Preliminary Evidence of a Genetic Association Between Tumor Necrosis Factor Alpha and the Severity of Sleep Disturbance and Morning Fatigue

Bradley E. Aouizerat, PhD, Marylin Dodd, RN, PhD, FAAN, Kathryn Lee, RN, PhD, FAAN, Claudia West, RN, MS, Steven M. Paul, PhD, Bruce A. Cooper, PhD, William Wara, MD, Patrick Swift, MD, Laura B. Dunn, MD, and Christine Miaskowski, RN, PhD, FAAN

Although fatigue and sleep disturbance are prevalent symptoms in oncology patients and their family caregivers, little is known about the factors that contribute to interindividual variability in symptom severity ratings as well as in their underlying biological mechanisms. In this study, we sought to determine whether a functional genetic variation in a prominent proinflammatory cytokine, tumor necrosis factor-alpha (TNFA-308G>A [rs1800629] promoter polymorphism) was associated with overall ratings of sleep disturbance and fatigue as well as with the trajectories of these symptoms. Over 6 months, participants completed standardized measures of sleep disturbance and fatigue. Multiple linear regression was used to assess the effect of the TNFA genotype and other covariates on mean sleep disturbance and fatigue scores. Hierarchical linear modeling was used to determine the effect of TNFA genotype on the trajectories of these symptoms. Common allele homozygotes reported higher levels of sleep disturbance ($p = .09$) and morning fatigue ($p = .02$) than minor allele carriers. Multivariate analyses demonstrated that age and genotype were predictors of both mean symptom scores and the trajectories of these symptoms. Findings provide preliminary evidence of an association between a functional promoter polymorphism in the TNFA gene and the severity of sleep disturbance and morning fatigue in oncology patients and their family caregivers.

Keywords: fatigue; sleep disturbance; tumor necrosis factor; genetics; family caregiver; cancer

Fatigue and sleep disturbance are common and distressing symptoms in patients undergoing cancer treatment. Recent reviews have noted that the prevalence of fatigue ranges from 25% to 99% depending on the patient population surveyed, the type of assessment performed, and whether or not the prevalence rates for moderate and severe fatigue were reported (Bower, 2007; Hofman, Ryan, Figueroa-Moseley, Jean-Pierre, & Morrow, 2007; Levy, 2008; Stone & Minton, 2008). Likewise, sleep disturbance is a common but often undiagnosed problem in patients with cancer, with prevalence rates in the range of 24–95% (Graci, 2005; Lee, Cho, Miaskowski, & Dodd, 2004; Savard & Morin, 2001). Although these symptoms have deleterious effects on patients’ mood and quality of life (Lee et al., 2004; Reich, Lesur, & Perdrizet-Chevallier, 2008; Vistad, Fossa, Kristensen, & Dahl, 2007), little is known about their underlying mechanisms.

In addition, although most of the research on fatigue and sleep disturbance in family caregivers (FCs) has been done with FCs of patients with dementia (Hosaka & Sugiyama, 1999; McCurry,
Tumor necrosis factor-alpha (TNF-A) is a proinflammatory cytokine involved in the regulation of a wide spectrum of biological processes including neuroprotection (Bruce et al., 1996; Quintana et al., 2005; Sullivan et al., 1999), sleep (Cavadini et al., 2007; Krueger & Majde, 2003), depression (Anisman, Merali, Poulter, & Hayley, 2005; Hayley, Poulter, Merali, & Anisman, 2005; Raison, Capuron, & Miller, 2006), and sickness behavior (Dantzer & Kelley, 2007; Konsman, Parret, & Dantzer, 2002). In addition, a common functional promoter polymorphism (-308G>A) in the TNFA gene is associated with inflammatory diseases (Lee, Ji, & Song, 2007; Lee, Woo et al., 2008) and sleep disturbances (Riha et al., 2005; Vgontzas & Chrousos, 2002; Vgontzas et al., 1997, 2004). Finally, although a recent meta-analysis of the association between fatigue and inflammatory biomarkers did not show a relationship between fatigue severity and serum levels of TNF-A (Schubert, Hong, Natarajan, Mills, & Dimsdale, 2007), several studies have suggested that the effects of TNF-A differ depending on the amount of cytokine produced and whether its action is within the central or peripheral nervous system (Abraham & Kroeger, 1999; Konsman, Drukarch, & Van Dam, 2007; Konsman et al., 2002; Kroeger, Carville, & Abraham, 1997; Kroeger, Steer, Joyce, & Abraham, 2000).

