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Inflammatory Pathway Genes Associated with Inter-Individual Variability in the Trajectories of Morning and Evening Fatigue in Patients Receiving Chemotherapy

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Abstract

Fatigue, a highly prevalent and distressing symptom during chemotherapy (CTX), demonstrates diurnal and interindividual variability in severity. Little is known about the associations between variations in genes involved in inflammatory processes and morning and evening fatigue severity during CTX. The purposes of this study, in a sample of oncology patients (N=543) with breast, gastrointestinal (GI), gynecological (GYN), or lung cancer who received two cycles of CTX, were to determine whether variations in genes involved in inflammatory processes were associated with inter-individual variability in initial levels as well as in the trajectories of morning and evening fatigue. Patients completed the Lee Fatigue Scale to determine morning and evening fatigue

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severity a total of six times over two cycles of CTX. Using a whole exome array, 309 single nucleotide polymorphisms among the 64 candidate genes that passed all quality control filters were evaluated using hierarchical linear modeling (HLM). Based on the results of the HLM analyses, the final SNPs were evaluated for their potential impact on protein function using two bioinformational tools. The following inflammatory pathways were represented: chemokines (3 genes); cytokines (12 genes); inflammasome (11 genes); Janus kinase/signal transducers and activators of transcription (JAK/STAT, 10 genes); mitogen-activated protein kinase/jun amino-terminal kinases (MAPK/JNK, 3 genes); nuclear factor-kappa beta (NFkB, 18 genes); and NFkB and MAP/JNK (7 genes). After controlling for self-reported and genomic estimates of race and ethnicity, polymorphisms in six genes from the cytokine (2 genes); inflammasome (2 genes); and NFkB (2 genes) pathways were associated with both morning and evening fatigue. Polymorphisms in six genes from the inflammasome (1 gene); JAK/STAT (1 gene); and NFkB (4 genes) pathways were associated with only morning fatigue. Polymorphisms in three genes from the inflammasome (2 genes) and the NFkB (1 gene) pathways were associated with only evening fatigue. Taken together, these findings add to the growing body of evidence that suggests that morning and evening fatigue are distinct symptoms.

Keywords

inflammation; genes; fatigue; hierarchical linear modeling; diurnal variability; cancer; chemotherapy

1. Introduction

During chemotherapy (CTX), over 45% of patients experience clinically meaningful levels of fatigue that decrease their ability to tolerate treatments, engage in social relationships, and maintain regular work activities [1]. However, a growing body of evidence demonstrates that inter-individual variability exists in fatigue severity across cancer diagnosis [2–4] and treatments [5, 6]. In addition, recent work from our group [7–11] and others [4, 12] demonstrates that morning and evening fatigue are distinct yet related symptoms. Some of this inter-individual variability is explained by different phenotypic characteristics that distinguish between higher levels of morning (e.g., higher body mass index (BMI), lack of regular exercise, higher state anxiety) and evening (e.g., being white, higher years of education, child care responsibilities) fatigue [9, 10]. In addition to phenotypic differences, preliminary evidence suggests that variations in cytokine genes are associated with inter-individual differences in morning (e.g., tumor necrosis factor alpha (*TNFA*) [13] and evening (e.g., interleukin (*IL*)*4* [14] and *IL6* [15]) fatigue severity.

While considered to be multi-factorial, a growing body of evidence suggests that cytokine dysregulation, as well as many other neuroinflammatory processes may modulate fatigue severity in a number of chronic conditions [16–19]. Increased knowledge of the mechanisms that underlie fatigue is essential for the development of effective treatments for this devastating symptom. However, no definitive conclusions can be drawn from studies that evaluated associations between fatigue severity and various biomarkers of cytokine dysregulation (for reviews see [18, 19]).

1.1 Associations between fatigue and serum markers of inflammation

To examine this cytokine dysregulation hypothesis, several studies evaluated the associations between fatigue severity and serum cytokine levels. To date, results are inconclusive, with some studies finding positive associations between fatigue severity and circulating levels of TNF-α [20, 21] and IL-6 [18, 20, 22–29] and others finding no associations with TNF-α[18, 22, 30–33], IL-6 [30, 34–38], and IL-4 [25, 36, 38]. These inconsistent results may be related to the challenges associated with the measurement of serum cytokines, as well as circadian variations in cytokine levels [39].

An alternative approach is to measure circulating levels of biomarkers of immune activation (e.g., cellular receptors) [40]. Again, these results are inconclusive. Some studies found positive associations between fatigue and changes in serum levels of IL-1 receptor antagonist (IL-1ra)[24, 35, 41, 42], soluble TNF receptor II (sTNF-RII) [41, 43, 44], sTNF-RI [45], and sIL-6R [37, 46, 47]. However, other studies found no associations between fatigue severity and changes in serum levels of IL-1ra [27, 34, 36], sTNF-RII [36, 42], and sIL-6R [46]. Of note, none of these studies evaluated for associations between diurnal variations in fatigue severity and changes in these serum markers.

1.2 Changes in gene expression and fatigue

Another approach to examine the role of inflammation in fatigue is to evaluate for changes in the expression of inflammatory genes. To date, seven studies have evaluated for changes in gene expression associated with fatigue severity in oncology patients [34, 48–53]. Five of these studies [34, 48, 50, 52, 53] examined changes in gene expression related to inflammation/immune function. In four of these studies higher levels of fatigue were associated with upregulation of genes that regulate cytokine production (i.e., interferon alpha-inducible protein 27 (*IFI27*) [48], α-synuclein (*SNCA*) [52], *IL1* [34], *IL6* [34], *IL4* [50]). In another study [53], differentially perturbed cytokine pathways were associated with higher levels of evening fatigue. However, across these studies only mean or evening fatigue scores were evaluated. In addition, the sample sizes for these studies were relatively small (i.e., 15 [49] to 137 [50] patients).

1.3 Associations between fatigue and variations in cytokine genes

A third approach that can be used to examine the role of inflammation in fatigue is to evaluate for associations between fatigue severity and variations in cytokine genes. Single nucleotide polymorphisms (SNP) in *TNFA* [54, 55], *IL6* [37, 56, 57], and *IL1RA* [58] were associated with increased levels of fatigue. To date, only three studies evaluated for associations between variations in cytokine genes and diurnal variations in fatigue severity [13–15]. In a study of oncology patients (n=185) and their family caregivers (n=103), SNPs in *TNFA* (i.e., rs1800629, rs3093662) and *IL6* (i.e., rs4719714) were associated with higher levels of morning and evening fatigue [13–15]. Additionally, a polymorphism in *IL4* rs2243248 was associated with lower levels of evening fatigue [14]. While the studies cited above provide preliminary evidence that variations in cytokine genes are associated with diurnal variations in fatigue severity, two of the studies evaluated only one polymorphism [13–15] and none of them evaluated oncology patients undergoing CTX.

While evidence exists for the role of cytokine dysregulation as a modulator of neuroinflammation, recent studies found other pathways and processes that contribute to the development of inflammation (e.g., the mitogen-activated protein kinase (MAPK) pathway [59], and inflammasomes [19, 60]). However, the contribution of these pathways to fatigue severity in oncology patients undergoing CTX has not been evaluated. Increased knowledge of whether additional inflammatory pathways are associated with diurnal variations in fatigue severity would enhance our understanding of the various mechanisms that contribute to this devastating symptom.

Recently, we identified common and distinct phenotypic characteristics for morning [9] and evening [10] fatigue severity in oncology patients undergoing CTX. This study extends these findings to identify associations between variations in genes associated with a variety of inflammatory processes and the severity of morning and evening fatigue. Since genes interact with one another [61], the polymorphisms that were evaluated were grouped into common inflammatory pathways to provide insights into the role of related genes and the severity of morning and evening fatigue. The purposes of this study, in a sample of oncology patients with breast, gastrointestinal (GI), gynecological (GYN), or lung cancer who received two cycles of CTX, were to determine whether variations in genes involved in inflammatory processes were associated with inter-individual variability in initial levels as well as in the trajectories of morning and evening fatigue.

2. Methods

2.1 Patients and settings

Some of the details of the phenotypic [9–11, 62] and genotypic [63, 64] methods used in this study are published elsewhere. In brief, patients were recruited from two comprehensive cancer centers, one Veteran's Affairs hospital, and four community-based oncology programs. Patients with a diagnosis of breast, GI, GYN, or lung cancer were eligible to participate if they were ≥18 years of age; had received CTX within the previous four weeks; were scheduled to receive at least two additional cycles of CTX; were able to read, write, and understand English; and gave written informed consent.

2.2 Instruments

Patients completed a demographic questionnaire, the Karnofsky Performance Status (KPS) scale [65], and the Self-Administered Comorbidity Questionnaire (SCQ) [66]. In addition, patients completed a number of questionnaires to evaluate anxiety [67], depression [68], and sleep disturbance [69].

Fatigue was evaluated using the 18 item Lee Fatigue Scale (LFS) that assesses physical fatigue and energy [70]. Each item was rated on a 0 to 10 numeric rating scale (NRS). Total fatigue and energy scores were calculated as the mean of the 13 fatigue and the 5 energy items, with higher scores indicating greater fatigue severity and higher levels of energy, respectively. Using separate LFS questionnaires, patients were asked to rate each item based on how they felt within 30 minutes of awakening (i.e., morning fatigue and morning energy) and prior to going to bed (i.e., evening fatigue and evening energy). The LFS has established

cutoff scores for clinically meaningful levels of fatigue (i.e., ≥3.2 for morning fatigue, ≥5.6 for evening fatigue) [70]. The LFS is easy to administer, relatively short, and has well established validity and reliability [70]. As noted in previous reports (9,10), the Cronbach's alphas were .95 for evening fatigue, .95 for morning fatigue, .93 for evening energy, and .95 for morning energy.

2.3 Study Procedures

Each of the sites' Institutional Review Board approved the study. All patients provided written informed consent. Patients completed study questionnaires in their homes a total of six times over two cycles of CTX (prior to CTX administration (i.e., recovery from previous CTX cycle, assessments 1 and 4), approximately 1 week after CTX administration (i.e., acute symptoms, assessments 2 and 5), approximately 2 weeks after CTX administration (i.e., potential nadir, assessments 3 and 6)).

3. Genomic Analyses

3.1 Blood collection and genotyping

Genomic deoxyribonucleic acid (DNA) was isolated from peripheral blood mononuclear cells (PBMCs), using the PUREGene DNA Isolation System (Invitrogen, Carlsbad, CA). DNA was quantitated with a Nanodrop Spectrophotometer (ND-1000) and normalized to a concentration of 50 nanograms/microliter (diluted in 10 mM Tris/1 mM EDTA). Genotyping was performed using the HumanExome Array-12 v1.1 on the Infinium Beadchip genotyping platform which provides focused coverage in the coding regions (i.e., exons) of genes (Illumina, San Diego, CA). Data were processed according to the standard protocol using GenomeStudio (Illumina, San Diego, CA).

3.2 Candidate gene and SNP selection

Candidate genes were selected based on evidence in the literature of an association between each gene and fatigue. SNPs representing these genes were selected from the genome-wide SNP array.

Quality control filtering excluded SNPs with call rates of <95%. SNPs with less than (i.e., monomorphic) or more than (i.e., tri- or tetra-allelic) two alleles were excluded. Allele counts at different loci were assumed to be independent. The 309 SNPs among the 64 candidate genes that passed all quality control filters and whose occurrence rates were evaluated in this sample are listed in Supplemental Table 1. The genes are grouped within their common inflammatory pathways based on a review of the literature as well as the description of each gene's function and pathway found in the National Center for Biotechnology Information (NCBI) gene database and pathcards (http://pathcards.genecards.org). The following inflammatory pathways are represented: chemokines (3 genes); cytokines (12 genes); inflammasome (11 genes); Janus kinase/signal transducers and activators of transcription (JAK/STAT, 10 genes); mitogen-activated protein kinase/jun amino-terminal kinases (MAPK/JNK, 3 genes); nuclear factor-kappa beta (NFkB, 18 genes); and NFkB and MAP/JNK (7 genes).

4. Statistical analyses

4.1 Demographic and clinical data

The sample's demographic and clinical characteristics and symptom severity scores at enrollment were determined with descriptive statistics and frequency distributions. These analyses were done using the Statistical Package for the Social Sciences (SPSS) version 22 [71].

4.2 Genetic data

Gene counting determined allele and genotype frequencies. To be included in subsequent evaluations, each SNP needed to have a total of six occurrences of the rare allele (i.e., heterozygous or homozygous) in order not to over- or under- estimate the effect of the rare allele. After applying this criterion, 93 SNPs among 49 genes were evaluated as potential predictors of inter-individual variability in morning and evening fatigue. Liability scores composed of the number of rare allele occurrences across all SNPs for each candidate gene were generated by summing the number of rare alleles carried by each patient.

To minimize confounding due to population stratification, ancestry informative markers (AIMs) identified with principal component (PC) analysis were used in subsequent analyses [72–75]. Approximately 3,468 AIMS were included in this analysis. To adjust for potential confounding due to population substructure (i.e., race/ethnicity) the first three PCs were included as covariates in the hierarchical linear modeling (HLM) analyses.

4.3 HLM Analysis

Details of the HLM analysis are published elsewhere [9, 10]. In brief, HLM based on full maximum likelihood estimation was performed in two stages to evaluate the effects of individual SNPs and liability scores on initial levels as well as on changes over time in the severity of morning and evening fatigue [76]. Morning and evening fatigue were evaluated in separate HLM analyses. Since the six assessments encompassed two cycles of CTX, a piecewise model strategy was employed to evaluate the pattern of change in morning and evening fatigue over time. The first piece (PW1) modeled change over time during the first CTX cycle (i.e., Assessments 1, 2, and 3). The second piece (PW2) modeled change during the second CTX cycle (i.e. Assessments 4, 5, and 6).

