, rs11674595, and 7570441, was associated with a 3-point increase in ratings of both trait and state anxiety. While no published associations were found between this haplotype and anxiety, in another study from the same sample, this haplotype was associated with a 2-fold increase in the odds of belonging to the group with higher levels of depressive symptoms [41]. In addition, in a sample of patients who were followed for six months after breast cancer surgery, a haplotype in IL1R2 that contained two of the same SNPs identified in this study (i.e., rs11674595 and rs7570441) was associated with 2-fold increase in the odds of belonging to the group with higher levels of sleep disturbance [69]. Given the fact that inflammation is one of the proposed mechanisms for anxiety [43,70] depression [71–73], and sleep disturbance [39,74,75,76], our findings across two independent samples suggest that IL1R2 may be a common mediator of these three common symptoms that are associated with cytokine-induced “sickness behavior” [77–80].

IL1R2 is an anti-inflammatory cytokine that inhibits inflammatory signalling by binding to IL1β and preventing its binding to IL1R1 [81]. Therefore, one can hypothesize that the activity of the SNPs in this haplotype, or an unmeasured SNP(s) in linkage disequilibrium, decreases IL1R2 expression, which would increase the amount of pro-inflammatory IL1-β bound to IL1R1 and result in higher levels of trait and state anxiety. While the functionality of each SNP in the haplotype is unknown, rs4141134 is located in the promoter region of the IL1R2 gene and may impact its expression. The other two SNPs in the haplotype are located in intronic regions of the gene that are evolutionarily conserved. Given the associations between the SNPs in this haplotype and higher levels of trait anxiety, state anxiety, depressive symptoms [41], and sleep disturbance [69], the role that IL1R2 plays in the regulation of inflammation and common symptoms experienced by individuals with chronic medical conditions warrants investigation in future studies. In addition, functional studies are needed to confirm the hypothesized mechanism proposed above for IL1R2 rs4141134.

IL1B rs1143629 and NFKB2 rs78989947 were associated with higher levels of trait anxiety. A growing body of evidence suggests that increased levels of IL1-β are associated with increased levels of anxiety in animals [82,83] and humans [38,84,85]. While the exact mechanisms by which inflammation results in anxiety are not completely understood, several lines of evidence suggest that IL1β may induce: alterations in serotonin metabolism [86–88]; changes in the activity of the hypothalamic-pituitary-adrenal axis [89]; and/or changes in the sensitivity of cannabinoid receptors [90]. Although the SNP in IL1B is intronic and has no function, it may be a surrogate marker in LD with other functional SNPs.

While in the bivariate analyses, NFKB2 rs7897947 was associated with increased levels of both trait and state anxiety, this relationship remained significant in the multivariate analyses only for trait anxiety. This SNP is located in an intronic region of the gene and has no known function. Of note, this SNP, in this same sample of patients and FCs, was associated with a 74% reduction in the odds of belonging to a group with higher levels of sleep disturbance [42]. In addition, another polymorphism in NFKB2 (i.e., rs1056890) was associated with a 47% reduction in the odds of belonging to a group with higher levels of sleep disturbance in a sample of patients who underwent surgery for breast cancer [69]. NFKB2 is a gene that

belongs to the nuclear factor-kappa beta family. This family is made up of transcription factors that regulate a variety of biological processes (e.g., immunity, stress responses, apoptosis, cellular differentiation) [91]. Given the fact that anxious individuals often report sleep disturbance, additional research is warranted on the mechanisms by which genetic variations in NFKB2 may result in decreased levels of anxiety and sleep disturbance.

