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Cytokine Gene Associations with Self-report Ratings of Morning and Evening Fatigue in Oncology Patients and Their Family Caregivers

Anand Dhruva, MD[1], **Bradley E. Aouizerat, PhD, MAS**[2,3], **Bruce Cooper, PhD**[2], **Steven M. Paul, PhD**[2], **Marylin Dodd, RN, PhD**[2], **Claudia West, RN, MS**[2], **William Wara, MD**[4], **Kathryn Lee, RN, PhD**[2], **Laura B. Dunn, MD**[1], **Dale J. Langford, PhD**[2], **John D. Merriman, RN, PhD**[2], **Christina Baggott, RN, PhD**[2], **Janine Cataldo, RN, PhD**[2], **Christine Ritchie, MD**[1], **Kord M. Kober, PhD**[2], **Heather Leutwyler, RN, PhD**[2], and **Christine Miaskowski, RN, PhD**[2]

[1]School of Medicine, University of California, San Francisco, California

[2]School of Nursing, University of California, San Francisco, California

[3]Institute for Human Genetics, University of California, San Francisco, California

[4]Kaiser Permanente, San Francisco, California

Abstract

The purpose of this study was to evaluate for differences in variations in pro- and anti-inflammatory cytokine genes between participants who were classified as having low and high levels of morning and evening fatigue and to evaluate for differences in phenotypic characteristics between these two groups. In a sample of 167 oncology outpatients with breast, prostate, lung, or brain cancer and 85 of their family caregivers, growth mixture modeling (GMM) was used to identify latent classes of individuals based on ratings of morning and evening fatigue obtained prior to, during, and for 4 months following completion of radiation therapy. Differences in single nucleotide polymorphisms (SNPs) and haplotypes in 15 cytokine genes were evaluated between the latent classes. Multiple logistic regression was used to assess the effect of phenotypic and genotypic characteristics on morning and evening fatigue class membership. Associations were found between morning fatigue and number of comorbidities as well as variations in *TNFA* rs1800629 and rs3093662. Evening fatigue was associated with caring for children at home and variations in *IL4* rs2243248 and *TNFA* rs2229094. Younger age and lower performance status was associated with both morning and evening fatigue. These findings suggest that inflammatory mediators are associated with the development of morning and evening fatigue. However, because different phenotypic characteristics and genomic markers are associated with diurnal variations in fatigue, morning and evening fatigue may be distinct but related symptoms.

Keywords

cytokines; genetics; morning fatigue; evening fatigue; breast cancer; tumor necrosis factor alpha; interleukin 4

Address correspondence to: Christine Miaskowski, RN, PhD, FAAN, Professor and Associate Dean, University of California, 2 Koret Way – Box 0610, San Francisco, CA 94143-0610, 415-476-9407, 415-476-8899, chris.miaskowski@nursing.ucsf.edu.

Fatigue is a frequent and disabling symptom (Lawrence, Kupelnick, Miller, Devine, & Lau, 2004) that occurs in approximately 80% of patients receiving radiation therapy (Henry et al., 2008; Hofman, Ryan, Figueroa-Moseley, Jean-Pierre, & Morrow, 2007) and in 24–30% of family caregivers of patients with cancer (Swore Fletcher, Dodd, Schumacher, & Miaskowski, 2008). Although authors have reported on the high prevalence and negative impact of this symptom for over 30 years, little is known about the mechanisms that underlie fatigue.

While the etiology of fatigue is undoubtedly multifactorial, a growing body of evidence suggests that inflammatory pathways are important in the development of this symptom (Barsevick, Frost, Zwinderman, Hall, & Halyard, 2010; Jager, Sleijfer, & van der Rijt, 2008; Reyes-Gibby et al., 2008; Schubert, Hong, Natarajan, Mills, & Dimsdale, 2007). In fact, several studies have evaluated for associations between serum cytokine levels and fatigue occurrence or severity. The findings of a quantitative review (Schubert et al., 2007) suggested a positive correlation between fatigue and circulating levels of inflammatory markers. However, of the 19 circulating inflammatory markers evaluated, only interleukin (IL)-6, IL1-receptor alpha (IL-1rα), and neopterin remained significant in the final analyses. These inconclusive findings may be due to the challenges inherent in the measurement of circulating cytokines and to circadian variability in cytokine levels (Gilbertson-White, Aouizerat, & Miaskowski, 2011).

