Cytokine polymorphisms are associated with fatigue in adults living with HIV/AIDS

Kathryn A. Lee[a], **Caryl L. Gay**[a,b,c], **Anners Lerdal**[b,d], **Clive R. Pullinger**[e,f], and **Bradley E. Aouizerat**[e,g]

[a]Department of Family Health Care Nursing, University of California, San Francisco, CA, USA

[b]Department of Research, Lovisenberg Diakonale Hospital, Oslo, Norway

[c]Lovisenberg Diakonale University of College, Oslo, Norway

[d]Department of Nursing Science, Institute of Health and Society, Faculty of Medicine, University of Oslo, Norway

[e]Department of Physiological Nursing, University of California, San Francisco, CA, USA

[f]Cardiovascular Research Institute, University of California, San Francisco, CA, USA

[g]Institute for Human Genetics, University of California, San Francisco, CA, USA

Abstract

Fatigue has been associated with inflammation and cytokine activity among adults, but this relationship has not been evaluated among adults living with HIV. Diurnal patterns of fatigue have been previously identified in adults with HIV/AIDS. Thus, the purpose of this study was to describe these fatigue patterns in relation to cytokine plasma concentrations and gene polymorphisms. A convenience sample of 317 adults living with HIV/AIDS completed a measure of fatigue in the morning and evening for three consecutive days; participants reporting low levels of both morning and evening fatigue (n=110) or high levels of fatigue in the morning and evening (n=114) were included in the analysis, resulting in a final sample of 224 adults (151 men, 55 women, and 18 transgender). Plasma cytokines were analyzed, and genotyping was conducted for 15 candidate genes involved in cytokine signaling: interferon-gamma (IFNG), IFNG receptor 1 (IFNGR1), interleukins (IL), nuclear factor of kappa light polypeptide gene enhancer in B cells (NFKB-1 and -2), and tumor necrosis factor alpha (TNFA). Demographic and clinical variables were evaluated as potential covariates. Controlling for genomic estimates of ancestry and self-reported race/ethnicity and gender, the high fatigue pattern was associated with five single nucleotide polymorphisms (SNPs): IL1B rs1071676 and rs1143627, IL4 rs2243274, and TNFA rs1800683 and rs1041981. The IL1B and TNFA polymorphisms were not associated with plasma

Corresponding Author: Kathryn A. Lee, RN, PhD, FAAN, School of Nursing, Box 0606, University of California, San Francisco, 2 Koret Way, Room N415Y, San Francisco, CA 94143-0606, (415) 476-4442 (phone) (415) 753-2161 (fax), Kathryn.lee@nursing.ucsf.edu.

levels of IL-1β or TNFα, respectively. This study strengthens the evidence for an association between inflammation and fatigue. In this chronic illness population, the cytokine polymorphisms associated with high levels of morning and evening fatigue provide direction for future personalized medicine intervention research.

Keywords

fatigue; cytokine; inflammation; genetic; biomarker; HIV

1. Introduction

Fatigue is a common symptom among individuals living with chronic illness, and there is growing evidence from animal models (Harrington, 2012) and adults with chronic illness (Bower et al., 2013; Miaskowski et al., 2010) that fatigue is associated with inflammation and cytokine activity in plasma. Tumor necrosis factor – alpha (TNF-α)(Aouizerat et al., 2009; Bower et al., 2013; Fung et al., 2013; Jim et al., 2012), interleukin 6 (IL6)(Inagaki et al., 2013; Rohleder et al., 2012; Schrepf et al., 2013; Schubert et al., 2007; Starkweather, 2013), and IL-1 beta (IL-1β) (Bower, 2007; Saligan and Kim, 2012; van Zuiden et al., 2012) exhibit the strongest relationships with fatigue in prior studies, although other cytokine-fatigue associations have also been reported (Bower et al., 2011; Liu et al., 2012; Piraino et al., 2012) and still other studies have yielded contradictory results (Cameron et al., 2012; Dirksen et al., 2013; Geinitz et al., 2004; Hamre et al., 2013). Both cytokine plasma concentrations and polymorphisms are implicated in fatigue, yet the nature of these relationships remains poorly understood.