Recently, we reported that considerable interindividual variability existed in the trajectories of fatigue in men who underwent radiation therapy (RT) for prostate cancer (Miaskowski et al., 2008) and in their spouse caregivers (Fletcher Schumacher et al., 2008). In addition, we found that oncology patients appear to vary in their innate susceptibility to experiencing symptoms with different levels of severity (Miaskowski et al., 2006; Pud et al., 2008). These findings have led us (Miaskowski & Aouizerat, 2007; Miaskowski et al., 2006; Pud et al., 2008) to suggest that the interindividual variability in fatigue and sleep disturbance that oncology patients and their FCs experience may be attributable in part to differences in their ability to respond to a variety of stressors with changes in proinflammatory cytokines and that these differences may be the result of genetic variation. In fact, recent work by Collado-Hidalgo and colleagues (Collado-Hidalgo, Bower, Ganz, Cole, & Irwin, 2006), in patients with a variety of other chronic medical conditions (Ackerman, Martino, Heyman, Moya, & Rabin, 1998; DeRijk et al., 1997; Papanicolaou, Wilder, Manolagas, & Chrousos, 1998; Vgontzas et al., 1997), and in FCs of patients with a variety of medical conditions (Glaser et al., 2001; Li et al., 2007; von Kanel, Dimsdale, Ancoli-Israel, et al., 2006; von Kanel, Dimsdale, Mills, et al., 2006; Wright et al., 2004). These changes in proinflammatory cytokines were associated in many cases with increased levels of depression, fatigue, and sleep disturbances.

Several lines of converging experimental and clinical evidence suggest that a variety of physical (e.g., inflammation, infection) and psychological (e.g., anxiety, depression, caregiver burden) stressors are associated with changes in neurohormonal and immune interactions and that many of these changes are mediated through proinflammatory cytokines (Calcagni & Elenkov, 2006; Elenkov, 2002; Elenkov & Chrousos, 2006; Elenkov, Iezzoni, Daly, Harris, & Chrousos, 2005; Glaser & Kiecolt-Glaser, 2005; Graham, Christian, & Kiecolt-Glaser, 2006; Leonard, 2006; McEwan, 2008; Steptoe, Hamer, & Chida, 2007). In fact, several studies have documented changes in proinflammatory cytokines in patients with cancer (Bower, 2007; Bower, Ganz, Aziz, & Fahy, 2002; Collado-Hidalgo, Bower, Ganz, Cole, & Irwin, 2006), in patients with a variety of other chronic medical conditions (Ackerman, Martino, Heyman, Moya, & Rabin, 1998; DeRijk et al., 1997; Papanicolaou, Wilder, Manolagas, & Chrousos, 1998; Vgontzas et al., 1997), and in FCs of patients with a variety of medical conditions (Glaser et al., 2001; Li et al., 2007; von Kanel, Dimsdale, Ancoli-Israel, et al., 2006; von Kanel, Dimsdale, Mills, et al., 2006; Wright et al., 2004). These changes in proinflammatory cytokines were associated in many cases with increased levels of depression, fatigue, and sleep disturbances.