While in the bivariate analyses, TNFA rs1800629 was associated with both trait and state anxiety, this relationship remained significant in the multivariate analyses only for state anxiety. In this same sample, this SNP was associated with a 57% reduction in the odds of belonging to a group with higher levels of depressive symptoms [41]. In addition, in this same sample, individuals who were heterozygous or homozygous for the rare A allele in rs1800629 reported lower levels of sleep disturbance and morning fatigue at the initiation of RT [92]. This SNP is a common functional promoter polymorphism (i.e., c.G-308A). However, investigations on the direction and magnitude of the gene's expression because of the minor allele have yielded conflicting results [93–95]. The findings on the association between this functional polymorphism and state anxiety warrant additional investigation given recent reports of associations between TNFα and its receptors and rodent models of anxiety [96,97].

Limitations

Limitations of this investigation must be acknowledged. While our sample size was adequate, these findings warrant replication in independent samples. During recruitment, the most common reasons for refusal were being too overwhelmed or too busy to participate. Therefore, the anxiety scores reported by study participants may be an underestimation. Larger samples with more heterogeneity in anxiety scores may identify additional genetic associations. Although valid and reliable self-report measures of trait and state anxiety were used in this investigation, a clinical diagnostic interview should be done to obtain a more comprehensive evaluation of the nature, severity, and time course of the patients' and FCs' level of both trait and state anxiety, as well as specific anxiety disorders. Furthermore, serum cytokine levels could be collected to support the genetic associations. Studies of genes that encode for proteins involved in other pathways (e.g., dopaminergic, serotonergic) will provide additional insights into the mechanisms that underlie anxiety in both patients and their FCs.