Although much of the research on inflammation, cytokines and fatigue focuses on adults with cancer, fatigue is a well-documented symptom among adults living with HIV, with an estimated 33%–88% of adults with HIV/AIDS experiencing fatigue (Barroso and Voss, 2013; daCosta DiBonaventura et al., 2012; Jong et al., 2010; Lee et al., 2009; Millikin et al., 2003; Pence et al., 2009; Sullivan and Dworkin, 2003). Fatigue was associated with some of the early immunotherapies for HIV/AIDS, such as interferon alpha (Finter et al., 1991) and IL2 (Grady et al., 1998) but otherwise, the relationship between inflammatory markers and fatigue in adults living with HIV is poorly understood. In our prior work, we identified several fatigue profiles based on an individual's diurnal fatigue patterns: low fatigue in both the morning and evening, high fatigue in both morning and evening, high fatigue in the morning only, and high fatigue in the evening only (Lerdal et al., 2011). The profile involving a high level of fatigue in both the morning and evening was associated with the greatest fatigue-related distress compared to individuals with consistently low levels of fatigue. Therefore, the aims of this study were to evaluate these two fatigue profiles in relation to cytokine plasma concentrations and polymorphisms and to describe the relative contributions of inflammatory markers in accounting for high levels of morning and evening fatigue among adults living with HIV.

2. Method

2.1 Participants and Setting

The Symptom and Genetic Study is a longitudinal study aimed at identifying biomarkers of symptom experience among HIV-infected adults (Lee et al., 2009). This analysis reports on cytokine-related biomarkers of fatigue. The Committee on Human Research at the University of California, San Francisco (UCSF) approved the study protocol. Participants were recruited using flyers posted at local HIV clinics and community sites. Participants provided written informed consent and signed a Health Insurance Portability and Accountability Act release to access their protected medical information for this research. Study visits were conducted at the UCSF Clinical Research Center (CRC).

Eligible participants were English-speaking adults at least 18 years of age who had been diagnosed with HIV at least 30 days before enrollment. To specifically address HIV-related symptom experience, potential participants were excluded if they currently used illicit drugs (as determined by self-report or by positive urine drug testing); worked nights (i.e., at least four hours between 12 $_{AM}$ and 6 $_{AM}$); reported having bipolar disorder, schizophrenia, or dementia; or were pregnant within the prior three months. Participants were not excluded for insomnia, but were excluded for other diagnosed sleep disorders, such as apnea and narcolepsy.

2.2 Measures

2.2.1 Demographic, clinical, and laboratory characteristics—A demographic questionnaire was used to collect information about the participant's age, gender, race/ethnicity, educational background, employment status, monthly income, and relationship status. Health history (i.e., time since HIV diagnosis, prior AIDS diagnosis) and current medication regimen were obtained by self-report. Medications were categorized as antiretroviral therapy (ART), sleep medication, anxiolytics, antidepressants, and opiates, based on the potential for such medications to impact fatigue. Trained research staff obtained measures of body mass index (i.e., weight in kilograms divided by squared height in meters) during a CRC visit. CD4+ T-cell count, HIV viral load values, and hemoglobin values were obtained from the most recent laboratory report in the patients' medical record.

2.2.2 Biomarkers—Fasting blood samples were obtained from each participant during the CRC visit. Blood was processed for genomic DNA and plasma and plasma samples were stored at −80C. An aliquot of each plasma sample was shipped to Biomarker Services (EMD Millipore, St. Charles, MO) on dry-ice for analysis. Plasma levels of six cytokines (i.e., IL-1β, IL-2, IL-6, IL-10, IL-13, TNFα) were assayed using the Luminex xMAP multiplex platform. IL-4 was also included in the assay panel, but the majority of the sample values were below the lower limit of detection for the IL-4 assay; thus IL-4 was excluded from subsequent analyses. Given its association with fatigue in prior studies (Klimas et al., 2012), C-reactive protein [CRP] plasma levels were also assayed.