Taken together, these converging lines of evidence suggest that a genetic variation in the TNFA gene might contribute to differences in the severity of fatigue and sleep disturbance experienced by patients and their FCs. In the study described here, we sought to determine in a sample of oncology patients and their FCs whether a functional genetic variation in TNFA was associated with overall ratings of sleep disturbance, evening fatigue, and morning fatigue as well as with the trajectories of these symptoms. We hypothesized that differences in sleep disturbance and fatigue would be associated with genetic variation in TNFA, as measured by the -308G>A functional promoter polymorphism.
Methods

Participants and Settings
For this longitudinal study, we recruited 288 participants (185 oncology outpatients with breast, prostate, lung, or brain cancer and 103 of their FCs). All patients met the following inclusion criteria: older than 18 years of age; able to read, write, and understand English; Karnofsky Performance Status (KPS) score of ≥60; and scheduled to receive primary or adjuvant RT. Patients were excluded if they had metastatic disease, had more than one cancer diagnosis, or had a diagnosed sleep disorder.

Following their recruitment, patients were asked to identify the person most involved in their care (i.e., their FC). FCs were eligible to participate if they were older than 18 years of age; were able to read, write, and understand English; had a KPS score of ≥60; were living with the patient; and did not have a diagnosed sleep disorder. Participants were recruited from RT departments located in a comprehensive cancer center and a community-based oncology program. This study was approved by the Institutional Review Boards at the University of California, San Francisco, and the community site.

Of the 472 patients approached, 185 consented to participate. The major reasons for refusal were being too overwhelmed with their cancer experience or too busy. In addition, 103 FCs consented to participate. Of the 288 participants, DNA could be recovered from the archived buffy coats of 253 (168 patients and 85 FCs). No differences were found in any demographic or clinical characteristics between patients who did and did not choose to participate in the study or in those participants for whom DNA could not be recovered from archived specimens.

Instruments
A demographic questionnaire provided information on age, gender, marital status, education, ethnicity, employment status, and presence of 26 comorbidities. In addition, participants completed the KPS score, which provides an indication of functional status (0 = dead to 100 = normal, no complaints or evidence of disease; Karnofsky, Abelmman, Craver, & Burchenal, 1948).

The General Sleep Disturbance Scale (GSDS) consists of 21 items that evaluate various aspects of sleep disturbance. Each item is rated on a scale that ranges from 0 (never) to 7 (every day). The 21 items are summed to yield a total score ranging from 0 (no disturbance) to 147 (extreme sleep disturbance). The GSDS has well established validity and reliability in shift workers, pregnant women, and patients with cancer and HIV (Humphreys, Lee, Neylan, & Marmar, 1999; Lee, 1992; Lee & DeJoseph, 1992; Lee, Portillo, & Miramontes, 2001; Miaskowski & Lee, 1999). In the current sample, the Cronbach’s alpha was .83.

A fatigue severity score was calculated as the mean of the 13 items on the Lee Fatigue Scale (LFS) and could range from 0 to 10, with higher scores indicating higher levels of fatigue. To assess diurnal variations in fatigue, participants completed the LFS within 30 min of awakening (morning fatigue) and prior to going to sleep (evening fatigue). The LFS has well established validity and reliability in healthy individuals and patients with cancer (Lee, Hicks, & Nino-Murcia, 1991; Miaskowski & Lee, 1999). In the current sample, the Cronbach’s alphas were .96 for morning and .94 for evening LFS ratings.

Study Procedures
At the time of the simulation visit, when the measurements are made for RT treatments (approximately 1 week prior to the start of RT), patients were approached by a research nurse to participate in the study. If the FC was present, the 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 study participation. The research nurse visited these FCs at home to complete the study procedures.

Participants completed the demographic questionnaire and the GSDS at this visit and had their blood drawn. In addition, they were taught to complete the LFS within 30 min of awakening and before going to bed each evening for two consecutive days. The LFS was to be completed 16 times—at the time of the simulation visit (i.e., baseline), weekly during the course of RT, and every 2 weeks for 2 months and once a month for 2 months following the completion of RT. The GSDS was to be completed 7 times—at baseline, at the midpoint of RT, at the end of RT, and once a month for 4 months after the completion of RT.