Conclusions

Despite these limitations, these findings suggest that an assessment of both trait and state anxiety in oncology patients and their FCs may more fully characterize these individuals' specific needs for psychosocial interventions. In addition, the genomic analyses suggest that inflammatory mechanisms are involved in both forms of anxiety, as well as in the development of other common symptoms (e.g., depression, fatigue, sleep disturbance) in both oncology patients and their FCs. An increased understanding of the common mechanisms that underlie the most frequently occurring symptoms in oncology patients and their FCs may lead to the identification of new therapeutic targets to reduce symptom burden in these individuals.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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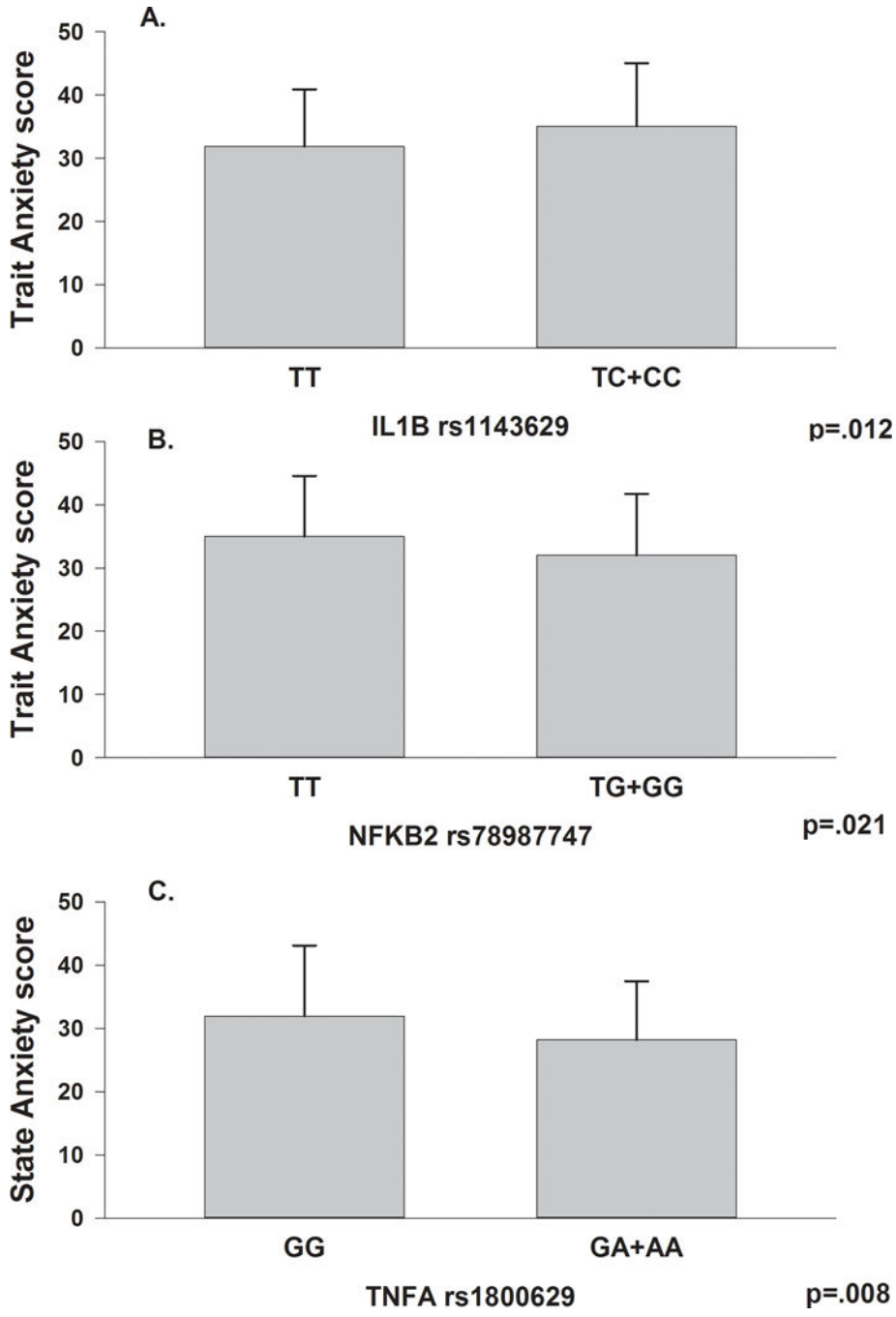
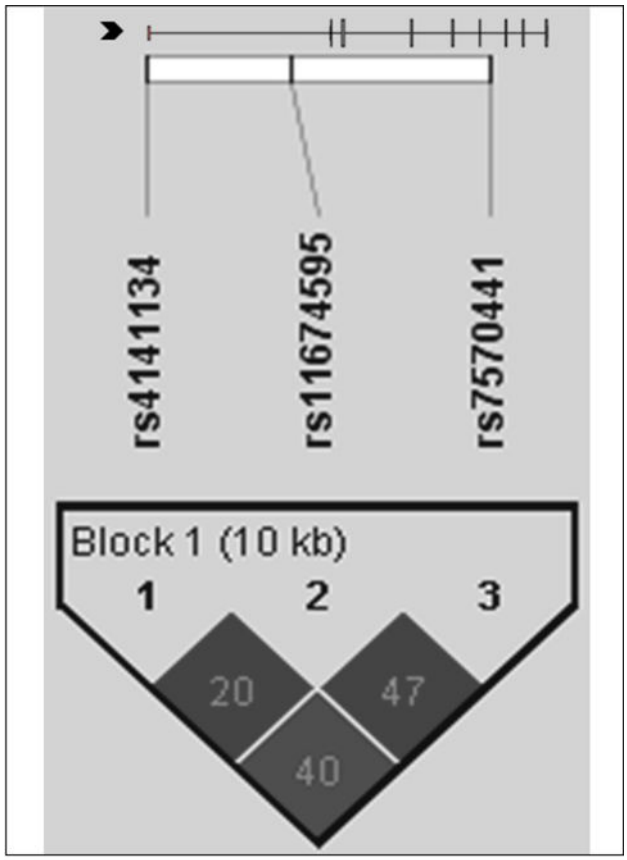


Figure 1.

Panel A – Differences in Trait Anxiety scores, not adjusting for covariates, between participants who were homozygous for the common T allele in Interleukin 1 beta (IL1B) rs1143629 and participants who were homozygous or heterozygous for the rare C allele. Panel B - Differences in Trait Anxiety, not adjusting for covariates, scores between participants who were homozygous for the common T allele in Nuclear Factor Kappa Beta 2 (NFKB2) rs78987747 and participants who were homozygous or heterozygous for the rare G allele.