In addition to the studies of serum cytokines, several studies have documented associations between variations in cytokine genes and fatigue (Aouizerat et al., 2009; Bower, Ganz, Irwin, Arevalo, & Cole, 2011; Collado-Hidalgo, Bower, Ganz, Irwin, & Cole, 2008; Hong et al., 1995; Miaskowski et al., 2010; Reinertsen et al., 2011). In a study of 33 fatigued and 14 nonfatigued breast cancer survivors, Collado-Hidalgo et al. (2008) found that, while *IL6* was not associated with fatigue, *IL1B* did show an association. In a study conducted by our research team (Miaskowski et al., 2010), oncology patients (n = 288) and family caregivers (n = 103) who were homozygotes for the common allele in *IL6* rs4719714 reported higher levels of morning and evening fatigue and sleep disturbance. In addition, in this same sample, individuals who were homozygous for the common allele in tumor necrosis factor alpha *(TNFA)* rs1800629 reported higher levels of morning fatigue and sleep disturbance (Aouizerat et al., 2009). The same single nucleotide polymorphism (SNP) in *TNFA* was associated with symptoms of exhaustion and higher C-reactive protein levels in patients with chronic fatigue syndrome (Jeanmonod, von Kanel, Maly, & Fischer, 2004). In another study, in which researchers compared fatigued (n = 11) and nonfatigued (n = 10) breast cancer survivors, nuclear factor kappa beta *(NFKB)* transcripts were shown to be increased among fatigued survivors (Bower et al., 2011). However, in a large study of breast cancer survivors, only one of seven SNPs in five cytokine genes (i.e., C-reactive protein *[CRP]* rs3091244) was associated with fatigue (Reinertsen et al., 2011).

One of the limitations of the research studies conducted to date is that diurnal variations in fatigue severity and associated phenotypic and genotypic characteristics were not evaluated in detail. In a recent paper from our research team that used growth mixture modeling (GMM) to identify latent classes of individuals with distinct morning and evening fatigue

trajectories among oncology patients and their family caregivers (Dhruva et al., 2013), we found phenotypic characteristics that distinguished among the morning and evening fatigue classes. For example, participants in the High Morning Fatigue class were more likely to be younger and have a lower functional status than participants in the Low Morning Fatigue class. In contrast, participants in the High Evening Fatigue class were more likely to be younger, female, caring for children at home, and a family caregiver than participants in the Low Evening Fatigue class. In addition, only 10.3% of the sample was classified as being in both the lowest morning and evening fatigue classes and only 41.3% of the sample was classified in both the highest morning and evening fatigue classes. Based on these findings, we suggested that morning and evening fatigue may be distinct but related symptoms.

In this paper, we extend these findings and evaluate for differences in a number of pro- and anti-inflammatory cytokine genes among those participants who were classified as having low and high levels of morning and evening fatigue.

Methods

Participants and Settings

Details of this study are published elsewhere (Aouizerat et al., 2009; Carney et al., 2011; Dhruva et al., 2012; Dunn et al., 2012; Miaskowski et al., 2010; Miaskowski et al., 2011). In brief, patients and their family caregivers (FCs) were recruited from two radiation-therapy departments located in a comprehensive cancer center and a community-based oncology program at the time of the patients' simulation visit.

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

We used a demographic questionnaire to obtain information on age, gender, marital status, education, ethnicity, employment status, and the presence of a number of comorbid conditions. We also reviewed medical records for disease and treatment information.

The Lee Fatigue Scale (LFS), which we used in the present study, consists of 13 items designed to assess physical fatigue (K. A. Lee, Hicks, & Nino-Murcia, 1991). A total fatigue score is calculated as the mean of the 13 items, with higher scores indicating greater fatigue severity. Participants are asked to rate each item based on how they feel “right now” within 30 min of awakening (morning fatigue) and prior to going to bed (evening fatigue). The LFS has well established validity and reliability. In the present study, Cronbach's alphas for evening and morning fatigue at enrollment were 0.96 and 0.95 for patients and 0.95 and 0.96 for FCs, respectively.

Study Procedures

The study was approved by the Committee on Human Research at the University of California, San Francisco, and the Institutional Review Board at the second site. We invited patients to participate in the study approximately 1 week prior to the start of radiation therapy (i.e., simulation visit when the measurements for radiation therapy are made). 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. Participants completed the LFS at enrollment, 4 weeks after the initiation of radiation therapy, at the completion of radiation therapy, and at 4, 8, 12, and 16 weeks after the completion of radiation therapy (i.e., seven assessments over 6 months).

Methods of Analysis for Phenotypic Data

Data were analysed using SPSS, version 19 (SPSS, 2010), and Mplus, version 6.11 (L. K. Muthen & Muthen, 1998-2010). Descriptive statistics and frequency distributions were generated on sample characteristics and fatigue severity scores. Independent sample t-tests, analyses of variance (ANOVA), and Chi-square analyses were done to evaluate for differences in demographic and clinical characteristics between the fatigue classes.

GMM with robust maximum likelihood estimation was used to identify latent classes (i.e., subgroups of participants) with distinct morning and evening fatigue trajectories over the 6 months of the study (B. O. Muthen & Kaplan, 2004). Because 65% of the participants were in patient–caregiver dyads, models were estimated with “dyad” as a clustering variable to ensure that any dependency between the morning and evening fatigue scores for patients and FCs in the same dyad was controlled for in the GMM analysis. It should be noted that after taking any dependency within dyads into account, no significant differences were found between patients and FCs in the parameter estimates for the various morning and evening fatigue GMM trajectories that were identified in the initial analysis.