Then, inter-individual differences in the piecewise trajectories of morning and evening fatigue were examined by modeling the individual change parameters (i.e., intercept and slope parameters) as a function of proposed predictors at level 2. First, each of the SNPs and liability scores that passed the quality control filters was evaluated in an exploratory analysis to determine whether it would result in a better fitting model if it alone was added as a predictor. To improve estimation efficiency and construct a parsimonious model, SNP predictors with a t value of <2.0 were excluded from subsequent model testing.

Each of the SNPs and liability scores, identified in the exploratory analyses (Tables 1 and 2), were entered into the model that controlled for self-reported and genomic estimates of race and ethnicity to predict each individual change parameter in morning or evening fatigue

severity. Only SNPs that maintained a statistically significant contribution were retained in the final models. A p-value of <.05 indicated statistical significance.

Consistent with our previous studies [14, 63, 77–80], recommendations from the literature [81, 82], rigorous quality controls for genomic data and the exploratory nature of our analyses, adjustments were not made for multiple testing. Since significant SNPs and liability scores identified in the exploratory analysis were evaluated further in the HLM analyses that controlled for population stratification (i.e., genomic and self-reported estimates of race and ethnicity), and other variations in the same gene, the significant independent genetic associations reported are unlikely to be due solely to chance.

4.4 Estimation of polymorphism function

Based on the results of the HLM analyses, the SNPs associated with inter-individual differences in the initial levels or trajectories of morning or evening fatigue were evaluated for their potential impact on protein function using two bioinformational tools (i.e., Sorting Intolerant From Tolerant (SIFT) algorithm [83] and Polymorphism Phenotyping v2 (PolyPhen-2) [84]). SIFT predicts whether a SNP in a coding region results in an amino acid substitution that may affect protein function. This prediction is based on an analysis of the conservation of amino acid residues in sequence alignments of closely related sequences [84]. PolyPhen-2 compares the SNP to sequence-based and structure-based predictive features to predict the functional significance of the SNP [84]. Results from the bioinformational tools are described in the discussion.

5. Results

5.1 Sample characteristics

As summarized in Table 3, of the 543 patients in the study, the majority of the patients were female, white, diagnosed with breast cancer, and were treated with CTX on a 21-day cycle. Most patients were well educated, married or partnered, and currently not employed. At enrollment, patients reported clinically meaningful sleep disturbance and anxiety levels. Morning and evening fatigue scores at enrollment were just below the cutoff for clinically meaningful levels (i.e., ≥3.2 for morning fatigue, ≥5.6 for evening fatigue) [70].

5.2 Changes in morning fatigue severity

HLM was used to examine how morning fatigue scores changed within the two cycles of CTX, controlling for self-reported and genomic estimates of race and ethnicity. The estimates for the initial piecewise model are presented in Table 4. Since the model was unconditional (i.e., no covariates included in the model), the average morning fatigue severity score at enrollment (i.e., 3.011 on a 0 to 10 scale) represents the intercept. The estimated linear piecewise rates of change were 1.192 and 0.532 (both p<.0001) for piecewise linear 1 and piecewise linear 2, respectively. The estimated quadratic piecewise rates of change were −.599 and −.153 (both p<.0001) for piecewise quadratic 1 and piecewise quadratic 2, respectively. Figure 1A displays the unconditional model for mean morning fatigue scores over the two cycles of CTX.

5.3 Genomic predictors of inter-individual differences in morning fatigue

Table 4 shows the final HLM models for morning fatigue. For the cytokine genes, two SNPs were associated with inter-individual differences in morning fatigue. Figure 1B illustrates the adjusted initial level of morning fatigue (i.e., intercept) based on a recessive model for *IL12B* rs3213094 (i.e., TT+TC vs. CC). Figure 1C illustrates the predicted changes in the trajectory of morning fatigue (i.e., slope) based on a dominant model for *TNFA* rs1041981 (i.e., CC vs. CA+AA).

For the inflammasome pathway genes, three SNPs were associated with changes in morning fatigue. Figure 1D illustrates the adjusted initial levels of morning fatigue based on the recessive model for nucleotide-binding oligomerization domain containing 2 (*NOD2*) rs2076756 (i.e., AA+AG vs. GG). Figures 1E and 1F respectively, illustrate the predicted changes in the trajectories of morning fatigue based on the recessive model for non-like receptor family, pyrin containing domain 5 (*NLRP5*) rs471979 (i.e., GG+GC vs. CC) and a dominant model for *NLRP6* rs74044411 (i.e., TT vs. TC+CC).

For the JAK/STAT pathway, the liability score for all *IL4R*, was associated with inter-individual differences in the slope of morning fatigue (Figure 2A).

For the NFkB pathway genes, two SNPs and four liability scores were associated with inter-individual differences in morning fatigue. Figure 2B illustrates the adjusted initial level of morning fatigue based on the dominant model for tumor necrosis factor receptor super family, member 14 (*TNFRSF14*) rs2234163 (i.e., AA vs. AG+GG). Figures 2C and 2D respectively, illustrate the adjusted initial levels of morning fatigue based on the liability scores for *IL17RB* and *TNFRSF21*. Figures 3A, 3B, and 3C, respectively, illustrate the predicted changes in the trajectories of morning fatigue for the additive model for *TNFRSF10A* rs17620 (i.e., TT vs. TC vs. CC) and the liability scores for *TNFRSF10D* and *TNFRSF11A*.

5.4 Changes in evening fatigue severity

HLM was used to examine how evening fatigue scores changed within the two cycles of CTX, controlling for self-reported and genomic estimates of race and ethnicity. The estimates for the initial piecewise model are presented in Table 5. Since the model was unconditional (i.e., no covariates), the intercept represents the average evening fatigue severity score at enrollment (i.e., 5.310 on a 0 to 10 scale). The estimated linear piecewise rates of change were 0.601 and 0.394 (both, $p<.0001$) for piecewise linear 1 and piecewise linear 2, respectively. The estimated quadratic piecewise rates of change were −.306 and −.113 (both, $p<.0001$) for piecewise quadratic 1 and piecewise quadratic 2, respectively. Figure 4A displays the unconditional model for mean evening fatigue scores over two cycles of CTX.

5.5 Genomic predictors of inter-individual differences in evening fatigue

Table 5 shows the final HLM models for evening fatigue. For the cytokine genes, two SNPs and one liability score were associated with inter-individual differences in evening fatigue. Figures 4B and 4C respectively, illustrate the adjusted initial level of evening fatigue based

on the recessive model for *IL12B* rs3213094 (i.e., TT+TC vs. CC) and the liability score for *IL12B*. Figure 4D illustrates the predicted changes in the trajectories of evening fatigue for a dominant model for *TNFA* rs1041981 (i.e., CC vs. CA+AA).

For the inflammasome pathway genes, four SNPs were associated with evening fatigue. Figures 5A, 5B, and 5C respectively, illustrate the adjusted initial levels of evening fatigue based on the additive model for capsase recruitment domain family member 6 (*CARD6*) rs10512747 (i.e., TT vs. TC vs. CC); the dominant model for *NLRP4* rs17857373 (i.e., CC vs. CG+GG) and the recessive model for *NOD2* rs2076756 (i.e., AA+AG vs. GG). Figure 5D illustrates the predicted changes in the trajectory of evening fatigue based on the dominant model for NLRP6 rs74044411 (i.e., TT vs. TC+CC).

For the MAP/JNK pathway genes, Figure 5E illustrates the adjusted initial level of evening fatigue based on the dominant model for *IL17RD* rs61742267 (i.e., AA vs. AG+GG).

For the NFkB pathway genes, three SNPs and one liability score were associated with inter-individual differences in evening fatigue. Figure 6A illustrates the adjusted initial level of evening fatigue based on the dominant model for *IL17RB* rs2232346 (i.e., TT vs. TC+CC) and the recessive model for *IL17RB* rs1043261 (i.e., TT+TC vs. CC). Figures 6B and 6C respectively, illustrate the adjusted initial level of evening fatigue based on the dominate model for *TNFRSF14* rs2234163 (i.e., AA vs. AG+GG) and the predicted change in the trajectory of evening fatigue based on the liability score for lymphotoxin beta receptor (*LTBR*).

6. Discussion

In our prior studies [9, 10], common and unique phenotypic predictors of morning and evening fatigue were identified that provided evidence that they are distinct but related symptoms. As summarized in Table 6, this study extends these findings by identifying common as well as unique genetic associations for morning and evening fatigue. Controlling for self-reported and genomic estimates of race and ethnicity, five SNPs were associated with inter-individual variability in both morning and evening fatigue. Three SNPs (i.e., *NOD2* rs2076756, *TNFRSF14* rs2234163, *IL12B* rs3213094) were associated with changes in the initial levels and two SNPs (i.e., *NLRP6* rs74044411, *TNFA* rs1041981) were associated with the trajectories of morning and evening fatigue severity. Two unique polymorphisms (i.e., *NLRP5* rs471979, *TNFRSF10A* rs17620) and five liability scores (i.e., *IL4R, IL17RB, TNFRSF10D, TNFRSF11A, TNFRSF21*) were associated with only morning fatigue. Five unique polymorphisms on four genes (i.e., *CARD6* rs10512747, *IL17RB* rs2232346, *IL17RB* rs1043261, *IL17RD* rs61742267, *NLRP4* rs17857373) and two liability scores (i.e., *IL12B, LTBR*) were associated with only evening fatigue.

In terms of the genes themselves, as summarized in Table 6, polymorphisms in six genes (i.e., *TNFA, IL12B, NLRP6, NOD2, TNFRSF14, IL17RB*) were associated with both morning and evening fatigue. Polymorphisms in six genes (i.e., *NLRP5, IL4R, TNFRSF10A, TNFRSF10D, TNFRSF11A, TNFRSF21*) were associated with only morning fatigue. Polymorphisms in three genes (i.e., *NLRP6, NLRP4, LTBR*) were associated with

only evening fatigue. Taken together, these findings add to the growing body of evidence that suggests that morning and evening fatigue are distinct but related symptoms.

One of the goals of this study was to evaluate the effects of polymorphisms in genes involved in inflammation on initial levels as well as on the trajectories of morning and evening fatigue. As noted in the introduction, while most studies evaluated for associations between cytokine dysregulation and fatigue, additional pathways are involved in the regulation of inflammatory processes. Therefore, the findings from this study are discussed in the context of each of the inflammatory pathways investigated in our study.

6.1 Cytokine genes

As key regulators of inflammation and immune responses, polymorphisms in cytokine genes were the most common variations evaluated as potential mechanisms for fatigue [14, 38, 55, 85–90]. While previous studies found associations between polymorphisms in a number of cytokine genes and fatigue (for reviews see [29, 91]), in our study, the same polymorphisms in *TNFA* and *IL12B* were associated with decreases in both morning and evening fatigue severity.

TNFA encodes for a pleiotropic cytokine that regulates immune responses, as well as cell proliferation and differentiation. For *TNFA* rs1041981, individuals who carried one or two doses of the rare A allele reported slightly lower morning and evening fatigue severity scores (Figures 1C and 4D). *TNFA* rs1041981 is located on chromosome 6p21.3. At this locus, the lymphotoxin-alpha (*LTA*) and *TNFA* genes are closely juxtaposed with the *LTA* coding region part of the *TNFA* early promoter region. While previous findings suggested that *LTA* shared a common signaling pathway with *TNFA* to mediate inflammatory responses [92], recent evidence suggests that the *LTA* and *TNFA* signaling pathways are unique [93, 94]. In the literature [95], rs1041981 is described as a SNP in both the *LTA* (i.e., coding region) and *TNFA* (i.e., promoter region) genes. When cited as a SNP in the *LTA* gene, rs1041981 was associated with inflammatory processes involved in atherogenesis [96], periodontal disease [97], and glucose intolerance [98]. When cited as a SNP in the promoter region of *TNFA*, rs1041981 was associated with sleep disturbance in patients with HIV [99]. These results suggest that as a promotor variant, this SNP may influence inflammatory responses through both *TNFA* and *LTA* signaling pathways.

In contrast to our findings, in the only study that evaluated the association between *TNFA* rs1041981 and morning and evening fatigue severity [100], patients with HIV disease who carried one or two doses of the rare A allele reported higher fatigue scores. These inconsistent findings may be related to differences in the way that the fatigue phenotypes were created. In the HIV study, mean scores were calculated. In our study, the effect of the polymorphism on initial levels as well as on the trajectories of morning and evening fatigue were evaluated. In addition, immune function in patients with HIV may have different signaling mechanisms than in oncology patients.

While, SIFT [83] and PolyPhen-2 [84] predicted a subtle impact of *TNFA* rs1041981 on function (i.e., tolerated by SIFT and benign by Polyphen-2), its impact on *TNFA* transcription is potentially more pronounced. The occurrence of the rare A allele changes the

profile of transcription factors that recognize this sequence. With the homozygous common allele genotype (CC), six transcription factors are predicted to recognize the C-containing sequence (i.e., binding sites) (i.e., c_Ets-1 68, C/EBPalpha, C/EBPbeta, FOXP3, HNF-4alpha2, HNF-4alpha1) compared to only three transcription factors when the rare A allele is present (i.e., RXR-alpha, ETF, c-Ets-1 68). Reduced transcription factor activity was linked to reduced inflammatory signaling and decreased levels of fatigue in breast cancer survivors [101] and may be a potential explanation for the decreases in morning and evening fatigue severity reported by patients with the CA or AA genotype.

IL12B encodes for a proinflamatory cytokine that initiates the release of interferon gamma. The *IL12B* rs3213094 polymorphism was associated with lower initial levels of both morning and evening fatigue. No predictions for the functional effects of *IL12B* rs3213094 were identified using SIFT or PolyPhen-2. While no studies were found on an association between this SNP and fatigue severity, previous research found an association with the development of psoriasis [102].