Panel C - Differences in State Anxiety scores, not adjusting for covariates, between participants who were homozygous for the common G allele in Tumor Necrosis Factor Alpha (TNFA) rs1800629 and participants who were homozygous or heterozygous for the rare A allele. All analyses were done using Independent Student's t-tests. All values are plotted as means ± standard deviations.



Haplotype	State Anxiety Mean (SD)	Trait Anxiety Mean (SD)
A1: T-T-G		
0 doses	30.8 (11.57)	33.8 (9.98)
1 dose	30.6 (9.21)	33.5 (9.42)
2 doses	33.4 (10.86)	38.5 (7.39)
A2: C-C-A		
0 doses	***29.9 (9.74)***	***32.8 (9.31)***
1 dose	***32.5 (12.07)***	***36.0 (10.02)***
2 doses	***39.9 (19.18)***	***40.2 (14.18)***
A4: C-T-A		
0 doses	33.2 (11.64)	35.5 (9.95)
1 dose	29.9 (10.65)	33.3 (10.01)
2 doses	27.8 (7.63)	31.5 (7.73)

Figure 2.

IL1R2 linkage disequilibrium-based heatmap and haplotype analysis. In the figure embedded in the top row of the table, an ideogram of interleukin 1 receptor 2 (IL1R2) is presented above the white bar that represents the physical distance along human chromosome 2 (position 31, 96,370,336 to 96,380,807; genome build 36.3, contig NT_022171.14). Exons are represented as tick marks. Gray lines connecting the exons represent introns. The black chevron indicates the direction of gene transcription. Reference sequence identifiers (rsID) for each single nucleotide polymorphism (SNP) are plotted both

in terms of their physical distance (i.e., the white bar at the top of the figure) and also equidistantly to render the pairwise linkage disequilibrium (LD) estimates that were calculated and visualized with Haploview 4.2. The gene structure for IL1R2 (i.e., reference sequence NM_004633) was rendered with FancyGene 1.4. The correlation statistics (r2 and D') are provided in the heatmap. LD-based haplotype block definition was based on the D' confidence interval method. The haploblock is indicated in a bolded triangle and its component SNPs are rendered in bold font. Pairwise D' values (range: 0-1, inclusive) were rendered in grey, with darker grey diamonds representing D' values approaching 1.0. When the r2 values (range of 0–100, inclusive) are not equal to 0 or 100, they are provided in a given diamond. The haplotypes observed in the haploblock are listed in each row, starting with the nucleotide composition across the two SNPs that compose the haplotype (i.e., rs4141134, rs11674595, rs7570441) and both the mean and standard deviation (SD) for trait and state anxiety for each of the three subgroups for a given haplotype (i.e., zero doses of the haplotype, one dose of the haplotype, two doses of the haplotype). The C-C-A haplotype (i.e., IL1R2 HapA2) identified in the bivariate analyses (Supplemental Table 1) that remained significant after controlling for relevant confounders is rendered in bold and italicized.

Table 1

Differences in Demographic and Clinical Characteristics Between Patients and Family Caregivers at Enrollment