As reported previously (Dhruva et al., 2013), three distinct latent classes were identified for morning and evening fatigue. For the candidate gene analyses reported in the present paper, the three morning fatigue classes were collapsed into two groups (i.e., Very Low [32.5%] versus Low and High [67.5%]) as were the three evening fatigue classes (i.e., Low [11.1%] versus Moderate and High [88.9%]). The rationale for this categorization is that very low levels of morning fatigue and low levels of evening fatigue might be expected in the general population. This extreme-phenotype approach is an effective strategy to identify potential candidate genes associated with symptoms or clinical conditions (Li, Lewinger, Gauderman, Murcray, & Conti, 2011).

Adjustments were not made for missing data. Therefore, the cohort for each analysis was dependent on the largest set of available data across groups. A p-value of $< .05$ was considered statistically significant.

Methods of Analysis for Genomic Data

Gene selection—Pro-inflammatory cytokines promote systemic inflammation and include interferon gamma (IFNγ), IFNγ receptor 1 (IFNγR1), IL-1R1, IL-2, IL-8, IL-17A, NFKB1, NFKB2, and TNF-α. Anti-inflammatory cytokines suppress the activity of pro-inflammatory cytokines and include IL-1R2, IL-4, IL-10, and IL-13. Of note, IFNγ1, IL-1β, and IL-6 possess pro- and anti-inflammatory characteristics (Seruga, Zhang, Bernstein, & Tannock, 2008).

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 253 participants (i.e., 168 patients and 85 FCs). Samples were genotyped using the GoldenGate genotyping platform (Illumina, San Diego, CA) and processed using GenomeStudio (Illumina, San Diego, CA).

Single nucleotide polymorphism (SNP) selection—We selected a combination of tagging SNPs and SNPs suggested by the literature for analyses. Tagging SNPs were required to be common (i.e., defined as having a rare allele frequency ≥ 0.05) in public databases. In order to ensure robust genetic association analyses, we performed quality control filtering of the SNPs. SNPs with call rates of < 95% or Hardy-Weinberg *p*-values of < .001 were excluded.

As shown in supplemental Table 1 <PRODUCTION: Please add link here to online table, if possible>, a total of 92 SNPs among the 15 candidate genes passed all quality control filters and were included in the genetic association analyses (*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). Potential functional roles of SNPs associated with specific symptoms were examined using PUPASuite 2.0 (Conde et al., 2006).

Statistical analyses—Allele and genotype frequencies were determined by gene counting. Hardy-Weinberg equilibrium was assessed by the Chi-square or Fisher's exact tests. Measures of linkage disequilibrium (LD; i.e., D' and r^2) were computed from the participants' genotypes with Haploview 4.2. LD-based haplotype block definition was based on D' confidence interval (Gabriel et al., 2002).

Haplotypes were constructed using the program PHASE, version 2.1 (Stephens, Smith, & Donnelly, 2001). Only haplotypes that were inferred with probability estimates of ≥ .85 across five iterations were retained for downstream analyses. Haplotypes were evaluated assuming a dosage model.

Ancestry informative markers (AIMS) were used to minimize confounding due to population stratification (Halder, Shriver, Thomas, Fernandez, & Frudakis, 2008; Hoggart et al., 2003; Tian, Gregersen, & Seldin, 2008). Homogeneity in ancestry among participants was verified by principal component analysis (Price et al., 2006) using Helix Tree (Golden Helix, Bozeman, MT). Included in the analysis were 106 AIMs. The first three PCs were

selected to adjust for potential confounding due to population substructure (i.e., race/ethnicity) by including the three covariates in all regression models.

For association tests, three genetic models were assessed for each SNP: additive, dominant, and recessive. Barring trivial improvements (i.e., delta < 10%), the genetic model that best fit the data, by maximizing the significance of the *p*-value, was selected for each SNP. Logistic regression analysis, which controlled for significant covariates as well as for race/ethnicity, was used to evaluate the association between genotype and fatigue-group membership. A backwards stepwise approach was used to create a parsimonious model. Except for self-reported race/ethnicity and AIMS, only predictors with a *p*-value of < .05 were retained in the final model. Genetic model fit and both unadjusted and covariate-adjusted odds ratios were estimated using STATA version 9.

As was done in our previous studies (Illi et al., 2012; McCann et al., 2012; C Miaskowski et al., 2012), based on recommendations in the literature (Hattersley & McCarthy, 2005; Rothman, 1990), 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 associations are reported for all SNPs passing quality control criteria in supplemental Table 1 to allow for subsequent comparisons and meta-analyses.

Results

Participant Characteristics

The total sample, which was 46.2% male and 53.8% female, consisted of 167 oncology outpatients and 85 FCs. The majority of the participants were well educated and Caucasian with a mean age of 61.5 years. The mean KPS score was 92 and the average participant had more than four comorbid conditions. Approximately 49% of the patients had prostate cancer, 38% had breast cancer, 7% had a brain tumor, and 6% had lung cancer. We found no significant differences between patients' and FCs' ratings of morning fatigue (2.3 ± 2.0 versus 2.3 ± 1.9, respectively) and evening fatigue (4.2 ± 2.0 versus 4.5 ± 2.0, respectively) at enrollment.