A higher liability score for *IL12B* was associated with lower initial levels of evening fatigue. When the six SNPs found on *IL12B* were evaluated, four were predicted to be tolerated or benign damaging by SIFT and PolyPhen-2 respectively, and two were not identified in these bioinformational tools. As shown in Figure 4C, for patients with zero occurrences of the rare alleles, evening fatigue scores were above the clinically meaningful cutoff score for four of the six assessments (i.e., assessments 2, 4, 5, and 6). In contrast, patients with only three doses of the rare allele had evening fatigue scores that were below the clinically meaningful cutoff score for all six assessments. While no studies were found on associations between polymorphisms in *IL12B* and fatigue, one potential explanation for the predicted lower levels of evening fatigue could be decreased release of interferon gamma. Lower levels of interferon gamma were associated with lower levels of fatigue in patients with Sjögren's syndrome [103].

It is important to note that cytokine dysregulation is associated with the co-occurrence of sleep disturbance, depressive symptoms, anxiety, and fatigue [56, 104]. Possible links among these co-occurring symptoms and polymorphisms in *TNFA* and *IL12B* require further study.

6.2 Inflammasome Pathway

Inflammasomes are multimeric protein complexes that serve as platforms for activation of the innate immune response through mediation of the NFkB and interferon signaling pathways [105, 106]. The formation of inflammasome complexes is activated by pattern recognition receptors (PRR) that detect danger-and pathogen-associated molecular patterns (i.e., DAMPs and PAMPs, respectively) [106]. These inflammasome complexes initiate a signaling pathway that activates capase-1 and transcription factor NFkB, which drives the expression of IL1β and IL18 [107]. To date, the most studied inflammasome complexes are activated by the Nod-Like Receptors (NLRs) family of PRRs.

In our study, five SNPs on five different NLR genes involved in inflammasome activation were associated with multiple effects on morning and evening fatigue. The same two SNPs,

NOD2 rs2076756 and *NLRP6* rs74044411 were associated with lower initial levels as well as with the trajectories of both morning and evening fatigue, respectively. One of the other SNPs (i.e., *NLRP5* rs471979) was associated with the trajectory of morning fatigue. The other two SNPs (i.e., *CARD6* rs10512747, *NLRP4* rs17857373) were associated with initial levels of evening fatigue.

NOD2 expression results in activation of the MAPK and NFkB inflammatory pathways and a reduction in toll-like receptor mediated inflammatory responses [105]. These effects appear to be dependent on the degree of *NOD2* expression [108]. While predictions of the functional effect of *NOD2* rs2076756 were not identified by SIFT or Poly-Phen-2, rs2076756 is strongly associated with increased cancer risk [109], Crohn's disease [110], and NOD2-associated autoinflammatory disease [111]. In addition, patients who are heterozygous (AG) or homozygous (GG) for the rare G allele are diagnosed with inflammatory bowel disease at a younger age [112]. In our study, patients who were homozygous (GG) for the rare G allele in *NOD2* rs2076756 were predicted to have morning and evening fatigue below clinically meaningful levels across all six assessments (Figures 1D and 5C, respectively). Explanations for this apparent protective effect of *NOD2* on fatigue severity in oncology patients are not readily apparent. Given that the effects of *NOD2* appear to be dependent on the degree of gene expression [108], future studies need to examine the effects of this specific polymorphism on differential gene expression in oncology patients with clinically meaningful differences in the severity of both morning and evening fatigue.

Recent evidence suggests that *NLRP6* inhibits inflammasome and non-inflammasome dependent inflammatory responses [113, 114]. *NLRP6* is associated with maintaining mucosal integrity of the gut and bacterial symbiosis [115]. *NLRP6* rs74044411 is a missense mutation that substitutes alanine for valine. The SNP is predicted to be tolerated by SIFT and benign by PolyPhen-2. Patients who were heterozygous or homozygous for the rare C allele reported levels of morning (Figure 1F) and evening (Figure 5D) fatigue that were below the clinically meaningful cutoff levels (i.e., 3.2 and 5.6 respectively) at five of the six assessments. Given recent evidence that dysbiosis of the gut membrane was associated with increased fatigue in oncology patients receiving pelvic radiation [116] and our findings, future studies need to evaluate the functional effects of this SNP as well as the inter-relationships between alterations in the gut microbiome and fatigue severity.

Only one polymorphism in a gene from the inflammasome pathway (i.e., *NLRP5* rs471979) predicted changes in the trajectory of morning fatigue. *NLRP5* encodes for a protein that plays a role for zygotes to progress beyond the first embryonic cell divisions and is essential for RNA stability. Functional effects of this SNP were not identified by SIFT or PolyPhen-2. In addition, no studies were founding linking it to fatigue. Variations in *NLRP5* were found to be associated with congenital disorders of growth and development [117], while higher *NLRP5* expression was associated with periodontal disease in older adults [118]. In our study, the morning fatigue scores of patients who were homozygous for the C allele of *NLRP5* rs471979 were predicted to be below the clinically meaningful cutoff score for five of the six assessments (Figure 1E).

Two SNPs from the inflammasome pathway (i.e., NLRP*4* rs17857373, *CARD6* rs10512747) were associated only with evening fatigue. *NLRP4* rs17857373, predicted lower initial levels of evening fatigue. *NLRP4* expression regulates type-1 interferon signaling and NF-kB activity induced by intracellular adapter proteins and kinases that functionally connect TNF and IL-1R receptors to NF-kB responses [105]. *NLRP4* rs17857373 is a missense mutation that substitutes aspartic acid for glutamic acid. This change is predicted to be tolerated by SIFT and benign by PolyPhen-2. While no studies were found that described the effect of *NLRP4* rs17857373, *NLRP4* overexpression was associated with a diagnosis of bladder cancer [119], with an increased potential for metastasis and CTX resistance in women with epithelial ovarian cancer [120], and with periodontal disease [118]. In our study, patients who were homozygous or heterozygous for the rare G allele were predicted to have evening fatigue scores below the clinically meaningful cutoff at all six assessments (Figure 5B).

CARD6 encodes for a protein that regulates the adaptive and innate immune responses through modulation of interferon and NFkB signaling [121]. *CARD6* appears to play a role in the development of GI cancers [122] and may protect cardiac muscle from hypertrophy [123]. *CARD6* rs10512747 is a missense mutation that substitutes leucine for serine. This change is predicted to be tolerated by SIFT and potentially damaging by PolyPhen-2. While no studies were found that described the effect of this polymorphism, in our study, each additional dose of the rare C allele was associated with lower predicted evening fatigue scores at enrollment (i.e., TT = 5.40, TC = 4.96, CC = 4.52) (Figure 5A).

The function of the inflammasomes is an evolving area of investigation. In our study, all of the SNPs across the five genes in this pathway were associated with decreases in fatigue severity. Additional research is warranted to determine if the functional effects of these polymorphisms prevent the initiation of the inflammasome complex which may decrease inflammatory responses. While no studies were found that discussed the role of inflammasomes in the development of fatigue, it is reasonable to hypothesize that dysregulation of inflammasomes could result in decreases in IL-1B activity and decreases in innate inflammatory responses, and subsequent decreases in fatigue severity [124]. Additional research is warranted on this potential new mechanism for fatigue in oncology patients.

6.3 JAK/STAT pathway

The pleiotropic JAK/STAT pathway signals a cascade of reactions to maintain homeostasis [125]. The liability score for SNPs in *IL4R* predicted changes in the trajectory of morning fatigue. *IL4R* encodes for a cellular receptor for IL-4 and IL-13 that coordinates inflammatory responses through cytokine receptor signal transduction [126]. Of the seven individual SNPs included in the liability score, four were predicted to be tolerated or possibly damaging by SIFT and PolyPhen-2, respectively and three were not predicted by either bioinformational tool. In our study, for those patients who carried three or more rare alleles, their morning fatigue levels were predicted to be below the clinically meaningful cutoff score for five of the six assessments (Figure 2A). While no studies reported on an association between *IL4R* polymorphisms and fatigue severity, increased expression of *IL4R* was correlated with higher levels of fatigue in a sample of breast cancer survivors [50]. One

potential explanation for the decreases in fatigue severity found in our study is that an increased number of polymorphisms in this gene changes the affinity of the cellular receptor which decreases the inflammatory responses associated with IL4 and IL13 signal transduction.

6.4 MAPK/JNK pathway

The MAPK/JNK pathway is both an upstream and downstream regulator of the expression of pro-inflammatory cytokines [127]. Only one SNP (i.e., *IL17RD* rs61742267) from the MAPK/JNK pathway was associated with evening fatigue. *IL17RD* (also called *Sef* [similar expression to fibroblast growth factor]) was found to be a tumor suppressor gene [128] and an inhibitor of toll-like receptor signaling [129]. *IL17RD* rs61742267 is a missense mutation that substitutes serine for proline. While no effect was predicted by PolyPhen-2, it was predicted by SIFT to have a potentially damaging effect on gene function. No studies were found that discussed the role of *IL17RD* in the development of fatigue. In our study, patients who are homozygous or heterozygous for the rare G allele were predicted to have evening fatigue at levels below the clinically meaningful cutoff at all six assessments (Figure 5E). One possible explanation for this finding could be that this polymorphism modulates toll-like receptor signaling which may decrease inflammatory responses that results in decreases in fatigue severity.

6.5 NFkB pathway

The NFkB pathway mediates cellular functions including apoptosis, cellular proliferation, and inflammatory responses through a complex signaling network [130]. This NFkB signaling network is regulated by canonical and non-canonical pathways. The canonical pathway is triggered by a variety of signals (e.g., members of the TNFRS family, *IL1-R*, toll-like receptors). The non-canonical NFkB pathway is triggered by specific members of the inhibitor of kappa B and TNFRSF families and *LTBR* [131]. Together, the canonical and non-canonical pathways regulate pro-and anti-inflammatory responses to prevent disorders that are associated with dysregulation of the NFkB pathway (e.g., rheumatoid arthritis, multiple sclerosis, inflammatory bowel disease) [132].

For the NFkB pathway, four SNPs and five liability scores across seven genes were associated with inter-individual differences in morning and evening fatigue. Only one SNP (i.e., *TNFRSF14* rs2234163) was associated with initial levels of both morning and evening fatigue. *TNFRSF14*, encodes for a protein that both activates and inhibits T-cells based on the cellular environment [133]. *TNFRSF14* rs2234163 is a missense mutation that substitutes threonine for alanine. It was predicted to be tolerated or possibly damaging by SIFT and PolyPhen-2, respectively. This SNP was associated with p53 mutations in Chinese women with GYN cancers [134]. In our study, compared to patients who were homozygous for the common allele (i.e., morning LFS score = 2.98, evening LFS score = 5.28), patients who were heterozygous or homozygous for the rare G allele of *TNFRSF14* rs2234163, were predicted to have higher morning (i.e., LFS = 4.41) and evening (i.e., LFS = 6.87) scores (Figures 2B and 6B). When these differences in fatigue scores were compared, caring one or two copies of the rare G allele was associated with clinically meaningful increases in both morning (d=.97) and evening (d=.75) fatigue severity. One possible explanation for higher

fatigue scores associated with this SNP could be increased signaling of the canonical NFkB pathway that results in increased inflammatory responses.

One SNP (i.e., *TNFRSF10A* rs17620) and four liability scores (i.e., *IL17RB, TNFRSF10D, TNFRSF11A* and *TNFRSF21*) were associated with inter-individual differences in morning fatigue severity. *TNFRSF10A* rs17620 predicted inter-individual differences in the trajectory of morning fatigue. *TNFRSF10A*, after activation by tumor necrosis factor-related apoptosis-inducing ligand (*TRAIL*), encodes for a protein that induces cellular apoptosis (http://www.ncbi.nlm.nih.gov/gene/8797). No predictions of the effects of this SNP on protein function were identified by SIFT or PolyPhen-2. While studies of the associations between *TNFRSF10A* rs17620 and fatigue were not found, *TRAIL* polymorphisms are associated with increased cancer risk [135, 136]. In our study, patients who were heterozygous or homozygous for the rare C allele were predicted to have a steeper trajectory of morning fatigue scores that suggests clinically meaningful increases in fatigue at four of the six assessments (i.e., assessments 2, 4, 5 and 6) (Figure 3A).

For *IL17RB*, the liability score for this gene and two SNPs in this gene (i.e., rs2232346, rs1043261) were associated with lower levels of morning and evening fatigue, respectively. *IL17RB* encodes for a protein that mediates the activation of the NFkB pathway and the production of C-X-C motif chemokine ligand 8 (CXCL8), a mediator of inflammatory responses. While no studies were found on an association between *IL17RB* polymorphisms and fatigue severity, overexpression of *IL17RB* is associated with increased tumorigenesis and metastasis [137, 138]. In our study, each additional copy of the rare allele for *IL17RB* was associated with lower predicted morning fatigue scores at enrollment (i.e., 0 alleles = 3.12, 1 allele = 2.77, 2 alleles = 2.42) (Figure 2C).

In terms of evening fatigue, two SNPs in *IL17RB* (i.e., rs2232346 and rs1043261) predicted the initial levels of evening fatigue at enrollment. *IL17RB* rs2232346 is a missense mutation that substitutes leucine for phenylalanine and is predicted to be tolerated by SIFT and benign by PolyPhen-2. No citations were identified for the effects of rs2232346. For *IL17RB* rs1043261, while no predictions of the functional effects were identified by the bioinformational tools, it was found to be associated with new-onset diabetes after renal transplantation [139].

Patients who were homozygous or heterozygous for the rare C allele of IL*17RB* rs2232346 or homozygous for rare C allele of *IL17RB* rs1043261 were predicted to have evening fatigue scores below clinically meaningful levels at all six assessments (Figure 6A). At enrollment, the effect size calculations for differences in predicted evening fatigue scores were in the moderate to large range for *IL17RB* rs2232346 (d = .4) and *IL17RB* rs1043261 (d = .7). Studies are needed to determine if the functional effects of these polymorphisms decrease inflammatory responses by inhibiting NFkB activation and decreasing the production of CXCL8.