Fifteen cytokine candidate genes were selected for analysis based on their known influence on inflammatory processes. Genomic DNA was extracted from peripheral blood

mononuclear cells using the PUREGene DNA Isolation System (Invitrogen, Carlsbad, CA) and maintained by the UCSF Genomic Markers of Symptoms Tissue Bank (Aouizerat et al., 2009; Miaskowski et al., 2010). DNA was isolated from 348 (99.4%) of the participants. Genotyping was performed blinded to clinical status and included positive and negative controls. DNA samples were quantitated with a Nanodrop Spectrophotometer (ND-1000) and normalized to a concentration of 50 ng/μL (diluted in 10 mM Tris/1 mM EDTA). Samples were genotyped using the GoldenGate genotyping platform (Illumina, San Diego, CA) and processed according to standard protocol using GenomeStudio (Illumina, San Diego, CA). Signal intensity profiles and resulting genotype calls for each single nucleotide polymorphism (SNP) were visually inspected by two blinded reviewers. Disagreements were adjudicated by a third reviewer.

A combination of tagging SNPs and literature driven SNPs (e.g., SNPs reported as being associated with altered function) were selected for analysis. Tagging SNPs were required to be common (defined as having a rare allele frequency 0.05) in public databases (e.g., HapMap). In order to ensure robust genetic association analyses, quality control filtering of SNPs was performed. All SNPs had call rates of > 95%, and two SNPs were excluded with Hardy- Weinberg P-values of < 0.001. To maximize the power to detect genetic associations due to common genetic risk factors, 22 SNPs with allele frequencies of less than 5% (n=7) or with less than 3 individuals homozygous for the rare allele (n=22) were excluded from analysis. As shown in Table 4, 80 SNPs among the 15 candidate genes passed all quality control filters and were included in the genetic association analyses. In order to control for potential confounding due to genomic ancestry, 106 ancestry informative marker (AIM) SNPs were also genotyped.

2.2.3 Fatigue severity—A 4-item version of the Lee Fatigue Scale (LFS)(Lee et al., 1991) was used to assess fatigue severity in the morning and evening. This measure focuses primarily on general fatigue and how participants are feeling 'right now' rather than specific dimensions of fatigue (e.g., mental or physical) over a broader timeframe. Participants completed the LFS within 30 minutes prior to going to sleep to measure fatigue on 3 consecutive evenings and within 30 minutes of waking on 3 consecutive mornings. Evening and morning fatigue scores were calculated as the mean of the 4 items across the 3 days and could range from 0 to 10, with higher scores indicating greater fatigue. Our prior study (Lerdal et al., 2011) used a median split of morning and evening fatigue ratings to distinguish four unique fatigue profiles: low fatigue in both the morning and evening, high morning fatigue only, high evening fatigue only, and high fatigue in both the morning and evening. The LFS has been used to measure fatigue in healthy individuals (Gay et al., 2004; Lee et al., 1991), as well as in patients with cancer (Miaskowski et al., 2008) and HIV (Lee et al., 1999) and has established validity and internal consistency. In this sample, the Cronbach alpha coefficients were .88 for evening fatigue ratings and .93 for morning fatigue ratings.

2.3 Statistical Analysis

Except where indicated below, analyses were conducted using Stata (version 12.0, College Station, TX). Descriptive statistics were used to summarize demographic, clinical, and

biomarker characteristics. Square root transformations were sufficient to normalize skewed distributions for CD4+ T-cell count, and log transformation was sufficient to normalize viral load values. CD4+ T-cell count and viral load were analyzed in clinically meaningful categories. Demographic and clinical differences between the fatigue groups were evaluated using analysis of variance with Scheffé post hoc tests or Chi-square test of independence. Allele and genotype frequencies were determined by gene counting. Hardy-Weinberg equilibrium was assessed by the Chi-square exact test.