Phenotypic Differences Between the Morning Fatigue Classes

Compared to those in the Very Low morning fatigue class (*n* = 82 [32.5%]), participants in the Low and High classes (combined *n* = 170 [67.5%]) were significantly younger and had a higher number of comorbidities and a significantly lower KPS score (Table 1). Within the Very Low morning fatigue class, we found no differences between patients' (0.7 ± 1.0) and FCs' (0.9 ± 0.9) ratings of morning fatigue at enrollment. Within the Low and High morning

fatigue classes, we found no differences between in patients' (3.1 ± 1.8) and FCs' (3.0 ± 2.0) ratings of morning fatigue at enrollment.

Candidate Gene Analyses for the Morning Fatigue Classes

Of the five SNPs that differed significantly between the two morning fatigue classes (Supplementary Table 1), two associations in one gene remained significant in the multivariate regression analyses (i.e., *TNFA* rs1800629, *TNFA* rs3093662). For both SNPs, a dominant model fit the data best (both $p = 0.006$).

Regression Analyses of Candidate Genes for Morning Fatigue Classes

In order to better estimate the magnitude (i.e., odds ratio, OR) and precision (95% confidence interval, CI) of genotype on morning fatigue class membership, multiple variable logistic regression models were fit to compare the two classes. In addition to genotype, the phenotypic characteristics that were included in the models were age, KPS score, and self-reported and genomic estimates of race/ethnicity (Table 2).

In the regression analysis for *TNFA* rs1800629, being heterozygous or homozygous for the rare A allele (GG versus GA+AA) was associated with a 52% decrease in the odds of belonging to the higher morning fatigue group (Figure 1A). In the regression analysis for *TNFA* rs309662, being heterozygous or homozygous for the rare G allele (AA versus AG +GG) was associated with a 6.59-fold increase in the odds of belonging to the higher morning fatigue group (Figure 1B).

Phenotypic Differences Between the Evening Fatigue Classes

Compared to the Low evening fatigue class, participants in the Moderate and High classes were significantly younger, had a significantly lower KPS score, and were more likely to have children living at home (Table 2). Within the Low evening fatigue class, we found no differences between patients' (1.2 ± 1.2) and FCs' (1.2 ± 0.8) ratings of evening fatigue at enrollment. Likewise, we found no differences within the Moderate and High evening fatigue classes between patients' (4.5 ± 1.8) and FCs' (4.8 ± 1.8) ratings of evening fatigue at enrollment.

Candidate Gene Analyses for the Evening Fatigue Classes

Of the eight SNPs that differed significantly between the two evening fatigue classes (Supplementary Table 1), one association in each of two genes remained significant in the multivariate regression analyses (i.e., *IL4* rs2243248, *TNFA* rs2229094). For both SNPs, a dominant model fit the data best.

Regression Analyses of Candidate Genes for Evening Fatigue Classes

In order to better estimate the magnitude (i.e., OR) and precision (95% CI) of genotype on evening fatigue class membership, multiple variable logistic regression models were fit to compare the two classes. In addition to genotype, the phenotypic characteristics that were included in the models were age and self-reported and genomic estimates of race/ethnicity (Table 3).

In the regression analysis for *IL4* rs2243248, being heterozygous or homozygous for the rare G allele (TT versus TG+GG) was associated with a 70% decrease in the odds of belonging to the higher evening fatigue class (Figure 2A). In the regression analysis for *TNFA* rs2229094, being heterozygous or homozygous for the rare C allele (TT versus TC+CC) was associated with a 3.75-fold increase in the odds of belonging to the higher evening fatigue class (Figure 2B).

Discussion

Findings from the present study support our prior work (Dhruva et al., 2013) that suggested that morning and evening fatigue are distinct but related symptoms. While we did find some overlap, different phenotypic and genotypic characteristics were associated with morning and evening fatigue. Among the phenotypic characteristics, we found that having a higher number of comorbid conditions was associated with more severe morning fatigue. In contrast, caring for children at home was associated with more severe evening fatigue. While these phenotypic differences warrant confirmation in future studies, they suggest that different biological and lifestyle factors are associated with diurnal variations in fatigue severity. The identification of distinct modifiable risk factors for morning and evening fatigue may lead to the development and testing of more targeted interventions.

This study is the first to identify genotypic differences in morning and evening fatigue. While the *TNFA* gene was associated with both morning and evening fatigue, we found that different SNPs were associated with morning (i.e., rs1800629, rs3093662) versus evening (i.e., rs2229094) fatigue. The presence of the rare allele in *TNFA* rs1800629, which is located in the promoter region of the gene, is known to alter gene expression. However, studies that evaluated the direction and magnitude of the changes in TNF-α due to the rare "A" allele have yielded conflicting results (Aouizerat et al., 2009; Kroeger, Carville, & Abraham, 1997; Kroeger, Steer, Joyce, & Abraham, 2000). In the current study, carrying the rare "A" allele decreased the odds of being categorized in the Moderate or High morning fatigue classes. Given the role that TNF-α plays in a number of inflammatory conditions (Bishehsari et al., 2012; Cerri et al., 2009; Y. H. Lee et al., 2008; Leung & Cahill, 2010; Raison et al., 2013), this finding suggests that changes in TNF-α may play an important role in the occurrence, severity, and maintenance of morning fatigue.