Genetic Analysis
DNA was amplified by whole genome amplification (GenomiPhi DNA Amplification Kit, GE Healthcare,
Piscataway, NJ) from archived buffy coat specimens. The TNFA-308G>A promoter polymorphism (rs 1800629) was screened by restriction assay (Duarte et al., 2005). Genomic DNA was amplified by polymerase chain reaction (PCR) using the manufacturer’s standard protocol. PCR products were subsequently size-fractionated on 5% polyacrylamide gel by electrophoresis, stained with ethidium bromide, and visualized by ultraviolet transillumination.

Statistical Analyses

Data were analyzed using SPSS version 15.0, Stata version 9.2, and hierarchical linear modeling (HLM) software (Raudenbush & Bryk, 2002; Raudenbush, Bryk, Cheong, & Congdon, 2004). Descriptive statistics and frequency distributions were generated on the sample characteristics and symptom severity scores. Independent sample t tests and Chi-square analyses were performed to evaluate for differences in demographic characteristics and mean symptom severity scores between patients and FCs.

The gene-counting method was used to determine allele and genotype frequencies. A test for deviation from Hardy-Weinberg expectation, a common indicator of genotype assay error, was evaluated by the goodness-of-fit χ² test (Gomes et al., 1999).

To estimate the association between TNFA gene variation and the average level of each symptom over the 6 months of the study, mean scores for sleep disturbance (i.e., GSDS scores) and for morning and evening fatigue (i.e., LFS scores) were calculated for 7 and 16 assessments, respectively. Independent sample t tests were used to evaluate for differences in mean GSDS scores and morning and evening LFS scores between genotype groups.

Multiple linear regression was used to assess the effect of TNFA genotype and other covariates on mean GSDS and morning and evening LFS scores. To model the effect of the nonindependence between patient and FC dyads, as compared with those patients who did not have an FC, a mixed effects model was fitted for each of the symptoms. Predictor variables included TNFA genotype, age, gender, ethnicity, patient versus FC status, and cancer diagnosis. Specific potential interactions, based on a biologic rationale or epidemiologic evidence, were examined between TNFA and the following variables: ethnicity, gender, and cancer diagnosis, as well as the interaction between age and gender, ethnicity and gender, and diagnosis and gender. Backwards stepwise selection was used to fit the model with the variable with the highest p value sequentially eliminated (with the exception of TNFA genotype because it was the primary variable of interest). Each predictor retained in the final model was required to have a p value of ≤.10. Although the criterion of p ≤ .05 is used as the formal threshold for significance for both the univariate and multivariate analyses, a p value of ≤.10 was adopted here in order not to miss a suggestive association between genotype and symptom severity score.

HLM, based on full maximum likelihood estimation, was used to evaluate the effects of TNFA genotype on the trajectories of sleep disturbance and fatigue (Raudenbush & Bryk, 2002; Raudenbush et al., 2004). With HLM, the repeated measures of the outcome variables (i.e., sleep disturbance, fatigue) are nested within individuals, and the analysis of change in symptom scores has two levels—within persons (Level 1) and between persons (Level 2). At Level 1, the outcome is conceptualized as varying within individuals and is a function of person-specific change parameters plus error. At Level 2, these person-specific change parameters are multivariate outcomes that vary across individuals. These Level 2 outcomes can be modeled as a function of demographic (e.g., age, gender), biologic (e.g., TNFA genotype), or clinical (e.g., number of comorbidities) characteristics that vary between individuals, plus an error associated with the individual. Combining Level 1 with Level 2 results in a mixed model with fixed and random effects (Li, 2005a, 2005b; Raudenbush & Bryk, 2002).

Separate two-stage HLM analyses were done to evaluate for changes over time in ratings of sleep disturbance and fatigue. First, intraperson variability in the symptom over time was examined. In this study, time in weeks refers to the length of time from the simulation visit to 4 months after the completion of RT (i.e., 6 months with a total of 7 or 16 assessments). Three Level 1 models, which represented that the patients’ symptom levels (a) did not change over time (i.e., no time effect), (b) changed at a constant rate (i.e., linear time effect), and (c) changed at a rate that accelerated or decelerated over time (i.e., quadratic effect) were compared. For Stage 1, the Level 2 model was constrained to be unconditional (i.e., no predictors) and likelihood ratio tests were used to determine the best model. These analyses identified the change parameters that best described individual changes in sleep disturbance and fatigue over time.