The liability score for *TNFRSF10D* predicted the trajectory of morning fatigue. *TNFRSF10D* encodes for a protein that protects against TRAIL-mediated apoptosis and is part of the canonical NFkB pathway (http://www.ncbi.nlm.nih.gov/gene/8793). While no

change was predicted in the trajectory of assessments 1, 2, and 3 (i.e., PW1), carrying more copies of the rare alleles lowered morning fatigue scores for assessments 4, 5, and 6 (i.e., PW2) (Figure 3B).

The liability score for *TNFRSF11A* predicted the trajectory of morning fatigue. *TNFRSF11A* encodes for a protein that is an activator of the non-canonical NFkB pathway to initiate inflammatory responses (http://www.ncbi.nlm.nih.gov/gene/8792). In our study, each additional copy of the rare allele of *TNFRSF11A* predicted lower morning fatigue scores across both piecewise models (Figure 3C).

The liability score for *TNFRSF21* predicted initial levels of morning fatigue (Figure 2D). *TNFRSF21* encodes for a protein that activates the canonical NFkB pathway and plays a role in neural cell apoptosis that is potentially related to the development of Alzheimer's disease [140]. In our study, each additional copy of the rare allele for *TNFRSF21* was associated with higher predicted morning fatigue scores at enrollment (i.e., 0 alleles = 2.92, 1 allele = 3.33, 2 alleles = 3.75).

The *LTBR* liability score was a unique predictor of evening fatigue. *LTBR* activates the non-canonical NFkB pathway and mediates cancer-associated inflammation [141]. While the two SNPs in the *LTBR* liability score were predicted to be tolerated by SIFT, no clinical studies of either SNP were identified. In our study, carrying the rare allele predicted a sharply decreased slope at assessment 2 (Figure 6C). Explanations for this trajectory at assessment 2 are not readily apparent and warrant further study to determine the effect of *LTBR* on the severity of evening fatigue.

An evaluation of the associations between polymorphisms in genes in the NFkB pathway identified in this study and changes in morning and evening fatigue severity reveals the complex nature of the mechanisms that underlie fatigue. While polymorphisms in four genes (i.e., *IL17RB, TNFRSF10D, TNFRSF11A, LTBR)* were associated with lower levels of morning and evening fatigue, polymorphisms in two genes (i.e. *TNFRSF10A, TNFRSF14, TNFRSF21*) were associated with higher levels of morning and evening fatigue. Future studies need to evaluate the functional effects of these polymorphisms and the interactions among the polymorphisms within and outside the NFkB pathway.

7. Limitations and strengths

Several limitations and strengths need to be acknowledged. While our sample size was adequate, these findings warrant replication. Because patients completed the questionnaires in their homes rather than in the clinic, it may have influenced their reports of fatigue severity. In our study, a liability score assumes that the rare alleles across all of the SNPs in a specific gene region carry a similar risk (e.g., all protective). It is possible that discordant allele effects could result in false negatives or bias the results obtained from using liability scores. Future analyses of the functional effects of each SNP could be aggregated into a liability score that might account for discordant allele effects. However, this large, representative sample of oncology outpatients undergoing CTX, the evaluation of morning and evening fatigue across two cycles of CTX, and the use of HLM to identify genetic

predictors of inter-individual variability in morning and evening fatigue are major strengths of this study. Our conceptual analysis of the genomic data within functional pathways contextualizes the results that can be used to inform future hypothesis of how functionally related genes collectively affect morning and evening fatigue severity.

8. Conclusion

This study extends the evidence that morning and evening fatigue are distinct yet related symptoms. In addition, new inflammatory pathways were identified that play potential roles in the complex mechanisms that are involved in the development of morning and evening fatigue. Future research with pathway analysis will help us to clarify the biological processes that contribute to inter-individual variability in the severity of morning and evening fatigue so that we can tailor interventions to prevent or alleviate these distinct but related symptoms.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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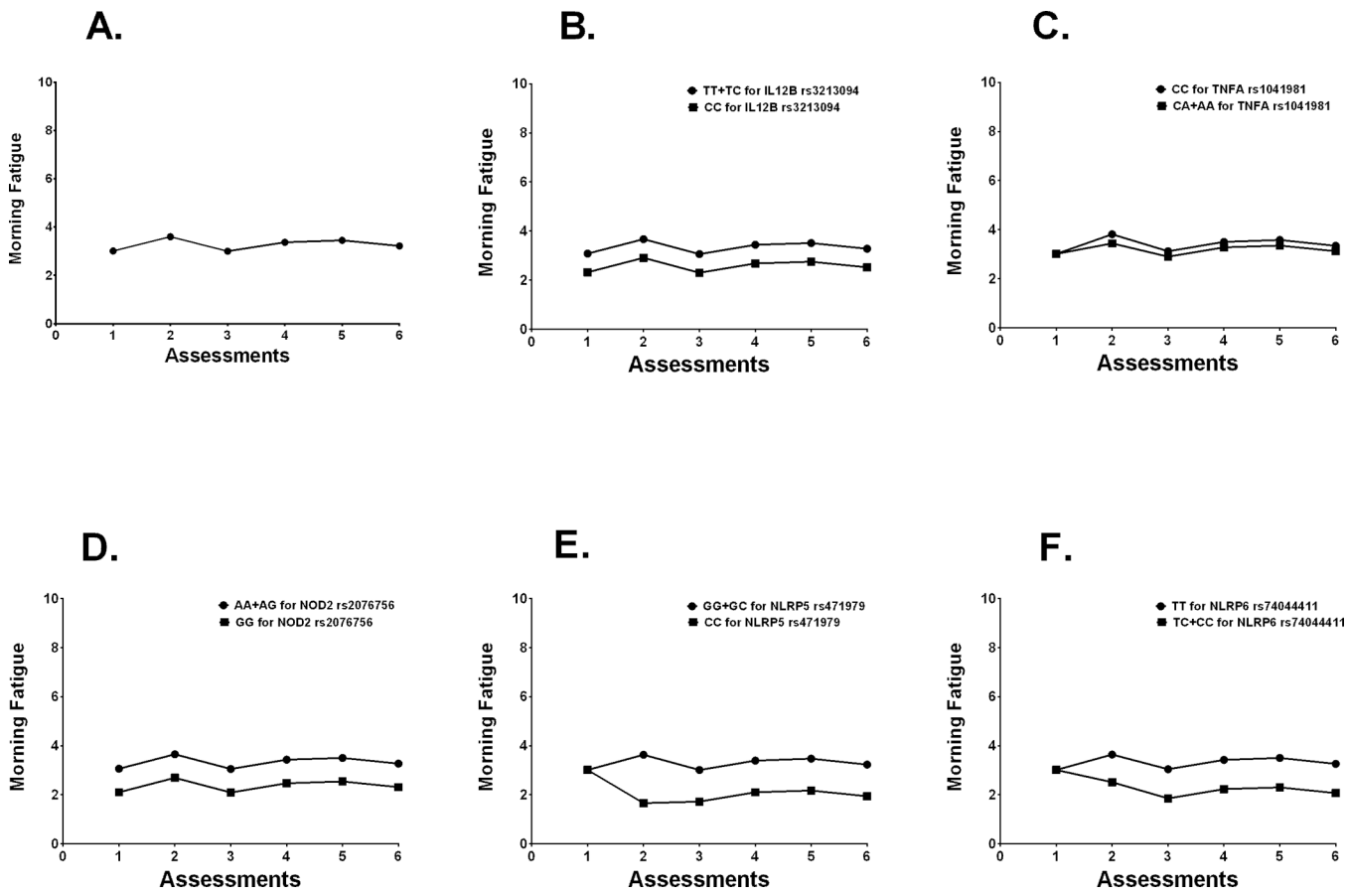
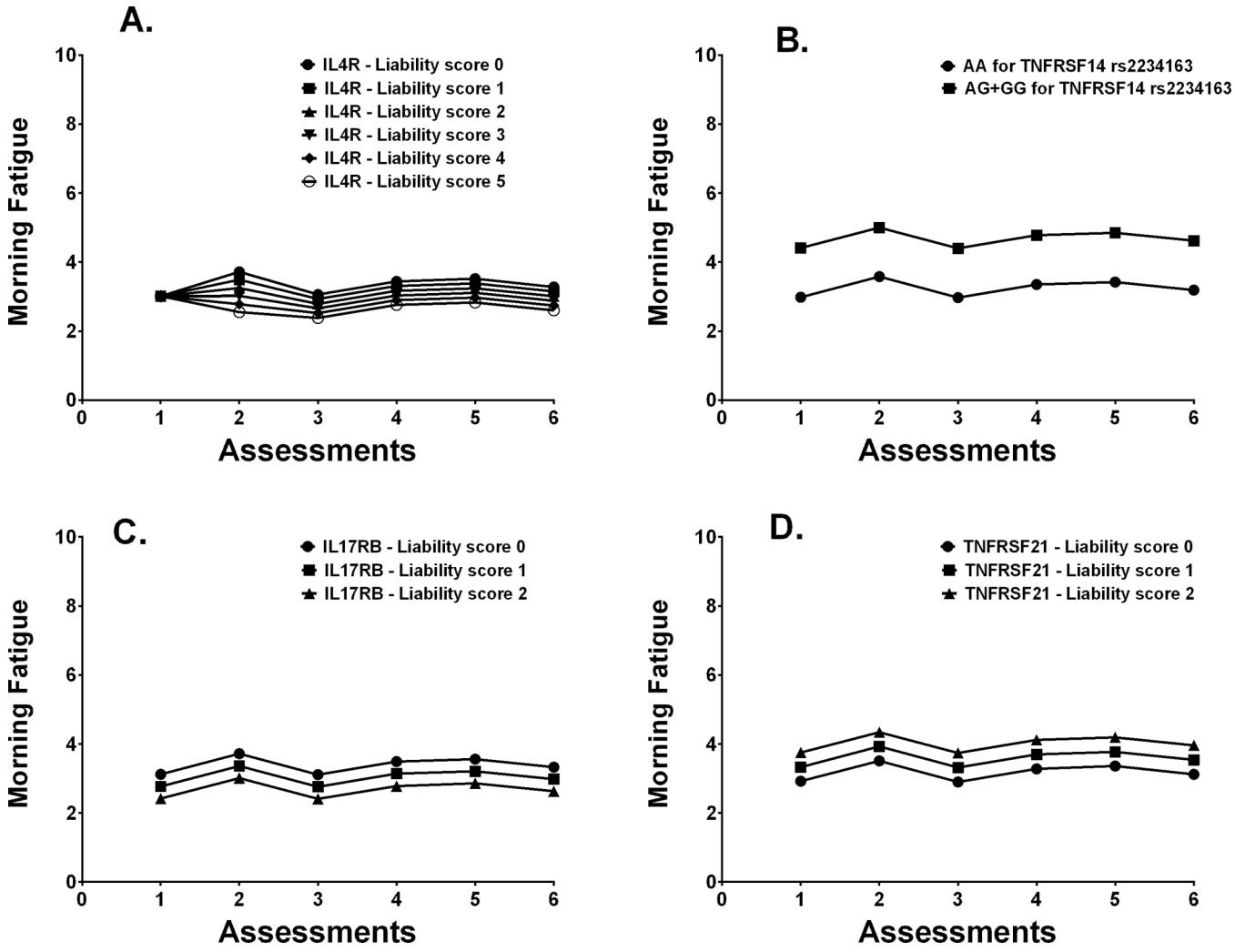
Highlights

- Morning and evening fatigue are distinct yet related symptoms.
- Multiple inflammatory pathways are associated with fatigue severity.
- Common and distinct polymorphisms are associated with morning and evening fatigue.



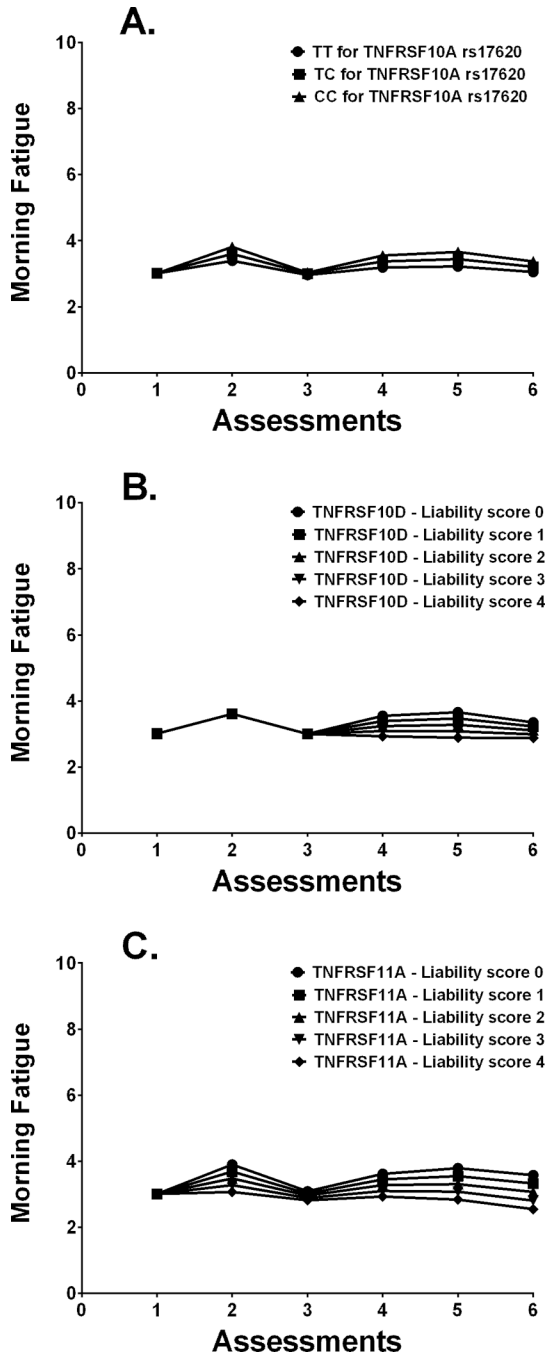
Figures 1.