TNFA rs2229094 lies in the promoter region of *TNFA* as well as within an exon of lymphotoxin alpha *(LTA)*. This SNP results in a missense mutation (i.e., an amino acid change from cysteine to arginine) in *LTA*. In addition, this SNP occurs in a DNase 1 hypersensitivity region that may influence both *TNFA* and *LTA* gene expression (Encode Project Consortium et al., 2012). Previous research has identified associations between this polymorphism and increased risks for proliferative vitreoretinopathy (Rojas et al., 2010) and cancer (Gallicchio et al., 2008; Takei et al., 2008). In addition, research has found associations between the rare "C" allele and increased risks for coronary artery disease (Y. Liu et al., 2011) and type 2 diabetes (Mahajan et al., 2010) and it appears to be a systemic marker of inflammation (i.e., C-reactive protein; Mahajan et al., 2010). In the aggregate, findings across these studies suggest that the rare "C" allele in *TNFA* is associated with increases in inflammation.

Findings from the current study suggest that carriers of the rare “C” allele in *TNFA* rs2229094 have a 3.75-fold increase in the odds of being classified in the two higher morning fatigue classes. Findings from studies done to date suggest that the biology of *TNFA/LTA* is extremely complex (Gallicchio et al., 2008; Mahajan et al., 2010; Oikari et al., 2013). This complexity may be due, in part, to the fact that the SNP lies both in the promoter region of *TNFA* and within an exon of *LTA.* Future studies are needed to determine if the association between higher levels of morning fatigue and rs2229094 is due to functional changes in LTα, differential gene expression of TNF-α, or both these mechanisms.

In terms of evening fatigue, participants who were heterozygous or homozygous for the rare “G” allele at rs3093662 were 3.8 times more likely to be in the higher evening fatigue classes. This SNP lies in an intronic region of *TNFA*, and research has found it to be associated with inflammation and poorer outcomes in renal transplant patients (Israni et al., 2008). In addition, this SNP occurs in both a DNase I hypersensitivity region and in a region that is differentially methylated (Encode Project Consortium et al., 2012). Finally, this region is differentially bound by DNA polymerase, which is required for gene expression (Encode Project Consortium et al., 2012). Taken together, our findings suggest that TNF-α plays a role in the mechanisms that underlie both morning and evening fatigue. Further study may elucidate the role of this complex multifunctional pro-inflammatory cytokine in the development and maintenance of morning and evening fatigue.

The other genetic association with evening fatigue was for *IL4* rs2243248. This SNP lies within the promoter region of the *IL4* gene, which suggests that it may have functional significance. In the current study, carrying one or two doses of the rare G allele was associated with a 70% decrease in the odds of being in the higher evening fatigue classes. While factors such as the target cell influence its biological effect (Biedermann & Rocken, 2005), IL-4 is primarily an anti-inflammatory cytokine. In previous studies (Brenner et al., 2007; Erdei et al., 2010), the rare “G” allele of *IL4* rs2243248 was associated with an increased risk for glioma and breast cancer. In addition, the “G” allele was associated with higher lytic titers (i.e., causing viral infected cell lysis) in patients with HHV-8 infection (Brown et al., 2006). Our own group reported an association with the “G” allele and an increased risk for belonging to an “All High” symptom cluster subgroup (i.e., oncology patients and their FCs who reported high levels of pain, fatigue, sleep disturbance and depression; Illi et al., 2012). In addition, this SNP occurs in a region of the *IL4* gene that displays differential heterochromatin structure. This region is differentially bound by RNA polymerase, which is required for gene expression (Encode Project Consortium et al., 2012). Taken together, these data suggest that this SNP influences the expression of IL-4. Of note, in several studies (Atkins et al., 1992; Gilleece et al., 1992; Majhail et al., 2004; Taylor et al., 2000; Vokes, Figlin, Hochster, Lotze, & Rybak, 1998; Whitehead et al., 2002; Whitehead et al., 1998), the therapeutic administration of IL-4 resulted in fatigue, which suggests that the association of IL-4 with evening fatigue may have clinical implications.

Prior studies (Bower et al., 2009; L. Liu et al., 2012; Wang et al., 2010), including work from our group (C. Miaskowski et al., 2010) have suggested that IL-6 is associated with fatigue. While in the bivariate analyses, only one SNP in *IL6* was significant for morning fatigue (i.e., rs4719714) and one SNP in *IL6* was significant for evening fatigue (i.e.,

rs1800796), none remained significant in the multivariate analyses. Studies with larger samples may identify SNPs in *IL6* that are associated with diurnal variations in fatigue.

Several limitations are worth noting. The relatively small sample size precluded the examination of gene–gene interactions. In addition, the genetic associations identified in this study require validation in an independent cohort. Finally, serum cytokine levels were not measured. Future studies need to evaluate for serum cytokines and changes in gene expression associated with these polymorphisms.