Stage 2 of the HLM analysis examined interindividual differences in the trajectories of sleep disturbance and fatigue by modeling the individual change
parameters (i.e., intercept, linear, and quadratic slopes) as a function of proposed predictors at Level 2. The predictors that were evaluated in the HLM analyses were those that were identified as significant in the regression analyses. Only predictors that maintained a significant contribution in conjunction with other variables were retained in the final model. Because these analyses were exploratory, a p value of < .10 was used to indicate statistical significance.

### Results

#### Participant Characteristics

As summarized in Table 1, the majority of participants were female, White, and well educated. No differences were found between patients and FCs in any demographic characteristics except gender and marital status. Compared to the patients, a higher percentage of the FCs was female and married/partnered.

#### Allelic and Genotypic Characteristics of the Sample

The frequency of the TNFA-308 A minor allele in the entire sample was 17.2%, which is consonant with population estimates available in public databases (dbSNP). The genotype distribution met Hardy-Weinberg expectations (p = .81). No gender differences were found in the distribution of TNFA-308 genotypes. Given the prohibitively low frequency of

---

**Table 1.** Comparison of the Demographic and Clinical Characteristics of Cancer Patients Undergoing Radiation Therapy and Family Caregivers

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Total Sample (n = 253) Mean (SD)</th>
<th>Patients (n = 168) Mean (SD)</th>
<th>Family Caregivers (n = 85) Mean (SD)</th>
<th>Statistical Tests</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>61.4 (11.3)</td>
<td>60.9 (11.6)</td>
<td>62.5 (10.5)</td>
<td>t = −1.03, p = .305</td>
</tr>
<tr>
<td>Education (years)</td>
<td>15.9 (3.0)</td>
<td>16.0 (2.9)</td>
<td>15.8 (3.2)</td>
<td>t = 0.56, p = .575</td>
</tr>
<tr>
<td>Number of comorbid conditions</td>
<td>4.6 (2.7)</td>
<td>4.8 (2.6)</td>
<td>4.2 (2.9)</td>
<td>t = 1.52, p = .131</td>
</tr>
<tr>
<td>Karnofsky performance score</td>
<td>92.0 (11.5)</td>
<td>91.1 (11.9)</td>
<td>93.7 (10.6)</td>
<td>t = −1.65, p = .100</td>
</tr>
<tr>
<td>Mean morning LFS score</td>
<td>2.4 (1.8)</td>
<td>2.4 (1.8)</td>
<td>2.4 (1.9)</td>
<td>t = 0.28, p = .783</td>
</tr>
<tr>
<td>Mean evening LFS score</td>
<td>4.6 (2.2)</td>
<td>4.5 (2.2)</td>
<td>4.8 (2.1)</td>
<td>t = −1.05, p = .296</td>
</tr>
<tr>
<td>Mean GSDS total score</td>
<td>39.4 (16.1)</td>
<td>40.7 (16.6)</td>
<td>37.0 (14.7)</td>
<td>t = 1.71, p = .089</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>%</th>
<th>%</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>46.2</td>
<td>55.4</td>
</tr>
<tr>
<td>Female</td>
<td>53.8</td>
<td>44.6</td>
</tr>
<tr>
<td>Race/ethnicity</td>
<td></td>
<td></td>
</tr>
<tr>
<td>African American</td>
<td>13.5</td>
<td>15.0</td>
</tr>
<tr>
<td>Asian or Pacific Islander</td>
<td>6.3</td>
<td>7.2</td>
</tr>
<tr>
<td>White</td>
<td>74.6</td>
<td>71.9</td>
</tr>
<tr>
<td>Other</td>
<td>5.6</td>
<td>5.9</td>
</tr>
<tr>
<td>Marital status</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Married/partnered</td>
<td>69.3</td>
<td>56.0</td>
</tr>
<tr>
<td>Other</td>
<td>30.7</td>
<td>44.0</td>
</tr>
<tr>
<td>Employment status: works for pay</td>
<td>46.4</td>
<td>47.0</td>
</tr>
<tr>
<td>Patient’s cancer diagnosis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Breast</td>
<td>32.8</td>
<td>38.1</td>
</tr>
<tr>
<td>Prostate</td>
<td>53.8</td>
<td>48.8</td>
</tr>
<tr>
<td>Lung</td>
<td>5.9</td>
<td>7.1</td>
</tr>
<tr>
<td>Brain</td>
<td>7.5</td>
<td>6.0</td>
</tr>
</tbody>
</table>