Characteristic	Total Sample	Patients	Family Caregivers	Statistic
	n=253	n=168	n=85	
	Mean (SD)	Mean (SD)	Mean (SD)	
Age (years)	61.4 (11.3)	60.9 (11.6)	62.5 (10.5)	t=−1.03, p=.305
Education (years)	15.9 (3.0)	16.0 (2.9)	15.8 (3.2)	t=0.56, p=.575
Number of comorbid conditions	4.6 (2.7)	4.8 (2.6)	4.2 (2.9)	t=1.52, p=.131
Karnofsky Performance Status score	92.0 (11.5)	91.1 (11.9)	93.7 (10.6)	t=−1.65, p=.100
STAI-T score	34.1 (9.9)	33.8 (10.0)	34.7 (9.7)	t=0.49, p=.484
STAI-S score	31.0 (10.8)	30.9 (10.9)	31.0 (10.7)	t=−0.06, p=.951
CES-D score	8.8 (8.2)	9.1 (8.7)	8.3 (7.2)	t=0.79, p=.429
	%	%	%	
Gender Male Female	46.2 53.8	55.4 44.6	28.2 71.8	FE, p<.0001
Race/Ethnicity White Asian/Pacific Islander Black Hispanic/Mixed Ethnic Background/Other	74.6 6.3 13.5 5.6	71.9 7.2 15.0 6.0	80.0 4.7 10.6 4.7	X^2=4.89, p=.429
Marital status Married/partnered Other	69.3 30.7	56.0 44.0	95.3 4.7	FE, p<.0001
Works for pay (% yes)	46.4	47.0	45.2	FE, p=.89
Children at home (% yes)	17.0	17.0	16.9	FE, p=1.00
Pain (% yes)	47.8	56.0	31.8	FE, p<.0001

Abbreviations: CES-D=Center for Epidemiologic Studies-Depression Scale; FE=Fisher's Exact; SD=standard deviation; STAI-T=State-Trait Anxiety Inventory-Trait; STAI-S=State-Trait Anxiety Inventory-State

Table 2

Relationships between Trait and State Anxiety Scores and Demographic and Clinical Characteristics

	Trait Anxiety		State Anxiety	
Characteristic	**Correlation**	***p* value**	**Correlation**	***p* value**
Age (years)	−.24	<.0001	−.21	.001
Education (years)	−.02	.756	−.04	.551
Number of comorbid conditions	.18	.005	.16	.015
Weight (pounds)	−.13	.043	−.13	.052
Karnofsky Performance Status score	−.27	<.0001	−.27	<.0001
	Mean (SD)	Statistic	Mean (SD)	Statistic
Gender Female Male	35.5 (10.7) 32.5 (8.6)	*t*=2.46, *p*=.015	33.0 (12.2) 28.6 (8.4)	*t*=3.36, *p*=.001
Ethnicity White Asian/Pacific Islander Black/African American Hispanic/Mixed Background/Other	33.4 (10.0) 40.6 (11.6) 34.4 (7.7) 35.8 (8.9)	F=2.88, *p*=.036	30.6 (11.0) 33.7 (13.3) 30.7 (9.8) 34.0 (8.0)	F=.80, *p*=.496
Lives Alone Yes No	34.5 (9.8) 33.4 (10.2)	*t*=.68, *p*=.500	31.9 (10.9) 30.5 (10.9)	*t*=.80, *p*=.424
Married or partnered Yes No	33.8 (10.3) 34.3 (8.9)	*t*=−.32, *p*=.748	30.5 (11.1) 31.9 (10.1)	*t*=−.95, *p*=.343
Work for pay Yes No	33.5 (8.7) 34.6 (10.8)	*t*=−.89, *p*=.375	29.9 (8.9) 31.6 (11.9)	*t*=−1.31, *p*=.193
Children at home Yes No	37.4 (10.2) 33.6 (9.4)	*t*=2.12, *p*=.036	34.1 (12.6) 30.5 (10.1)	*t*=1.83, *p*=.068
Older adult at home Yes No	38.4 (13.0) 34.1 (9.5)	*t*=1.18, *p*=.238	32.9 (15.7) 31.0 (10.5)	*t*=.−44, *p*=.659
Patient/FC Patient Family caregiver	33.8 (10.0) 34.7 (9.7)	*t*=−.70, *p*=.484	30.9 (10.9) 31.0 (10.7)	*t*=−.06, *p*=.951