Overall, the results of the current study suggest that polymorphisms in *TNFA* influence both morning and evening fatigue. However, only evening fatigue was associated with a polymorphism in *IL4*. Many cytokines display circadian variability in serum levels (Schubert et al., 2007). It may be that variability in cytokine genes leads to circadian variability in serum levels. Further research will help to clarify the relationships among circadian variability in serum cytokine levels, genetic variability, and the severity of morning and evening fatigue. In addition, while numerous studies have suggested a role for a number of pro- and anti-inflammatory cytokines in the development of fatigue (Bower, Ganz, Irwin, Kwan, et al., 2011; Bower et al., 2009; Dantzer et al., 2008; Myers, 2008), the fact that only two out of the fifteen candidate genes evaluated in the current study were associated with morning and/or evening fatigue suggests that additional mechanisms (e.g., neurotransmitter pathways) may be involved in this clinically significant symptom. We are evaluating this hypothesis in another study.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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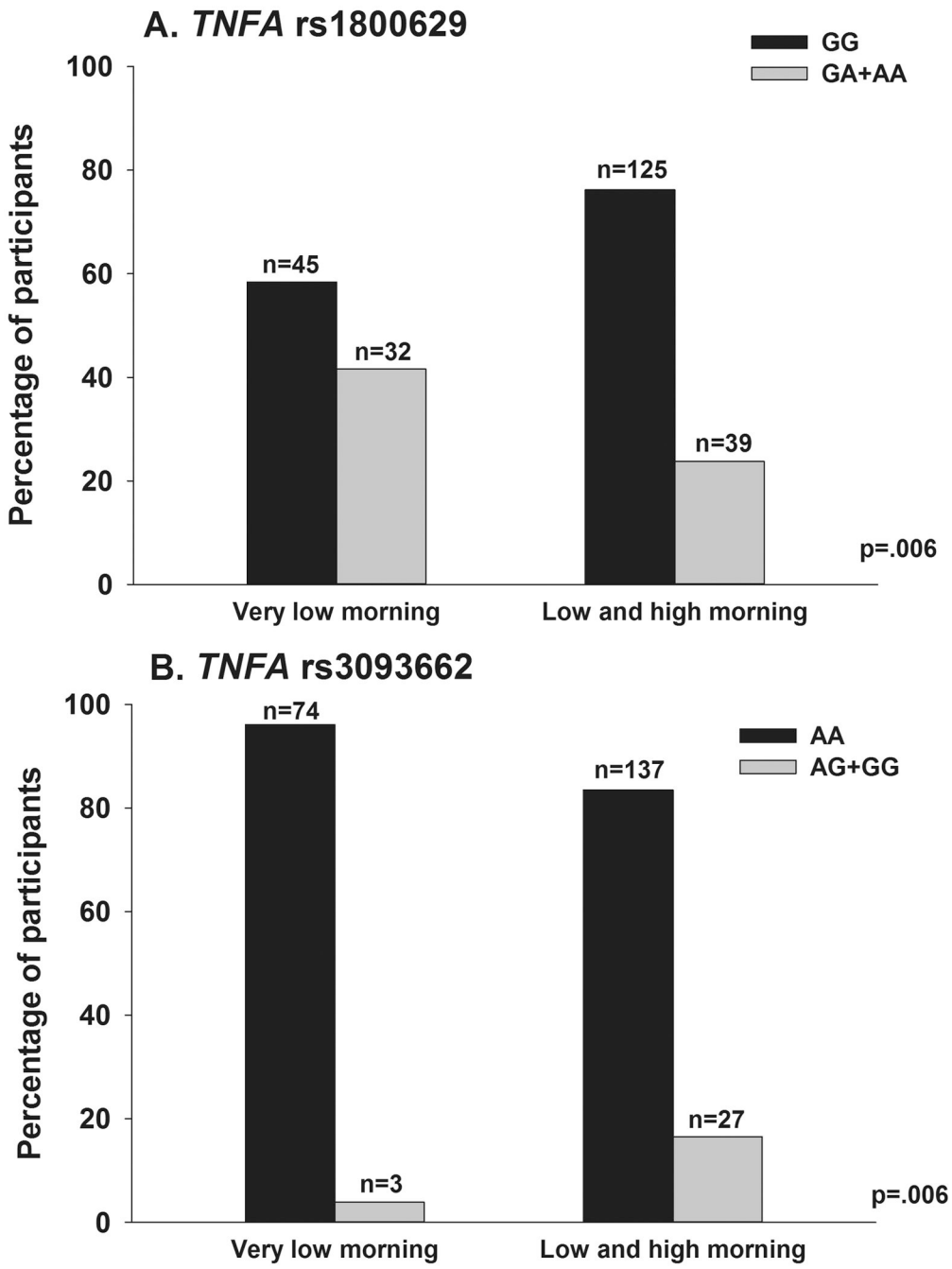

Figure 1.