NOTE: Bold text indicates significant difference. GSDS = General Sleep Disturbance Scale; LFS = Lee Fatigue Scale.
the rare allele homozygote group \((n = 9)\), for all of the subsequent analyses, the TNFA genotypes were collapsed into 78 (31%) carriers of the rare allele \((GA + AA)\) genotypes and 175 (69%) common allele homozygotes \((GG)\).

### Differences in Mean Sleep Disturbance and Fatigue Scores

As shown in Table 1, no differences were found in mean morning LFS, evening LFS, and GSDS scores between patients and FCs. The mean symptom severity scores for morning and evening fatigue and sleep disturbance in the two genotype groups are illustrated in Figure 1A and B. Common allele homozygotes (i.e., \(GG\)) reported higher morning LFS scores \((t = -2.22, p = .02)\) and higher total GSDS scores \((t = -1.69, p = .09)\) than minor allele carriers (i.e., \(GA + AA\)), but there were no differences in evening fatigue scores \((t = -0.64, p = .52)\). Because no differences were found in evening fatigue scores, this variable was not included in any additional analyses.

### Multivariate Analysis of TNFA Genotype and Symptom Severity

Table 2 lists the results of the multiple regression analyses for sleep disturbance and morning fatigue.
For both symptoms, none of the interactions that were tested were significant.

In the model fitted for sleep disturbance (i.e., mean GSDS score), TNFA genotype and age were the only predictors retained in the final model ($p < .0008$). Controlling for age, common allele homozygotes had a mean GSDS score that was $3.91 \pm 2.10$ points higher than minor allele carriers (95% confidence interval CI: $-0.214, 8.027, p = .063$). Controlling for TNFA genotype, for every 1 year increase in age, mean GSDS score decreased by $-0.299 \pm 0.091$ points (95% CI: $-0.477, -0.121, p = .001$).

In the model fitted for morning fatigue (i.e., mean morning LFS score), TNFA genotype and age were the only predictors retained in the final model ($p < .0001$). Controlling for age, common allele homozygotes had a mean morning LFS score that was $0.521 \pm 0.225$ points higher than minor allele carriers (95% CI: $-0.080, -0.962, p = .021$). Controlling for TNFA genotype, for every 1 year increase in age, the mean morning LFS score decreased by $-0.055 \pm 0.010$ points (95% CI: $-0.075, -0.036, p < .0001$).

### Trajectories of Sleep Disturbance and Morning Fatigue

The first HLM analyses examined how sleep disturbance and morning fatigue levels changed from the time of the simulation visit to 4 months after the completion of RT. The second stage of the HLM analyses tested the hypothesis that the pattern of change over time in sleep disturbance and morning fatigue varied based on predictor variables that were identified in the regression analyses.

#### Sleep disturbance.

The estimates of the linear change model are presented in Table 3 (unconditional model). Because the model had no covariates (i.e., unconditional), the intercept represents the estimated amount of sleep disturbance (41.05 on a 0–107 scale) at the time of the simulation visit. The estimated linear rate of change in sleep disturbance for each week was $-0.144$ ($p < .0001$).