Abbreviation: SD = standard deviation

Table 3

Multiple Linear Regression Analyses for Interleukin 1B (IL1B) rs1143629, Interleukin 1R2 (IL1R2) HapA2, and Nuclear Factor Kappa, Beta 2 Subunit (NFKB2) rs7897947 to Predict Low to High Trait Anxiety (n=234)

Predictor of Low to High Trait Anxiety	β Coefficient	Standard Error	95% CI	t	p-value
IL1B rs1143629	2.980	1.248	.520, 5.440	2.39	.018
Age	−.875	.283	−1.433, −.318	−3.10	.002
Functional status	−1.843	.529	−2.884, −.801	−3.49	.001
Number of comorbidities	.588	.231	.132, 1.044	2.54	.012
Overall model fit: $F_{(10,223)}$ = 5.31, p <.0001 R^2 = .1923					
IL1R2 HapA2 genotype	2.744	1.236	.308, 5.180	2.22	.027
Age	−.875	.283	−1.434, −.317	−3.09	.002
Functional status	−1.766	.528	−2.808, −.725	−3.34	.001
Number of comorbidities	.563	.232	.105, 1.020	2.42	.016
Overall model fit: $F_{(10,223)}$ = 5.22, p <.0001 R^2 = .1895					
NFKB2 rs78979947	−2.698	1.217	−5.096, −.301	−2.22	.028
Age	−.897	.283	−1.454, −.340	−3.17	.002
Functional status	−1.826	.529	−2.869, −.783	−3.45	.001
Number of comorbidities	.534	.233	.074, .994	2.29	.023
Overall model fit: $F_{(10,223)}$ = 5.21, p<.0001 R^2 = .1895					

For this model, the first three principle components identified from the analysis of ancestry informative markers as well as self-reported race/ethnicity (White, Asian/Pacific Islander, Black, Hispanic/Mixed Ethnic Background/Other) were retained to adjust for potential confounding due to race or ethnicity (data not shown). Predictors evaluated in the model included genotype (IL1B rs1143629: TT versus CT +CC; IL1R2 HapA2: composed of IL1R2 rs4141134 [rare “C” allele], rs11674595 [common “T” allele], and rs7570441 [common “G” allele]; and NFKB2 rs7897947: TT versus TG+GG), age (in 5-year increments), functional status (Karnofsky Performance Status score, in 10-point increments), and number of comorbidities.

Table 4

Multiple Linear Regression Analyses for Interleukin 1R2 (IL1R2) Haplotype A2 (HapA2) and Tumor Necrosis Factor Alpha (TNFA) rs1800629 to Predict Low to High State Anxiety (n=231)

Predictor of Low to High State Anxiety	β Coefficient	Standard Error	95% CI	t	p-value
IL1R2 HapA2 genotype	3.014	1.380	.294, 5.735	2.18	.030
Age	−.968	.314	−1.587, −.349	−3.08	.002
Functional status	−2.210	.573	−3.338, −1.081	−3.86	<.001
Overall model fit: $F_{(9, 221)} = 4.45$, p <.0001 $R^2 = .1534$					
TNFA rs1800629	−3.679	1.462	−6.560, −.797	−2.52	.013
Age	−1.031	.312	−1.647, −.415	−3.30	.001
Functional status	−2.070	.574	−3.202, −.939	−3.61	<.001
Overall model fit: $F_{(9, 221)} = 4.65$, p <.0001 $R^2 = .1592$					

For this model, the first three principle components identified from the analysis of ancestry informative markers as well as self-reported race/ethnicity (White, Asian/Pacific Islander, Black, Hispanic/Mixed Ethnic Background/Other) were retained to adjust for potential confounding due to race or ethnicity (data not shown). Predictors evaluated in the model included genotype (IL1R2 HapA2: composed of IL1R2 rs4141134 [rare “C” allele], rs11674595 [common “T” allele], and rs7570441 [common “G” allele]; and TNFA rs1800629: GG versus GA+AA), age (in 5-year increments), and functional status (Karnofsky Performance Status score, in 10-point increments).