Differences between the morning fatigue latent classes in the percentages of participants who were A) homozygous for the common allele (GG) or heterozygous or homozygous for the rare allele (GA+AA) for rs1800629 and B) homozygous for the common allele (AA) or heterozygous or homozygous for the rare allele (AG+GG) for rs3093662 in tumor necrosis factor alpha *(TNFA)*. Values are plotted as unadjusted proportions with corresponding *p*-value.

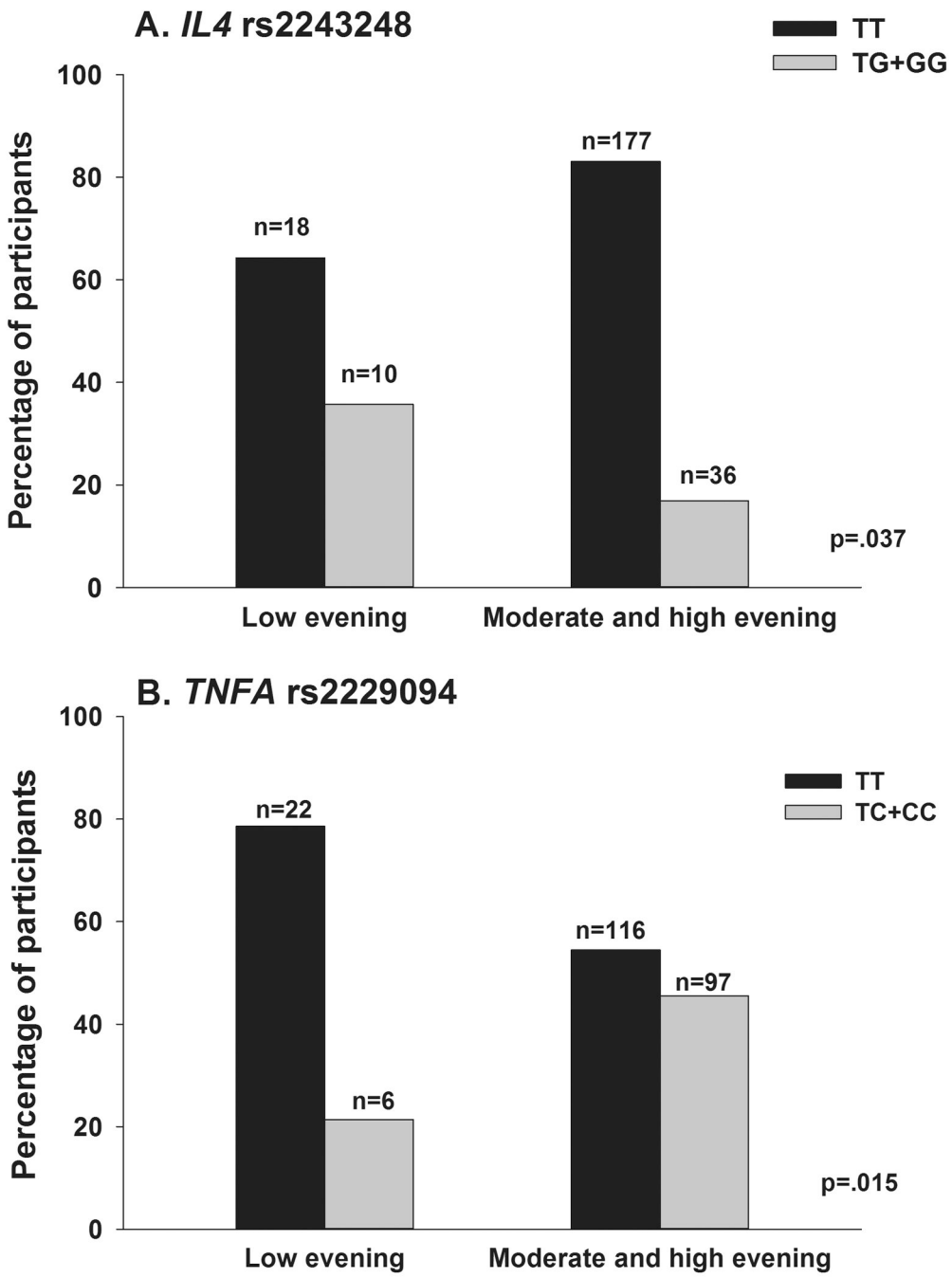

Figure 2.

Differences between the evening fatigue latent classes in the percentages of participants who were A) homozygous for the common allele (TT) or heterozygous or homozygous for the rare allele (TG+GG) for rs2243248 in interleukin 4 *(IL4)* and B) homozygous for the common allele (TT) or heterozygous or homozygous for the rare allele (TC+CC) for rs2229094 in tumor necrosis factor alpha *(TNFA)*. Values are plotted as unadjusted proportions with corresponding *p*-value.