A–F – Unconditional piecewise model of mean morning fatigue scores for six assessment points over two cycles of chemotherapy (A). Influence of the recessive model (TT+TC vs. CC) of the rare C allele in *IL12B* rs3213094 on the inter-individual differences in the intercept for morning fatigue (B). Influence of the dominant model (CC vs. CA+AA) of the rare A allele in *TNFA* rs1041981 on the slope parameters for morning fatigue (C). Influence of the recessive model (AA+AG vs. GG) of the rare G allele in *NOD2* rs2076756 on the inter-individual differences in the intercept for morning fatigue (D). Influence of the recessive model (GG+GC vs. CC) of the rare C allele in *NLRP5* rs471979 on the slope parameters for morning fatigue (E). Influence of the dominant model (TT vs. TC+CC) of the rare C allele in *NLRP6* rs74044411 on the slope parameters for morning fatigue (F).



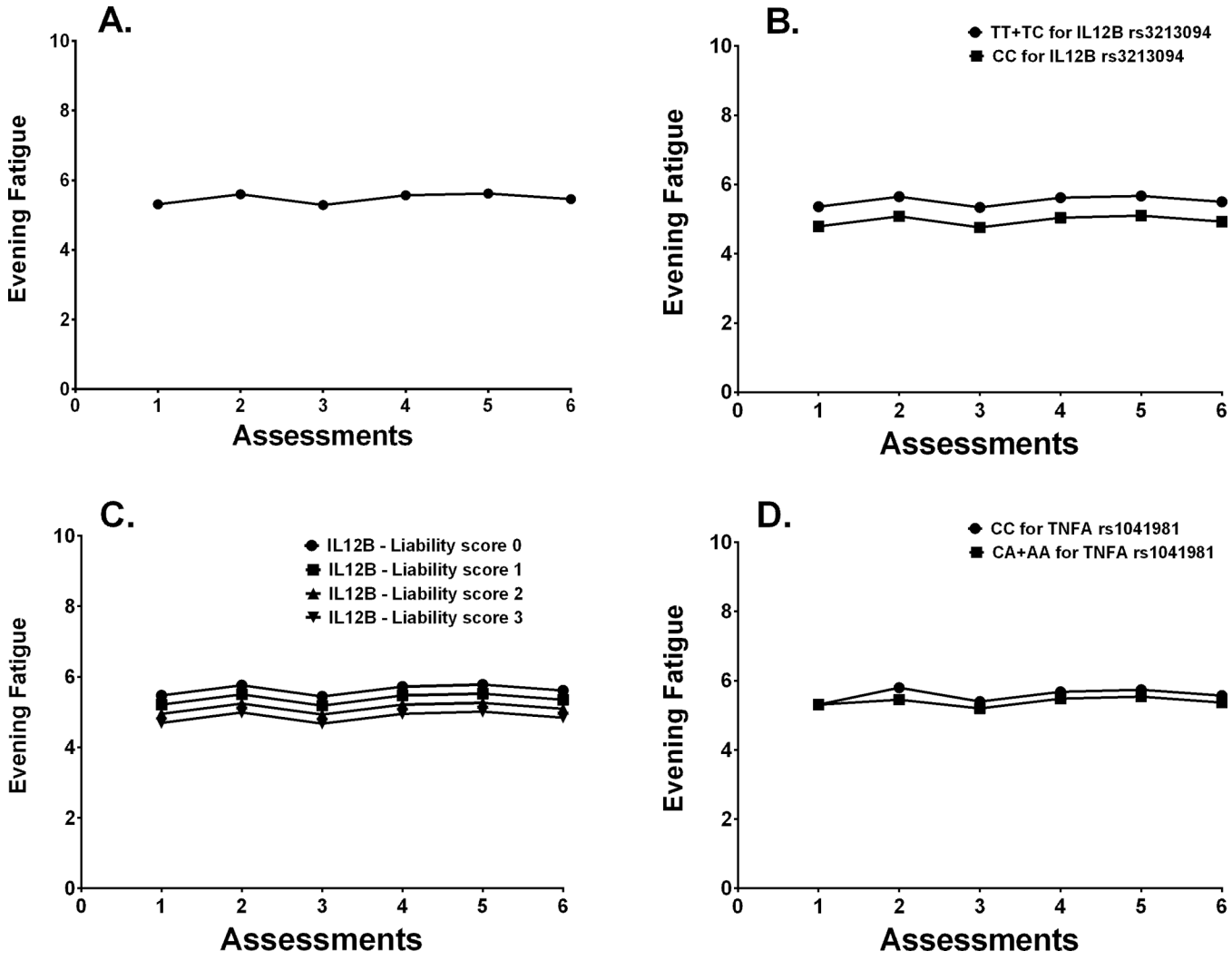
Figures 2.

A–D – Influence of the liability score for the sum of the occurrence of the rare alleles in *IL4R* on the slope parameters for morning fatigue (A). Influence of the dominant model (AA vs. AG+GG) of the rare G allele for *TNFRSF14* rs2234163 on the inter-individual differences in the intercept for morning fatigue (B). Influence of the liability score for the sum of the occurrence of the rare alleles in *IL17RB*, on the inter-individual differences in the intercept for morning fatigue (C). Influence of the liability score for the sum of the occurrence of the rare alleles in *TNFRSF21* on the inter-individual differences in the intercept for morning fatigue (D).



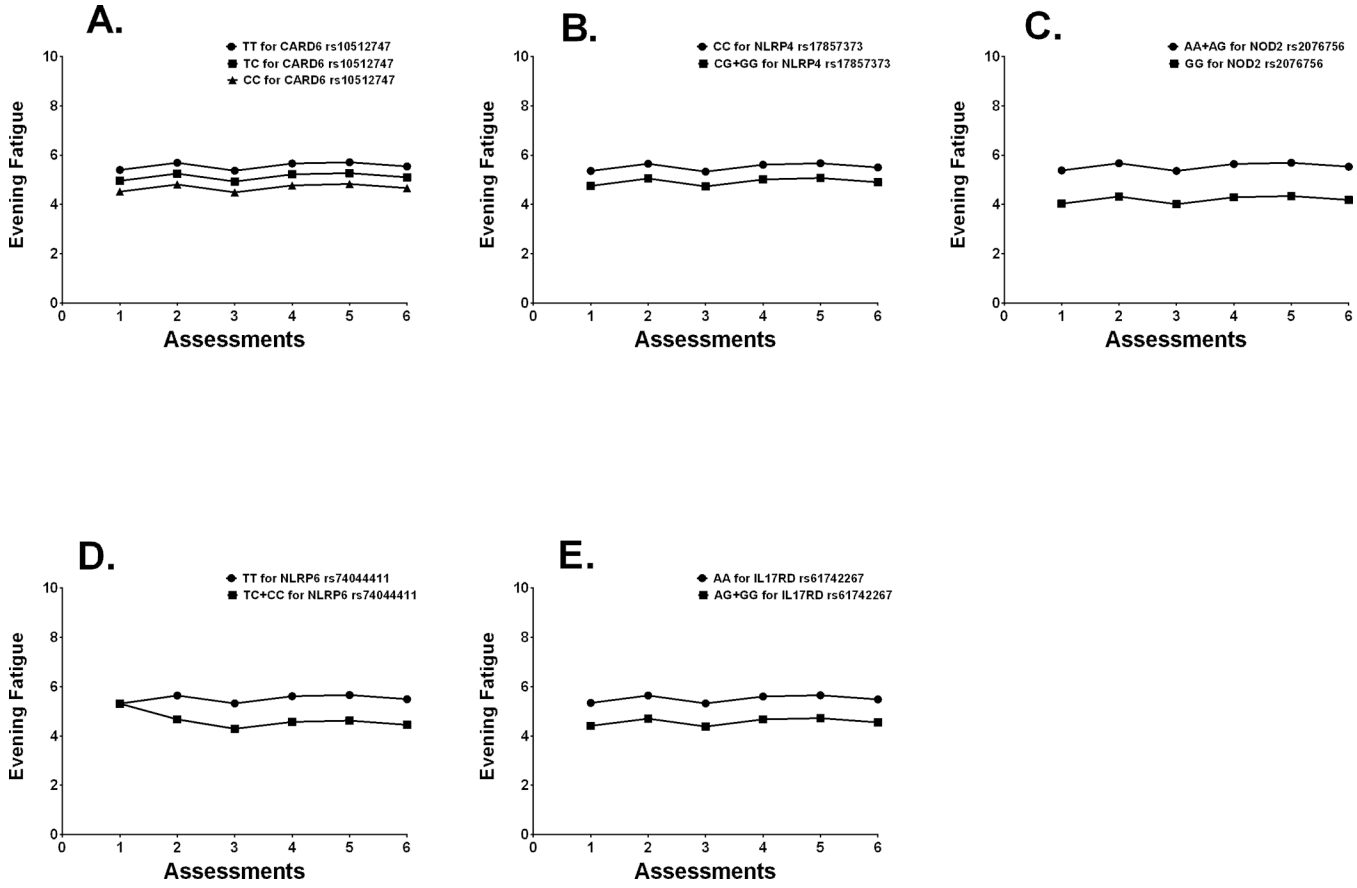
Figures 3.

A–C – Influence of the additive model (TT vs. TC vs. CC) of the rare C allele for *TNFRSF10A* rs17620 on the slope parameters for morning fatigue (A). Influence of the liability score for the sum of the occurrence of the rare alleles in *TNFRSF10D* on the slope parameters for morning fatigue (B). Influence of the liability score for the sum of the occurrence of the rare alleles in *TNFRSF11A* on the slope parameters for morning fatigue (C).



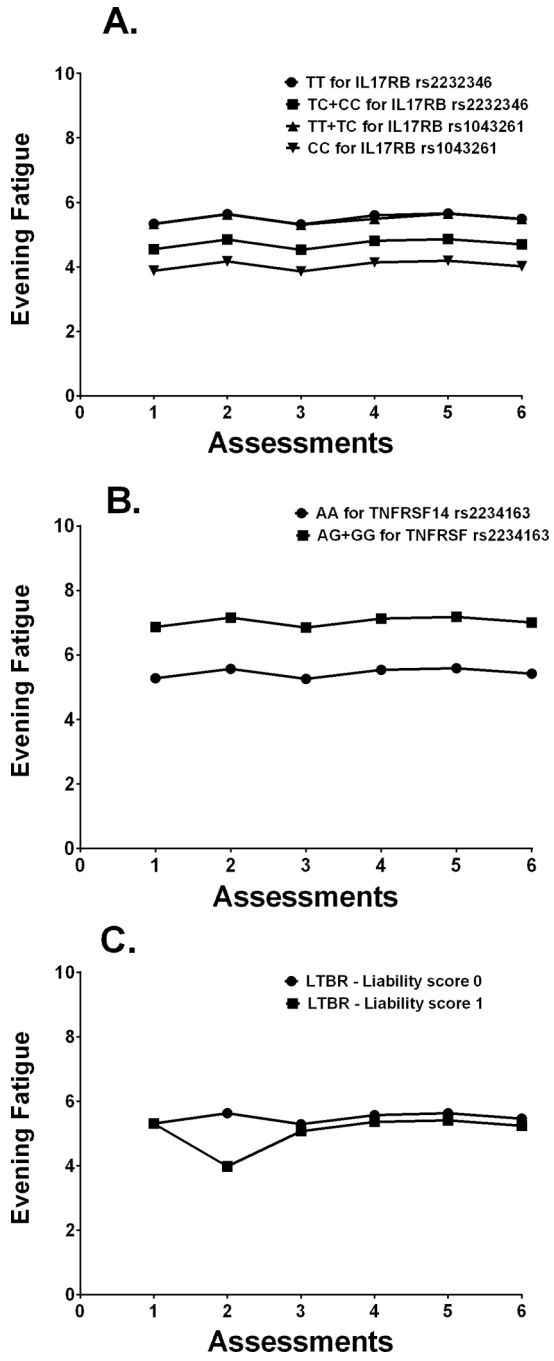
Figures 4.

A–D – Unconditional piecewise model of mean evening fatigue scores for six assessment points over two cycles of chemotherapy (A). Influence of the recessive model (TT+TC vs. CC) of the rare C allele in *IL12B* rs3213094 on the inter-individual differences in the intercept for evening fatigue (B). Influence of the liability score for the sum of the occurrence of the rare alleles in *IL12B* on the inter-individual differences in the intercept for evening fatigue (C). Influence of the dominant model (CC vs. CA+AA) of the rare A allele in *TNFA* rs1041981 on the slope parameters for evening fatigue (D).



Figures 5.

A–E – Influence of the additive model (TT vs. TC vs. CC) of the rare C allele in *CARD6* rs10512747 on the inter-individual differences in the intercept for evening fatigue (A). Influence of the dominant model (CC vs. CG+GG) of the rare G allele for *NLRP4* rs17857373 on the inter-individual differences in the intercept for evening fatigue (B). Influence of the recessive model (AA+AG vs. GG) of the rare G allele for *NOD2* rs2076756 on the inter-individual differences in the intercept for evening fatigue (C). Influence of the dominant model (TT vs. TC+CC) of the rare C allele for NLRP6 rs74044411 on the slope parameters for evening fatigue (D). Influence of the dominant model (AA vs. AG+GG) for the rare G allele for *IL17RD* rs61742267 on the inter-individual differences in the intercept for evening fatigue (E).



Figures 6.

A–C – Influence of the dominant model (TT vs. TC+CC) of the rare C allele for *IL17RB* rs2232346 and the recessive model (TT+TC vs. CC) of the rare C allele for *IL17RB* rs1043261 on the inter-individual differences in the intercept for evening fatigue (A). Influence of the dominant model (AA vs. AG+GG) of the rare G allele for *TNFRSF14* rs2234163 on the inter-individual differences in the intercept for evening fatigue (B).

Influence of the liability score for the sum of the occurrence of the rare alleles in *LTBR* on the slope parameters for evening fatigue (C).