As shown in the final model in Table 3, the two variables that predicted interindividual differences in the slope for sleep disturbance were baseline level of sleep disturbance. To illustrate the effects of each of the predictors on participants’ trajectories of sleep disturbance, Figure 2 displays the adjusted change curves of sleep disturbance that were estimated based on differences in age (i.e., younger or older based on 1 standard deviation above and below the mean age of the participants), genotype (GG or GA/AA), and baseline level of sleep disturbance (i.e., lower sleep disturbance or higher sleep disturbance calculated based on 1 standard deviation above and below the mean baseline GSDS score). Figure 3 illustrates the effects of all three of the predictors on participants’ trajectories of sleep disturbance.

#### Morning fatigue.

The estimates of the quadratic change model are presented in Table 3 (unconditional model). Because the model had no covariates (i.e., unconditional), the intercept represents the estimated amount of morning fatigue (2.43 on a scale of 0–10) at the time of the simulation visit. The estimated linear rate of change in morning fatigue for each additional week was $0.035$ ($p \leq .01$), and the estimated quadratic rate of change per week was $-0.002$ ($p < .0001$). It is important to note that it is the weighted combination of the linear and quadratic terms that defines each curve.

As shown in the final model in Table 3, the two variables that predicted interindividual differences in the intercept for morning fatigue were age and genotype. Baseline morning fatigue was entered in Level 2 as a predictor of the slope parameters to control for interindividual differences in morning fatigue at baseline. The two variables that predicted interindividual differences in the slope parameters for morning fatigue were age and baseline level of morning fatigue.

To illustrate the effects of each of the predictors on participants’ trajectories of morning fatigue, Figure 4 displays the adjusted change curves of morning fatigue that were estimated based on differences in age (i.e., younger or older based on 1 standard deviation above and below the mean age of the participants), genotype (GG or GA/AA), and baseline level of morning fatigue (i.e., lower fatigue or higher fatigue calculated based on 1 standard deviation above and below the mean baseline morning LFS score). Figure 5 illustrates the effects of all three of the predictors on the participants’ trajectories of morning fatigue.
This study is the first to provide preliminary evidence of a genetic association between a functional promoter polymorphism in the TNFA gene and the severity of sleep disturbance and morning fatigue in a sample of oncology patients and their FCs. Carriers of the TNFA minor allele reported lower overall levels of sleep disturbance and morning fatigue. In addition, the various trajectories of morning fatigue and sleep disturbance associated with the TNFA minor allele were characterized by lower levels of symptom severity than the complementary trajectory associated with the common allele homozygotes.

For example, as illustrated in Figure 5, the worst fatigue trajectory over the 6 months of the study was associated with younger age, being homozygous for the common allele (GG), and having lower baseline morning fatigue scores compared to the trajectory associated with being younger, having a lower level of baseline fatigue, but being a carrier of the minor allele, GA/AA. Similar relationships are noted when...
complementary trajectories of morning fatigue and sleep disturbance are paired by the other two predictors (i.e., age and baseline level of symptom severity) and compared by genotype (i.e., \(GG\) vs. \(GA/AA\)). The fact that complementary trajectories were found rather than all of the trajectories associated with the homozygous common allele having the four worst overall symptom trajectories may provide evidence of genetic predisposition to experiencing higher levels of both sleep disturbance and morning fatigue.

These findings support our a priori hypothesis that a \(TNFA\) gene variation would be associated with differences in symptom severity. This hypothesis was based on findings from clinical and experimental studies in humans and animals that suggested that increased in vivo concentrations of \(TNFA\) resulted in more severe disease or increased symptoms (Cavadini et al., 2007; Cleeland et al., 2003; Dantzer & Kelley, 2006, 2007; Vgontzas et al., 1997). In addition, recent studies have found that the administration of competitive antagonists to \(TNFA\) results in decreased symptoms in patients with a number of chronic medical conditions including arthritis (Lee, Rho, Choi, Ji, & Song, 2006) and obstructive sleep apnea (Vgontzas et al., 2004).