Differences in cytokine plasma levels between the fatigue groups were assessed using Mann-Whitney U Test, using Bonferroni-adjusted p-values to correct for multiple comparisons. Adjusted associations were evaluated using logistic regression models predicting membership in the high fatigue group and controlling for relevant demographic and clinical covariates. A model was fit for each plasma cytokine to estimate its unique contribution to membership in the high fatigue group when adjusting for relevant covariates. All adjusted regression models controlled for genomic estimates of ancestry (described below), self-reported race/ethnicity (i.e., Caucasian, African-American, other), and gender, given the potential of these variables to confound genetic associations and therefore downstream biomarkers (i.e., protein product of a gene). In addition, all demographic and clinical variables associated with fatigue group ($p < 0.10$) were evaluated as potential covariates. Variables were retained as covariates in all adjusted models if their significance was $p < 0.05$ prior to including cytokine plasma level in the model.

Unadjusted genetic associations were determined using logistic regression models predicting membership in the high fatigue group. Three genetic models were tested (i.e., additive, dominant, and recessive), and the model that best fit the data (barring improvements of delta < 10%) was reported for each SNP. Genetic markers were further evaluated in multiple variable logistic regression models controlling for relevant covariates. As in the unadjusted regression analyses, the adjusted models predicted membership in the high fatigue group. A model was fit for each genetic marker to estimate its unique contribution to fatigue group when adjusting for relevant covariates. The same covariates used for the adjusted plasma cytokine models were used in the adjusted genetic association models. Each polymorphism associated with fatigue group in adjusted analyses was evaluated with respect to its impact on cytokine plasma levels using linear regression models (1000 draws), controlling for relevant covariates as described above. Models were bootstrapped with 1000 draws due to non-normal distributions of the cytokine plasma levels that could not be adequately corrected by transformation.

Ancestry informative markers (AIMs) are used to minimize bias due to population substructure (Halder et al., 2008; Hoggart et al., 2003; Tian et al., 2008). Homogeneity in ancestry among participants was estimated by principal component analysis with orthogonal rotation (Price et al., 2006) using HelixTree software (GoldenHelix, Bozeman, MT). With 106 AIMs included in this analysis, principal components (PC) were sought that distinguished the major racial/ethnic groups in the sample (i.e., Caucasian, African-American, other) by visual inspection of scatter plots of orthogonal PCs (PC1 versus PC2, PC2 versus PC3). This procedure was repeated until no discernible clustering of participants by self-reported race/ethnicity was possible. The first three PCs were included in all adjusted

regression models to control for potential confounding due to genomic differences in ancestry.

3. Results

3.1 Sample characteristics

A convenience sample of 350 adults with HIV was enrolled in the study, and 33 participants were excluded prior to analysis for either screening positive for illicit drugs (n=31) or being unable to submit a urine or blood sample (n=2). Of the remaining 317 participants, the 224 with relatively high (n=114) or relatively low (n=110) levels of fatigue in both the morning and evening were included in the analysis. Due to the small sample sizes, participants discordant in their reported of experience of morning fatigue only (n=47) or evening fatigue only (n=46) were excluded. Demographic and clinical characteristics for the 224 participants included in the final sample are presented in Table 1. The sample was ethnically diverse and predominantly male, reflecting the local population of adults with HIV. Participants had been living with HIV for an average of 12.4 ± 6.8 years; 51% had been diagnosed with AIDS, but only 29% of those with an AIDS diagnosis had current CD4+ T-cell counts below 200 cells/mm^3. Most (75%) were receiving medical disability assistance, 69% were currently receiving ART, and they were taking an average of 6.1 ± 4.2 medications (median 5, range 0–22).

Plasma cytokine levels did not differ by gender, but there were slight differences by race/ethnicity, with African-Americans having lower levels of IL-6 (21.2 ± 40.6 vs. 25.2 ± 35.5 pg/mL, $p = .003$), IL-10 (23.5 ± 55.9 vs. 28.6 ± 54.8 pg/mL, $p = .008$), and TNFα (10.5 ± 10.4 vs. 13.8 ± 12.1 pg/mL, $p = .038$) than participants in the 'other' race/ethnicity group. Plasma cytokine levels also differed by several HIV disease indicators: lower TNFα plasma levels were associated with ART (10.6 ± 9.5 vs. 16.6 ± 14.4 pg/mL, $p = 0.002$), CD4+ T-cell counts of at least 200 cells/mm3 (11.9 ± 11.4 vs. 16.3 ± 12.3 pg/mL, $p = 0.006$), and undetectable viral loads (9.4 ± 8.7 vs. 16.2 ± 13.5 pg/mL, $p < 0.001$).