Table 1

Candidate Genotype Predictors of Intercept, Piecewise 1 and Piecewise 2 Linear and Quadratic Components for Morning Fatigue

Gene Symbol	SNP ID	Position	Chr	Alleles	Model	Genotype Counts[1]	I	PW1-L	PW1-Q	PW2-L	PW2-Q
Cytokine genes											
IL10	rs3024493	206943968	1	G>T	R	411, 117, 15	■				
IL12B	rs3213094	158750769	5	T>C	R	292, 202, 49	■				
TNFA	rs1041981	31540784	6	C>A	D	233, 241, 69		■	■		
Inflammasome pathways genes											
NLRC5	rs28438857	57060353	16	T>C	D	380, 132, 31	■				
NLRC5	rs7185320	57101373	16	A>G	R	484, 54, 5	■				
NLRP5	rs471979	56538976	19	G>C	R	417, 119, 7		■			
NLRP6	rs74044411	284477	11	T>C	D	521, 20, 2		■	■		
NOD2	rs2076756	50756881	16	A>G	R	347, 167, 29	■				
JAK/STAT pathway genes											
IL4R	Liability Score					0: 356 1: 124 2: 42 3: 18 4:2 5:1		■	■		
IFNGR2	rs4986958	34787294	21	C>G	D	528, 14, 1		■			
IL10RB	rs2834167	34640788	21	A>G	R	277, 212, 54	■	■	■		
MAP/JNK pathway genes											
PSMD2	rs11545169	184020542	3	T>G	R	408, 123, 12	■				
NFkB pathway genes											
LTBR	Liability Score					0: 536 1: 7		■			
IL17RB	Liability Score					0: 391 1: 129 2: 23	■				
TNFRSF1A	rs4149584	6442643	12	T>C	D	525, 17, 1				■	■
TNFRSF1A	rs4149637	6443001	12	A>G	D	537, 6, 0				■	■
TNFRSF10A	rs17620	23060256	8	T>C	A	143, 238, 162			■		■
TNFRSF10D	Liability Score					0: 134 1: 243				■	■

Gene Symbol	SNP ID	Position	Chr	Alleles	Model	Genotype Counts[1]	I	PW1-L	PW1-Q	PW2-L	PW2-Q
						2: 138 3: 23 4: 5					
TNFRSF11A	Liability Score					0: 99 1: 196 2: 185 3: 46 4: 17		■	■	■	■
TNFRSF14	rs2234163	2491306	22	A>G	D	533, 10, 0	■				
TNFRSF21	Liability Score					0: 435 1: 94 2: 14	■				
MAP/JNK and NFkB pathway genes											
TRAF1	rs3761847	123690239	9	A>G	D	176, 265, 102		■	■		■
TRAF4	rs35932778	27075334	17	A>G	D	535, 8, 0				■	■

■ = From morning fatigue exploratory analysis had a *t*-value of ≥2.0

[1] = The order of the genotypes for which counts are provided is homozygous common, heterozygote, and homozygous rare.
Liability Score= The total number of rare allele occurrences across all SNPs and models tested for a given gene in the sample.

For the liability scores - The total number of rare allele occurrences across all SNPs for a given gene are coded as follows for *IL4R as an example* 0: 356 means that 356 patients did not have any doses of the rare alleles across *IL4R*; 1: 124 means that 124 patients had 1 dose of the rare alleles across *IL4R*; 2: 42 means that 42 patients had 2 doses of the rare alleles across *IL4R*; 3: 18 means that 18 patients had 3 doses of the rare alleles across *IL4R*; and 4: 2 means that 2 patients had 4 doses of the rare alleles across *IL4R*.

Abbreviations: A = additive model; Chr = chromosome; D = dominant model; I = intercept; *IFNGR2* = Interferon gamma receptor 2; *IL4R* = Interleukin 4 receptor; *IL10* = Interleukin 10; *IL10RB* = Interleukin 10 receptor subunit beta; *IL12B*= Interleukin 12B; *IL17RB* = Interleukin 17 receptor B; JAK/STAT = Janus kinase/signal transducers and activators of transcription; JNK = jun amino-terminal kinases; *LTBR* = Lymphotoxin beta receptor; MAP = mitogen-activated protein kinase; NFkB = nuclear factor-kappa beta; *NLRC5* = Nod-Like receptor family, caspase recruitment domain containing 5; *NLRP5* = Nod-Like receptor family, pyrin domain containing 5; *NLRP6* = Nod-Like receptor family, pyrin domain containing 6; *NOD2* = Nucleotide-Binding oligomerization domain containing 2; *PSMD2* = Proteasome 26S subunit non-ATPase 2; PW1-L = piecewise 1 linear component; PW1-Q = piecewise 1 quadratic component; PW2-L = piecewise 2 linear component; PW2-Q = piecewise 2 quadratic component; R = recessive model; rs = reference SNP cluster identification number; SNP= single nucleotide polymorphism; *TNFA* = Tumor necrosis factor alpha; *TNFRSF1A* = Tumor necrosis factor receptor superfamily member 1A; *TNFRSF10A* = Tumor necrosis factor receptor superfamily member 10A; *TNFRSF10D* = Tumor necrosis factor receptor superfamily member 10D; *TNFRSF11A* = Tumor necrosis factor receptor superfamily member 11A; *TNFRSF14* = Tumor necrosis factor receptor superfamily member 14; *TNFRSF21* = Tumor necrosis factor receptor superfamily member 21; *TRAF1* = Tumor necrosis factor receptor associated factor 1; *TRAF4* = Tumor necrosis factor receptor associated factor 4

Table 2

Candidate Genotype Predictors of Intercept, Piecewise 1 and Piecewise 2 Linear and Quadratic Components for Evening Fatigue

Gene Symbol	SNP ID	Position	Chr	Alleles	Model	Genotype Counts[1]	I	PW1-L	PW1-Q	PW2-L	PW2-Q
					Cytokine genes						
IL12B	rs3213094	158750769	5	T>C	R	292, 202, 49	■				
IL12B	Liability Score					0: 275 1: 211 2: 49 3: 8	■				
IL17F	rs11465553	52101758	6	T>C	D	513, 30, 0				■	
IL17	Liability Score					0: 511 1: 32				■	■
TNFA	rs1041981	31540784	6	C>A	D	233, 241, 69			■		
					Inflammasome pathways genes						
CARD6	rs10512747	40841741	5	T>C	A	441, 92, 10	■				
CARD6	Liability Score					0: 401 1: 127 2: 15	■		■		
NLRC5	rs16965150	57059484	16	T>C	D	514, 27, 2				■	■
NLRP4	rs17857373	56369908	19	C>G	D	500, 42, 1	■				
NLRP5	rs471979	56538976	19	G>C	R	417, 119, 7	■				
NLRP6	rs74044411	284477	11	T>C	D	521, 20, 2		■	■		
NOD2	rs2076756	50756881	16	A>G	R	347, 167, 29	■				
					JAK/STAT pathway genes						
IFNGR2	rs4986958	34787294	21	C>G	D	528, 14, 1		■	■	■	■
IL2RA	rs791587	6088699	10	A>G	R	149, 268, 126		■	■		
IL2RB	rs2284033	37534034	22	A>G	D	180, 261, 102		■			
					MAP/JNK pathway genes						
IL17RD	rs61742267	57132035	3	A>G	D	528, 18, 0	■				
					NFkB pathway genes						
IL17RB	rs2232346	53892830	3	T>C	D	520, 23, 0	■				
IL17RB	rs1043261	53899276	3	T>C	R	437, 96, 10	■				
LTBR	Liability Score					0: 536 1: 7			■		

Gene Symbol	SNP ID	Position	Chr	Alleles	Model	Genotype Counts[1]	I	PW1-L	PW1-Q	PW2-L	PW2-Q
TNFRSF14	rs2234163	2491306	22	A>G	D	533, 10, 0	■				

■ = From evening fatigue exploratory analysis had a *t*-value of ≥2.0

[1] = The order of the genotypes for which counts are provided is homozygous common, heterozygote, and homozygous rare.
Liability Score= The total number of rare allele occurrences across all SNPs and models tested for a given gene in the sample.

For the liability scores - The total number of rare allele occurrences across all SNPs for a given gene are coded as follows for *IL12B as an example* 0: 275 means that 275 patients did not have any doses of the rare alleles across *IL12B*; 1: 211 means that 211 patients had 1 dose of the rare alleles across *IL12B*; 2: 49 means that 49 patients had 2 doses of the rare alleles across *IL12B*; 3: 8 means that 8 patients had 3 doses of the rare alleles across *IL12B.*

Abbreviations: A = additive model; *CARD6* = Caspase recruitment domain family member 6; Chr = chromosome; D = dominant model; I = intercept; *IFNGR2* = Interferon gamma receptor 2; *IL2RA* = Interleukin 2 receptor alpha; *IL2RB* = Interleukin 2 receptor beta; *IL12B*= Interleukin 12B; *IL17* = Interleukin 17; *IL17F* = Interleukin 17F; *IL17RB* = Interleukin 17 receptor B; *IL17RD* = Interleukin 17 receptor D; JAK/STAT = janus kinase/signal transducers and activators of transcription; JNK = jun amino-terminal kinases; *LTBR* = Lymphotoxin beta receptor; MAP = mitogen-activated protein kinase; NFkB = nuclear factor-kappa beta; *NLRC5* = Nod-Like receptor family, caspase recruitment domain containing 5; *NLRP4* = Nod-Like receptor family, pyrin domain containing 4; *NLRP5* = Nod-Like receptor family, pyrin domain containing 5; *NLRP6* = Nod-Like receptor family, pyrin domain containing 6; *NOD2* = Nucleotide-Binding oligomerization domain containing 2; PW1-L = piecewise 1 linear component; PW1-Q = piecewise 1 quadratic component; PW2-L = piecewise 2 linear component; PW2-Q = piecewise 2 quadratic component; R = recessive model; rs = reference SNP cluster identification number; SNP= single nucleotide polymorphism; *TNFA* = Tumor necrosis factor alpha; *TNFRSF14* = Tumor necrosis factor receptor superfamily member 14.

Table 3

Sample Characteristics (n=543)

Characteristics	Mean (SD)
Age (years)	57.1 (11.7)
Education (years)	16.3 (3.0)
Body mass index (kg/m^2)	26.3 (5.8)
Hemoglobin (gm/dL)	11.7 (1.4)
Karnofsky Performance Status score	80.7 (11.8)
Self-administered Comorbidity Questionnaire score	5.5 (3.0)
Time since cancer diagnosis (years)	2.5 (4.4)
Number prior cancer treatments	1.9 (1.6)
Number of metastatic sites including lymph node involvement	1.4 (1.3)
Number of metastatic sites excluding lymph node involvement	0.9 (1.2)
Symptom scores at enrollment	
Lee Fatigue Scale: morning fatigue	3.0 (2.2)
Lee Fatigue Scale: evening fatigue	5.3 (2.1)
Lee Fatigue Scale: morning energy	4.5 (2.2)
Lee Fatigue Scale: evening energy	3.5 (1.9)
Center for Epidemiological Studies-Depression Scale	12.5 (9.3)
General Sleep Disturbance Scale	51.9 (19.3)
Trait Anxiety	35.0 (10.3)
State Anxiety	33.3 (11.9)
	% (N)
Gender (female)	80.8 (439)
Ethnicity	
White	69.4 (377)
Black	6.8 (77)
Asian/Pacific Islander	12.7 (69)
Hispanic/Mixed/Other	11.0 (60)
Married or partnered	68.0 (369)
Lives alone	19.5 (106)
Currently employed	34.3 (186)
Child care responsibilities (% yes)	23.6 (128)
Exercise on a regular basis (% yes)	70.7 (413)
Cancer diagnosis	
Breast	43.6 (237)
Gastrointestinal	26.0 (141)
Gynecological	21.0 (114)
Lung	9.4 (51)
Chemotherapy cycle length	
14 days	36.1 (196)

Characteristics	Mean (SD)
21 days	54.9 (298)
28 days	9.0 (49)
Previous cancer treatments	89.9 (450)
Pain present (% yes)	72.9 (396)

Abbreviations: gm/dL = grams per deciliter; kg/m^2 = kilograms per meter squared; SD = standard deviation

Table 4

Hierarchical Linear Models for Morning Fatigue

Cytokine Genes		
Morning Fatigue and *IL12B* rs3213094	**Coefficient (SE)**	
	Unconditional Model	**Final Model**
Fixed effects		
Intercept	3.011 (.099)+	3.008 (.099)+
Ethnicity[a]		
Black versus White	−.845 (.917)	−.735 (.913)
Asian versus White	.214 (.675)	.265 (.672)
Hispanic versus White	.328 (.331)	.348 (.329)
Ancestry informative markers principal components[b]		
PC1	−.102 (.104)	−.105 (.103)
PC2	−.052 (.088)	−.031 (.088)
PC3	.008 (.039)	.008 (.039)
PW1 – linear	1.192 (.148)+	1.191 (.148)+
PW1 – quadratic	−.599 (.071)+	−.599 (.071)+
PW2 – linear	.532(.096)+	.531 (.096)+
PW2 – quadratic	−.153 (.031)+	−.153 (.031)+
Time invariant covariates		
Intercept - *IL12B* rs3213094		−.760 (.319)*
Variance components		
In intercept	3.552+	3.512+
Goodness-of-fit deviance (parameters estimated)	11026.531695 (13)+	11020.881398 (14)
Model comparison χ^2 (df)		5.650 (1)*
Morning Fatigue and *TNFA* rs1041981		Coefficient (SE) Final Model
Fixed effects		
Intercept		3.011 (.099)+
Ethnicity[a]		
Black versus White		−.807 (.917)
Asian versus White		.187 (.675)
Hispanic versus White		.304 (.331)
Ancestry informative markers principal components[b]		
PC1		−.106 (.103)
PC2		−.045 (.088)
PC3		.015 (.039)
PW1 – linear		1.186 (.148)+
PW1 – quadratic		−.597 (.071)+