Table 1

Differences in demographic and clinical characteristics at enrollment between participants (*N* = 167 patients and 85 FCs) by fatigue class

Characteristic	Very Low Morning Fatigue (*n* = *82*) Mean (SD)	Low and High Morning Fatigue (*n* = 170) Mean (SD)	Statistics for Morning Fatigue	Low Evening Fatigue (*n* = 28) Mean (SD)	Moderate and High Evening Fatigue (*n* = 224) Mean (SD)	Statistics for Evening Fatigue
Age (years)	64.9 (10.6)	59.0 (11.2)	$t = 3.36$, $p = .001$	66.5 (9.0)	60.9 (11.4)	$t = 2.52$, $p = .012$
Education (years)	15.8 (3.0)	16.0 (3.0)	NS	15.4 (3.2)	16.0 (3.0)	NS
Number of comorbid conditions	4.1 (2.7)	4.9 (2.7)	$t = -2.10$, $p = .029$	3.9 (2.9)	4.7 (2.8)	NS
Weight (lb)	178.5 (34.1)	173.6 (41.2)	NS	180.0 (36.3)	174.8 (39.5)	NS
KPS score	96.5 (7.9)	89.8 (12.4)	$t = 5.14$, $p < .0001$	95.7 (8.4)	91.4 (11.8)	$t = 2.40$, $p = .020$
	n (%)	*n* (%)		*n* (%)	*n* (%)	
Gender (female)	39 (47.6)	96 (56.5)	NS	11 (39.3)	124 (55.4)	NS
Ethnicity (White)	66 (80.5)	122 (71.8)	NS	19 (67.9)	169 (75.4)	NS
Lives alone (yes)	14 (26.4)	39 (34.2)	NS	10 (52.6)	43 (29.1)	NS
Married or partnered (yes)	61 (74.4)	113 (67.3)	NS	17 (60.7)	157 (70.7)	NS
Children at home (yes)	9 (12.9)	27 (19.1)	NS	0 (0.0)	36 (19.1)	FE = .017
Older adult at home (yes)	3 (4.3)	4 (2.8)	NS	2 (8.7)	5 (2.6)	NS
Works for pay (yes)	34 (42.0)	81 (48.8)	NS	10 (35.7)	105 (47.9)	NS
Patient/FC (patient)	53 (64.6)	114 (67.1)	NS	19 (67.9)	148 (66.1)	NS

Note. FC = family caregiver; FE = Fisher's Exact; KPS = Karnofsky Performance Status; NS = not significant; SD = standard deviation.

Table 2

Multiple logistic regression analyses for morning fatigue groups and *TNFA* candidate genes

Predictor	Odds Ratio	Standard Error	95% CI	*Z*	*p*-value
TNFA rs1800629	0.48	0.157	0.252, 0.910	-2.25	0.025
Age	0.79	0.063	0.673, 0.922	-2.97	0.003
KPS score	0.54	0.102	0.371, 0.782	-3.26	0.001
Overall model fit: $\chi^2 = 39.39$, $p < 0.0001$, $R^2 = 0.1342$					
TNFA rs3093662	6.59	4.369	1.796, 24.171	2.84	0.004
Age	0.76	0.062	0.645, 0.889	-3.39	0.001
KPS score	0.53	0.103	0.365, 0.780	-3.24	0.001
Overall model fit: $\chi^2 = 45.43$, $p < 0.0001$, $R^2 = 0.1548$					

Note. For each model, the first three principal components identified from the analysis of ancestry informative markers as well as self-reported race/ethnicity (White, Asian/Pacific Islander, Black, Hispanic/mixed background/other) were retained in all models to adjust for potential confounding due to race or ethnicity (data not shown). Predictors evaluated in the model included genotype (*TNFA* rs1800629: GG versus GA +AA; *TNFA* rs3093662: AA versus AG + GG), age (5-year increments), and functional status (KPS score, 10-point increments). CI = confidence interval; KPS = Karnofsky Performance Status; *TNFA* = tumor necrosis factor alpha gene.

Table 3

Multiple logistic regression analyses for evening fatigue groups and cytokine candidate genes

Predictor	Odds Ratio	Standard Error	95% CI	*Z*	*p*-value
IL4 rs2243248	0.30	0.143	0.120, 0.762	-2.54	0.011
Age	0.73	0.082	0.583, 0.910	-2.79	0.005
Overall model fit: $\chi^2 = 15.86$, $p = 0.04$, $R^2 = 0.0917$					
TNFA rs2229094	3.75	1.897	1.389, 10.110	2.61	0.009
Age	0.71	0.081	0.571, 0.891	-2.98	0.003
Overall model fit: $\chi^2 = 17.86$, $p = 0.02$, $R^2 = 0.1033$					

Note. For each model, the first three principal components identified from the analysis of ancestry informative markers as well as self-reported race/ethnicity (White, Asian/Pacific Islander, Black, Hispanic/Mixed background/other) were retained in all models to adjust for potential confounding due to race or ethnicity (data not shown). Predictors evaluated in the model included genotype (*IL4* rs2243248: TT versus TG+GG; *TNFA* rs2229094: TT versus TC+CC) and age (5-year increments). CI = confidence interval; *IL4* = interleukin 4 gene; *TNFA* = tumor necrosis factor alpha gene.