It should be noted that while findings from in vitro studies suggest that the \(TNFA\) -308G>A polymorphism is functional and not simply a surrogate marker for a linked \(TNFA\) polymorphism, investigations of the direction and magnitude of the gene’s expression because of the minor allele have yielded conflicting results (Abraham & Kroeger, 1999; Kroeger et al., 1997, 2000). For example, a recent meta-analysis of the \(TNFA–308G>A\) polymorphism and susceptibility to rheumatoid arthritis concluded that this polymorphism may represent a significant risk factor for rheumatoid arthritis in Latin Americans but not in Europeans.

Figure 2. Influence of age (A) and genotype (B) on interindividual differences in the intercept for sleep disturbance, and influence of baseline level of sleep disturbance (C) on the slope parameter for sleep disturbance. GSDS = General Sleep Disturbance Scale.
Additional research is warranted to determine the mechanisms that underlie the higher levels of sleep disturbance and morning fatigue associated with the more common TNFA allele homozygotes.

Several other findings from this study are worth noting. First, the differences in mean morning fatigue scores between the TNFA minor allele carriers (GA + AA) and common allele homozygotes (GG) represent not only statistically but also clinically significant differences in fatigue based on calculations of effect sizes (i.e., $d = 0.25$). This conclusion is based on reports that suggest that minimally important differences in morning fatigue are in the range of 0.20–0.50 standard deviation units (Osoba, 1999; Sloan & Dueck, 2004). In addition, consistent with previous studies (Miaskowski et al., 2006; Pud et al., 2008), regardless of genotype, younger age was associated with more fatigue and sleep disturbance.

Another interesting finding is that no differences in mean fatigue and sleep disturbance scores were found between patients and FCs. In both groups, evening fatigue and sleep disturbance scores were in the moderate range. This finding adds support to the idea that both patients and FCs are affected by the cancer experience and require support with symptom management (Fletcher et al., 2008; Passik & Kirsh, 2005). Finally, it is not clear why evening fatigue was not associated with the TNFA genotype. Additional studies are needed to determine if different cytokines or combinations of cytokines are involved in the development of evening fatigue as well as the genetic markers that may predict the development of evening fatigue.

Several limitations of this study need to be acknowledged. Because of the relatively small sample size, additional research is warranted to validate this
candidate gene association in a large, independent sample and to test for Gene × Environment interactions, as well as for Gene × Gene interactions (i.e., epistasis). In addition, the measurement of plasma TNFA levels may allow for more definitive conclusions to be drawn about the association between TNFA genotype and symptom severity. This type of analysis might help to determine the underlying mechanisms for sleep disturbance and morning fatigue in oncology patients and FCs.

In conclusion, these findings suggest that the identification of genetic determinants that modulate sleep disturbance and morning fatigue may be applicable not only to patients with a chronic illness like cancer but also to individuals in the general population that experience symptoms as a result of stressful situations (e.g., being an FC of an oncology patient). Additional research on the association between a variety of other candidate genes and symptoms is warranted to test this hypothesis.

**Acknowledgments**

This research was supported by a grant from the National Institute of Nursing Research (NINR, NR04835). Dr. Aouizerat is funded through the National Institutes of Health Roadmap for Medical Research Grant (K12RR023262). Dr. Dunn is supported in part by funds from the Mount Zion Health
Fund. Additional support for the corresponding author’s program of research was provided through unrestricted grants from Endo Pharmaceuticals, PriCara Unit of Ortho-McNeil Inc., and Purdue Pharma LP.

References


Bruce, A. J., Boling, W., Kindy, M. S., Peschon, J., Kraemer, P. J., Carpenter, M. K. et al. (1996). Altered neuronal and microglial responses to excitotoxic and

Figure 5. Trajectories of morning fatigue by age, genotype, and baseline level of morning fatigue.
ischemic brain injury in mice lacking TNF receptors. Nature Medicine, 2, 788-794.


Wright, R. J., Finn, P., Contreras, J. P., Cohen, S., Wright, R. O., Staudenmayer, J. et al. (2004). Chronic caregiver stress and IgE expression, allergen-induced proliferation, and cytokine profiles in a birth cohort predisposed to atopy. *Journal of Allergy and Clinical Immunology*, 113, 1051-1057.