Cytokine Genes		
Morning Fatigue and *IL12B* rs3213094	**Coefficient (SE)**	
	Unconditional Model	**Final Model**
PW2 – linear		.533 (.096) +
PW2 – quadratic		−.153 (.031) +
Time invariant covariates		
PW1 – linear - *TNFA* rs1041981		−.641 (.278) *
PW1 – quadratic - *TNFA* rs1041981		.264 (.131) *
Variance components		
In intercept		3.549 +
Goodness-of-fit deviance (parameters estimated)		11019.804052 (15)
Model comparison χ^2 (df)		6.728 (2) *
Inflammasome Pathway Genes		
Morning Fatigue and *NLRP5* rs471979		Coefficient (SE) Final Model
Fixed effects		
Intercept		3.011 (.099) +
Ethnicity[a]		
Black versus White		−.852 (.918)
Asian versus White		.212 (.676)
Hispanic versus White		.310 (.332)
Ancestry informative markers principal components[b]		
PC1		−.101 (.104)
PC2		−.051 (.088)
PC3		.004 (.039)
PW1 – linear		1.190 (.148) +
PW1 – quadratic		−.598 (.071) +
PW2 – linear		.533 (.096) +
PW2 – quadratic		−.153 (.031) +
Time invariant covariates		
PW1 – linear - *NLRP5* rs471979		−3.292 (1.258) *
PW1 – quadratic - *NLRP5* rs471979		1.323 (.595) *
Variance components		
In intercept		3.566 +
Goodness-of-fit deviance (parameters estimated)		11016.309635 (15)
Model comparison χ^2 (df)		10.222 (2) *
Morning Fatigue and *NLRP6* rs74044411		Coefficient (SE) Final Model
Fixed effects		
Intercept		3.010 (.099) +

Cytokine Genes		
Morning Fatigue and *IL12B* rs3213094	**Coefficient (SE)**	
	Unconditional Model	**Final Model**
Ethnicity[a]		
Black versus White		−.727 (.914)
Asian versus White		.229 (.672)
Hispanic versus White		.339 (.330)
Ancestry informative markers principal components[b]		
PC1		−.128 (.103)
PC2		−.068 (.087)
PC3		.011 (.039)
PW1 – linear		1.186 (.148)+
PW1 – quadratic		−.597 (.071)+
PW2 – linear		.534 (.096)+
PW2 – quadratic		−.153 (.031)+
Time invariant covariates		
Intercept		
PW1 – linear - *NLRP6* rs74044411		−1.675 (.785)*
PW1 – quadratic - *NLRP6* rs74044411		.539 (.373)
Variance components		
In intercept		3.522+
Goodness-of-fit deviance (parameters estimated)		11010.712889 (15)
Model comparison χ^2 (df)		15.819 (2)**
Morning Fatigue and *NOD2* rs2076756		Coefficient (SE)
		Final Model
Fixed effects		
Intercept		3.010 (.099)+
Ethnicity[a]		
Black versus White		−.916 (.912)
Asian versus White		.280 (.671)
Hispanic versus White		.359 (.329)
Ancestry informative markers principal components[b]		
PC1		−.096 (.103)
PC2		−.068 (.087)
PC3		.009 (.039)
PW1 – linear		1.192 (.148)+
PW1 – quadratic		−.599 (.071)+
PW2 – linear		.532 (.096)+
PW2 – quadratic		−.153 (.031)+
Time invariant covariates		

Cytokine Genes	
Morning Fatigue and *IL12B* rs3213094	**Coefficient (SE)**
	Unconditional Model / **Final Model**
Intercept - *NOD2* rs2076756	−.960 (.383) *
Variance components	
In intercept	3.507 +
Goodness-of-fit deviance (parameters estimated)	11020.293155 (14)
Model comparison χ^2 (df)	6.239 (1) *
JAK/STAT Pathway Genes	
Morning Fatigue and *IL4R* Liability Score	Coefficient (SE) / Final Model
Fixed Effects	
Intercept	3.010 (.099) +
Ethnicity[a]	
Black versus White	−.772 (.917)
Asian versus White	.233 (.674)
Hispanic versus White	.336 (.331)
Ancestry informative markers principal components[b]	
PC1	−.103 (.103)
PC2	−.061 (.088)
PC3	.012 (.039)
PW1 – linear	1.191 (.148) +
PW1 – quadratic	−.599 (.071) +
PW2 – linear	.532 (.096) +
PW2 – quadratic	−.153 (.031) +
Time invariant covariates	
Intercept	
PW1 – linear - *IL4R* Liability Score	−.401(.168) *
PW1 – quadratic - *IL4R* Liability Score	.167 (.079) *
Variance components	
In intercept	3.548 +
Goodness-of-fit deviance (parameters estimated)	11019.542001 (15)
Model comparison χ^2 (df)	6.990 (2) *
NFkB pathway Genes	
Morning Fatigue and *IL17RB* Liability Score	Coefficient (SE) / Final Model
Fixed effects	
Intercept	3.011 (.099) +
Ethnicity[a]	
Black versus White	−.843 (.913)

Cytokine Genes		
Morning Fatigue and *IL12B* rs3213094	**Coefficient (SE)**	
	Unconditional Model	**Final Model**
Asian versus White		.256 (.672)
Hispanic versus White		.324 (.330)
Ancestry informative markers principal components[b]		
PC1		−.111 (.103)
PC2		−.037 (.088)
PC3		.013 (.039)
PW1 – linear		1.191 (.148)+
PW1 – quadratic		−.599 (.071)+
PW2 – linear		.531 (.096)+
PW2 – quadratic		−.153 (.031)+
Time invariant covariates		
Intercept - *IL17RB* Liability Score		−.352 (.162)*
Variance components		
In intercept		3.519+
Goodness-of-fit deviance (parameters estimated)		11021.825332 (14)
Model comparison χ^2 (df)		4.706 (1)*
Morning Fatigue and *TNFRSF10A* rs17620		Coefficient (SE) Final Model
Fixed effects		
Intercept		3.010 (.099)+
Ethnicity[a]		
Black versus White		−.873 (.916)
Asian versus White		.235 (.674)
Hispanic versus White		.330 (.331)
Ancestry informative markers principal components[b]		
PC1		−.099 (.103)
PC2		−.073 (.088)
PC3		.012 (.039)
PW1 – linear		1.194 (.148)+
PW1 – quadratic		−.601 (.071)+
PW2 – linear		.535 (.096)+
PW2 – quadratic		−.154 (.031)+
Time invariant covariates		
PW1 – linear - *TNFRSF10A* rs17620		.402 (.189)*
PW1 – quadratic - *TNFRSF10A* rs17620		−.193 (.093)*
PW2 – linear - *TNFRSF10A* rs17620		.197 (.127)
PW2 – quadratic - *TNFRSF10A* rs17620		−.051 (.041)

Cytokine Genes		
Morning Fatigue and *IL12B* rs3213094	**Coefficient (SE)**	
	Unconditional Model	**Final Model**
Variance components		
In intercept		3.544 +
Goodness-of-fit deviance (parameters estimated)		11018.710558 (17)
Model comparison χ^2 (df)		7.821 (4)
Morning Fatigue and *TNFRSF10D* Liability Score		Coefficient (SE) Final Model
Fixed effects		
Intercept		3.011 (.099) +
Ethnicity[a]		
Black versus White		−.810 (.917)
Asian versus White		.203 (.675)
Hispanic versus White		.323 (.331)
Ancestry informative markers principal components[b]		
PC1		−.106 (.104)
PC2		−.048 (.088)
PC3		.008 (.039)
PW1 – linear		1.195 (.148) +
PW1 – quadratic		−.601 (.071) +
PW2 – linear		.529 (.096) +
PW2 – quadratic		−.152 (.031) +
Time invariant covariates		
PW2 – linear - *TNFRSF10D* Liability Score		−.211 (.094) *
PW2 – quadratic - *TNFRSF10D* Liability Score		.057 (.033)
Variance components		
In intercept		3.552 +
Goodness-of-fit deviance (parameters estimated)		11018.923385 (15)
Model comparison χ^2 (df)		7.608 (2) *
Morning Fatigue and *TNFRSF11A* Liability Score		Coefficient (SE) Final Model
Fixed effects		
Intercept		3.011 (.099) +
Ethnicity[a]		
Black versus White		−.866 (.914)
Asian versus White		.172 (.673)
Hispanic versus White		.330 (.330)
Ancestry informative markers principal components[b]		
PC1		−.107 (.103)
PC2		−.046 (.087)

Cytokine Genes		
Morning Fatigue and *IL12B* rs3213094	**Coefficient (SE)**	
	Unconditional Model	**Final Model**
PC3		.005 (.039)
PW1 – linear		1.193 (.148)+
PW1 – quadratic		−.600 (.071)+
PW2 – linear		.535 (.096)+
PW2 – quadratic		−.154 (.031)+
Time invariant covariates		
PW1 – linear - *TNFRSF11A* Liability Score		−.381 (.141)*
PW1 – quadratic - *TNFRSF11A* Liability Score		.174 (.070)*
PW2 – linear - *TNFRSF11A* Liability Score		−.130 (.097)
PW2 – quadratic - *TNFRSF11A* Liability Score		.022 (.031)
Variance components		
In intercept		3.532+
Goodness-of-fit deviance (parameters estimated)		11009.606255 (17)
Model comparison χ^2 (df)		16.925 (4)*
Morning Fatigue and *TNFRSF14* rs2234163		Coefficient (SE)
		Final Model
Fixed effects		
Intercept		3.011 (.099)+
Ethnicity[a]		
Black versus White		−.903 (.913)
Asian versus White		.112 (.673)
Hispanic versus White		.311 (.330)
Ancestry informative markers principal components[b]		
PC1		−.104 (.103)
PC2		−.055 (.087)
PC3		.010 (.039)
PW1 – linear		1.192 (.148)+
PW1 – quadratic		−.599 (.071)+
PW2 – linear		.532 (.096)+
PW2 – quadratic		−.153 (.031)+
Time invariant covariates		
Intercept - *TNFRSF14* rs2234163		1.426 (.638)*
Variance components		
In intercept		3.514+
Goodness-of-fit deviance (parameters estimated)		11021.551571 (14)
Model comparison χ^2 (df)		4.980 (1)*
Morning Fatigue and *TNFRSF21* Liability Score		Coefficient (SE)

Cytokine Genes		
Morning Fatigue and *IL12B* rs3213094	**Coefficient (SE)**	
	Unconditional Model	**Final Model**
		Final Model
Intercept		3.012 (.099)+
Ethnicity[a]		
Black versus White		−.815 (.913)
Asian versus White		.281 (.672)
Hispanic versus White		.310 (.330)
Ancestry informative markers principal components[b]		
PC1		−.104 (.103)
PC2		−.069 (.088)
PC3		.001 (.039)
PW1 – linear		1.194 (.148)+
PW1 – quadratic		−.601 (.071)+
PW2 – linear		.531 (.096)+
PW2 – quadratic		−.153 (.031)+
Time invariant covariates		
Intercept *TNFRSF21* Liability Score		.416 (.183)*
Variance components		
In intercept		3.517+
Goodness-of-fit deviance (parameters estimated)		11021.369941 (14)
Model comparison χ^2 (df)		5.162 (1)*

[a]Self-reported ethnicity – represented by three “dummy” coded variables

[b]Ancestry informative markers - represented by the first three PCs (i.e., PC1, PC2, PC3)

*p<.05,

**p<.001,

+p<.0001

Abbreviations: df = degrees of freedom; *IL4R* = Interleukin 4 receptor; *IL12B*= Interleukin 12B; *IL17RB* = Interleukin 17 receptor B; JAK/STAT = Janus kinase/signal transducers and activators of transcription; NFkB = nuclear factor-kappa beta; *NLRC5* = Nod-Like receptor family, caspase recruitment domain containing 5; *NLRP5* = Nod-Like receptor family, pyrin domain containing 5; *NLRP6* = Nod-Like receptor family, pyrin domain containing 6; *NOD2* = Nucleotide-Binding oligomerization domain containing 2; PC=principal component; *PW1* = piecewise 1 PW2 = piecewise 2; rs = reference SNP cluster identification number; SE = standard error; *TNFA* = Tumor necrosis factor alpha; *TNFRSF10A* = Tumor necrosis factor receptor superfamily member 10A; *TNFRSF10D* = Tumor necrosis factor receptor superfamily member 10D; *TNFRSF11A* = Tumor necrosis factor receptor superfamily member 11A; *TNFRSF14* = Tumor necrosis factor receptor superfamily member 14; *TNFRSF21* = Tumor necrosis factor receptor superfamily member 21

Table 5

Hierarchical Linear Models for Evening Fatigue

Cytokine Genes		
Evening Fatigue and *IL12B* rs3213094	**Coefficient (SE)**	
	Unconditional Model	**Final Model**
Fixed effects		
Intercept	5.310 (.090)+	5.307 (.090)+
Ethnicity[a]		
Black versus White	−1.584 (.824)	−1.501 (.822)
Asian versus White	−.269 (.608)	−.231 (.606)
Hispanic versus White	−.348 (.298)	−.332 (.297)
Ancestry informative markers principal components		
PC1	−.070 (.093)	−.072 (.093)
PC2	−.044 (.079)	−.029 (.079)
PC3	−.009 (.035)	−.008 (.035)
PW 1 – linear	.601 (.137)+	.601 (.137)+
PW 1 – quadratic	−.306 (.066)+	−.306 (.066)+
PW 2 – linear	.394 (.088)+	.394 (.088)+
PW 2 – quadratic	−.113 (.028)+	−.113 (.028)+
Time invariant covariates		
Intercept *IL12B* rs3213094		−.573 (.288)*
Variance components		
In intercept	2.867+	2.843+
Goodness-of-fit deviance (parameters estimated)	10399.632349 (13)+	10395.680232 (14)
Model comparison χ^2 (df)		3.952 (1)*
Evening Fatigue and *IL12B* Liability Score		Coefficient (SE) Final Model
Fixed effects		
Intercept		5.308 (.090)+
Ethnicity[a]		
Black versus White		−1.579 (.820)
Asian versus White		−.322 (.605)
Hispanic versus White		−.347 (.297)
Ancestry informative markers principal components[b]		
PC1		−.086 (.093)
PC2		−.021 (.079)
PC3		−.003 (.035)
PW 1 – linear		.600 (.137) +
PW 1 – quadratic		−.306 (.066) +

Cytokine Genes		
Evening Fatigue and *IL12B* rs3213094	**Coefficient (SE)**	
	Unconditional Model	**Final Model**
PW 2 – linear		.393 (.088) +
PW 2 – quadratic		−.112 (.028) +
Time invariant covariates		
Intercept - *IL12B* Liability Score		−.258 (.115) *
Variance components		
In intercept		2.838 +
Goodness-of-fit deviance (parameters estimated)		10394.643652 (14)
Model comparison χ^2 (df)		4.989 (1) *
Evening Fatigue and *TNFA* rs1041981		Coefficient (SE)
		Final Model
Fixed effects		
Intercept		5.309 (.090) +
Ethnicity[a]		
Black versus White		−1.548 (.822)
Asian versus White		−.293 (.606)
Hispanic versus White		−.369 (.298)
Ancestry informative markers principal components[b]		
PC1		−.073 (.093)
PC2		−.038 (.079)
PC3		−.002 (.035)
PW 1 – linear		.596 (.137) +
PW 1 – quadratic		−.304 (.066) +
PW 2 – linear		.395 (.088) +
PW 2 – quadratic		−.113 (.028) +
Time invariant covariates		
Intercept		
PW 1 – linear - *TNFA* rs1041981		−.602 (.256) *
PW 1 – quadratic - *TNFA* rs1041981		.252 (.121) *
Variance components		
In intercept		2.852 +
Goodness-of-fit deviance (parameters estimated)		10392.936781 (15)
Model comparison χ^2 (df)		6.696 (2) *
Inflammasome Pathway Genes		
Evening Fatigue and *CARD6* rs10512747		Coefficient (SE)
		Final Model
Fixed effects		
Intercept		5.309 (.089) +

Cytokine Genes	
Evening Fatigue and *IL12B* rs3213094	**Coefficient (SE)**
	Unconditional Model / **Final Model**
Ethnicity[a]	
Black versus White	−1.591 (.819)
Asian versus White	−.350 (.605)
Hispanic versus White	−.397 (.297)
Ancestry informative markers principal components[b]	
PC1	−.057 (.093)
PC2	−.043 (.078)
PC3	−.008 (.035)
PW 1 – linear	.599 (.137)+
PW 1 – quadratic	−.306 (.066)+
PW 2 – linear	.394 (.088)+
PW 2 – quadratic	−.113 (.028)+
Time invariant covariates	
Intercept - *CARD6* rs10512747	−.441 (.176)*
Variance components	
In intercept	2.829+
Goodness-of-fit deviance (parameters estimated)	10393.393966 (14)
Model comparison χ^2 (df)	6.238 (1)*
Evening Fatigue and *NLRP4* rs17857373	Coefficient (SE)
	Final Model
Fixed effects	
Intercept	5.308 (.090)+
Ethnicity[a]	
Black versus White	−1.600 (.821)
Asian versus White	−.298 (.605)
Hispanic versus White	−.365 (.297)
Ancestry informative markers principal components[b]	
PC1	−.061 (.093)
PC2	−.046 (.079)
PC3	−.003 (.035)
PW 1 – linear	.601 (.137)+
PW 1 – quadratic	−.306 (.066)+
PW 2 – linear	.394 (.088)+
PW 2 – quadratic	−.113 (.028)+
Time invariant covariates	
Intercept - *NLRP4* rs17857373	−.603 (.291)*
Variance components	

Cytokine Genes	
Evening Fatigue and *IL12B* rs3213094	**Coefficient (SE)**
	Unconditional Model / **Final Model**
In intercept	2.841 +
Goodness-of-fit deviance (parameters estimated)	10395.340962 (14)
Model comparison χ^2 (df)	4.291 (1) *
Evening Fatigue and *NLRP6* rs74044411	Coefficient (SE)
	Final Model
Fixed effects	
Intercept	5.309 (.090) +
Ethnicity[a]	
Black versus White	−1.491 (.822)
Asian versus White	−.257 (.606)
Hispanic versus White	−.339 (.297)
Ancestry informative markers principal components[b]	
PC1	−.093 (.093)
PC2	−.060 (.079)
PC3	−.007 (.035)
PW 1 – linear	.598 (.136) +
PW 1 – quadratic	−.306 (.066) +
PW 2 – linear	.395 (.088) +
PW 2 – quadratic	−.113 (.028) +
Time invariant covariates	
Intercept	
PW 1 – linear - *NLRP6* rs74044411	−1.417 (.682) *
PW 1 – quadratic - *NLRP6* rs74044411	.449 (.322)
Variance components	
In intercept	2.847 +
Goodness-of-fit deviance (parameters estimated)	10385.256709 (15)
Model comparison χ^2 (df)	14.376 (2) **
Evening Fatigue and *NOD2* rs2076756	Coefficient (SE)
	Final Model
Fixed effects	
Intercept	5.308 (.089) +
Ethnicity[a]	
Black versus White	−1.684 (.813) *
Asian versus White	−.177 (.599)
Hispanic versus White	−.304 (.294)
Ancestry informative markers principal components[b]	
PC1	−.061 (.092)

Cytokine Genes		
Evening Fatigue and *IL12B* rs3213094	**Coefficient (SE)**	
	Unconditional Model	**Final Model**
PC2		−.067 (.078)
PC3		−.008 (.035)
PW 1 – linear		.601 (.137)+
PW 1 – quadratic		−.306 (.066)+
PW 2 – linear		.394 (.088)+
PW 2 – quadratic		−.113 (.028)+
Time invariant covariates		
Intercept - *NOD2* rs2076756		−1.350 (.342)+
Variance components		
In intercept		2.777+
Goodness-of-fit deviance (parameters estimated)		10384.268252 (14)
Model comparison χ^2 (df)		15.364 (1)+
MAP/JNK Pathway Genes		
Evening Fatigue and *IL17RD* rs61742267		Coefficient (SE)
		Final Model
Fixed effects		
Intercept		5.310 (.090)+
Ethnicity[a]		
Black versus White		−1.539 (.821)
Asian versus White		−.265 (.605)
Hispanic versus White		−.319 (.297)
Ancestry informative markers principal components[b]		
PC1		−.059 (.093)
PC2		−.044 (.079)
PC3		−.013 (.035)
PW 1 – linear		.601 (.137)+
PW 1 – quadratic		−.306 (.066)+
PW 2 – linear		.393 (.088)+
PW 2 – quadratic		−.112 (.028)+
Time invariant covariates		
Intercept - *IL17RD* rs61742267		−.933 (.429)*
Variance components		
In intercept		2.840+
Goodness-of-fit deviance (parameters estimated)		10394.915944 (14)
Model comparison χ^2 (df)		4.716 (1)*
NfKB Pathway Genes		
Evening Fatigue and *IL17RB* rs2232346 and *IL17RB* rs1043261		Coefficient (SE)

Cytokine Genes		
Evening Fatigue and *IL12B* rs3213094	**Coefficient (SE)**	
	Unconditional Model	**Final Model**
		Final Model
Fixed effects		
Intercept		5.308 (.089) [+]
Ethnicity[a]		
Black versus White		−1.573 (.817)
Asian versus White		−.179 (.603)
Hispanic versus White		−.344 (.296)
Ancestry informative markers principal components[b]		
PC1		−.065 (.092)
PC2		−.037 (.078)
PC3		−.009 (.035)
PW 1 – linear		.600 (.137) [+]
PW 1 – quadratic		−.306 (.066) [+]
PW 2 – linear		.394 (.088) [+]
PW 2 – quadratic		−.113 (.028) [+]
Time invariant covariates		
Intercept - *IL17RB* rs2232346		−.791 (.388) [*]
Intercept - *IL17RB* rs1043261		−1.456 (.584) [*]
Variance components		
In intercept		2.809 [+]
Goodness-of-fit deviance (parameters estimated)		10389.389046 (15)
Model comparison χ^2 (df)		10.243 (2) [*]
Evening Fatigue and *LTBR* Liability Score		Coefficient (SE)
		Final Model
Fixed effects		
Intercept		5.309 (.090) [+]
Ethnicity[a]		
Black versus White		−1.562 (.826)
Asian versus White		−.277 (.609)
Hispanic versus White		−.346 (.299)
Ancestry informative markers principal components[b]		
PC1		−.072 (.093)
PC2		−.044 (.079)
PC3		−.010 (.035)
PW 1 – linear		.604 (.136) [+]
PW 1 – quadratic		−.308 (.066) [+]
PW 2 – linear		.396 (.088) [+]

Cytokine Genes		
Evening Fatigue and *IL12B* rs3213094	**Coefficient (SE)**	
	Unconditional Model	**Final Model**
PW 2 – quadratic		−.113 (.028) +
Time invariant covariates		
Intercept		
PW 1 – linear - *LTBR* Liability Score		−3.188 (1.080) *
PW 1 – quadratic - *LTBR* Liability Score		1.540 (.513) *
Variance components		
In intercept		2.885 +
Goodness-of-fit deviance (parameters estimated)		10390.660476 (15)
Model comparison χ^2 (df)		8.972 (2) *
Evening Fatigue and *TNFRSF14 rs2234163*		Coefficient (SE)
		Final Model
Fixed effects		
Intercept		5.309 (.090) +
Ethnicity[a]		
Black versus White		−1.648 (.818)
Asian versus White		−.383 (.605)
Hispanic versus White		−.366 (.296)
Ancestry informative markers principal components[b]		
PC1		−.072 (.092)
PC2		−.048 (.078)
PC3		−.007 (.035)
PW 1 – linear		.600 (.137) +
PW 1 – quadratic		−.306 (.066) +
PW 2 – linear		.394 (.088) +
PW 2 – quadratic		−.113 (.028) +
Time invariant covariates		
Intercept - *TNFRSF14 rs2234163*		1.590 (.572) *
Variance components		
In intercept		2.821 +
Goodness-of-fit deviance (parameters estimated)		10391.966279 (14)
Model comparison χ^2 (df)		7.666 (1) *

[a] Self-reported ethnicity – represented by three “dummy” coded variables

[b] Ancestry informative markers - represented by the first three PCs (i.e., PC1, PC2, PC3)

* p<.05,

** p<.001,

+ p<.0001

Abbreviations: *CARD6* = Caspase recruitment domain family member 6; df = degrees of freedom; *IL12B*= Interleukin 12B; *IL17RB* = Interleukin 17 receptor B; *IL17RD* = Interleukin 17 receptor D; JAK = jaunts kinase transducers and activators of transcription; *LTBR* = Lymphotoxin beta receptor; MAP = mitogen-activated protein kinase; NFkB = nuclear factor-kappa beta; *NLRP4* = Nod-Like receptor family, pyrin domain containing 4; *NLRP6* = Nod-Like receptor family, pyrin domain containing 6; *NOD2* = Nucleotide-Binding oligomerization domain containing 2; PC=principal component; PW1 = piecewise 1; PW2 = piecewise 2; rs = reference SNP cluster identification number; SE = standard error; *TNFA* = Tumor necrosis factor alpha; *TNFRSF14* = Tumor necrosis factor receptor superfamily member 14.

Table 6

Comparison of Genes Associated with Interindividual Differences in Morning and Evening Fatigue in Patients Receiving Chemotherapy

Pathway	Morning Fatigue	Evening Fatigue
Cytokine genes	*TNFA* rs1041981	*TNFA* rs1041981
	IL12B rs3213094	*IL12B* rs3213094
		IL12B liability score
Inflammasome pathway genes	*NLRP6* rs74044411	*NLRP6* rs74044411
	NOD2 rs2076756	*NOD2* rs2076756
	NLRP5 rs471979	*CARD6* rs10512747
		NLRP4 rs1785737
JAK/STAT pathway genes	*IL4R* liability score	None
MAP/JNK pathway genes	None	*IL17RD* rs61742267
NfKB pathway genes	*TNFRSF14* rs2234163	*TNFRSF14* rs2234163
	IL17RB liability score	*IL17RB* rs2232346
	TNFRSF10A rs17620	*IL17RB* rs1043261
	TNFRSF10D liability score	*LTBR* liability score
	TNFRSF11A liability score	
	TNFRSF21 liability score	

Abbreviations: *CARD6* = caspase recruitment domain family member 6; *IL4R* = interleukin 4 receptor; *IL12B* = interleukin 12B; *IL17RB* = interleukin 17 receptor B; *IL17RD* = interleukin 17 receptor D: JAK/STAT = Janus kinase/signal transducers and activators of transcription; *LTBR* = lymphotoxin beta receptor; MAP = mitogen-activated protein kinase; NFkB = nuclear factor-kappa beta; *NLRC5* = nod-like receptor family, caspase recruitment domain containing 5; *NLRP4* = nod-like receptor family, pyrin domain containing 4; *NLRP5* = nod-like receptor family, pyrin domain containing 5; *NLRP6* = nod-like receptor family, pyrin domain containing 6; *NOD2* = nucleotide-binding oligomerization domain containing 2; rs = reference SNP cluster identification number; *TNFA* = tumor necrosis factor alpha; *TNFRSF10A* = tumor necrosis factor receptor superfamily member 10A; *TNFRSF10D* = tumor necrosis factor receptor superfamily member 10D; *TNFRSF11A* = Tumor necrosis factor receptor superfamily member 11A; *TNFRSF14* = tumor necrosis factor receptor superfamily member 14; *TNFRSF21* = tumor necrosis factor receptor superfamily member 21