3.2 Fatigue characteristics

The mean fatigue ratings were 3.70 ± 2.54 in the morning and 5.11 ± 2.43 in the evening, and scores for both had a range of 0 to 10. Participants in the low fatigue group (n=110) had a mean morning rating of 1.49 ± 1.03 and a mean evening rating of 3.00 ± 1.45, while the high fatigue group (n=114) had a mean morning rating 5.84 ± 1.54 and a mean evening rating of 7.15 ± 1.08. As shown in Table 1, the demographic variables that differed ($p < 0.10$) by fatigue group included self-reported race/ethnicity, education level, and income, with African-American race/ethnicity being associated with low fatigue and higher education and income being associated with high fatigue. Of the clinical variables, participants taking ART or with an AIDS diagnosis were more likely to be in the high fatigue group. The fatigue groups did not differ with respect to hemoglobin level or BMI even when stratified by gender or race/ethnicity. A correlation matrix of these potential covariates is included in Table 2.

3.3 Plasma cytokines

As shown in Table 3, the fatigue groups differed with respect to two of the cytokine plasma levels: IL-6 and IL-10. To estimate the plasma cytokines' effect on fatigue group when adjusting for relevant covariates, multiple linear regression models were fit for each plasma cytokine. To control for population substructure, genomic estimates of ancestry, as well as self-reported race/ethnicity and gender, were forced into all models. Education, income, ART, and AIDS diagnosis were evaluated as potential covariates but did not meet the criterion for retention ($p < 0.05$) in the final models. In the multivariable analyses adjusting for genomic estimates of ancestry, self-reported race/ethnicity, and gender, none of the cytokine plasma levels evaluated was significantly associated with fatigue group.

3.4 Genetic associations

Of the 80 SNPs examined, 31 SNPs mapping to 13 of the 15 evaluated candidate genes were significantly associated with fatigue group in bivariate analyses (Table 4). To estimate genotype's effect on fatigue group when adjusting for relevant covariates, multiple linear regression models were fit for these 31 SNPs using the same approach and covariates as in the plasma level models described above. After adjusting for genomic estimates of ancestry, self-reported race/ethnicity, and gender, three SNPs remained associated with fatigue group: IL1B rs1071676 and rs1143627 and IL4 rs2243274 (Table 4). In addition, two SNPs in TNFA (i.e., rs1800683, rs1041981) that were not associated with fatigue in bivariate analyses were significantly associated with fatigue after controlling for the confounding effects of genomic ancestry, self-reported race/ethnicity, and gender.

The IL1B polymorphisms (i.e., rs1143627, rs16944) were not associated with plasma levels of IL-1β, and the TNFA polymorphisms (i.e., rs1800683, rs1041981) were not associated with plasma levels of TNFα in bootstrapped regression analyses adjusting for genomic estimates of ancestry, self-reported race/ethnicity, and gender. Because the IL-4 plasma assay failed quality control, IL-4 plasma levels could not be evaluated in relation to the IL4 polymorphism (i.e., rs2243274).

3.5 Analyses by racial/ethnic sub-group

Given the significant role of race/ethnicity in predicting fatigue group and attenuating the effect of genotype in multivariable analyses, the regression models were repeated separately for African-American and Caucasian sub-groups, while still controlling for genomic ancestry and gender. As shown in Table 5, only one of the race-specific models indicated a significant association between cytokine genotype and fatigue group (i.e. TNFA rs1800683 among African-Americans), which may be due to the limited statistical power of the sub-group analyses given that the magnitude of the estimates were often similar to (or stronger than) those in the overall analyses. However, examination of the genetic associations across the African-American and Caucasian sub-groups indicates that, at least for some SNPs, the associations may be stronger in one racial/ethnic sub-group than the other. In addition, IL4 rs2243266 (and IL4 rs2243267 which was in perfect linkage disequilibrium) was significantly associated with fatigue group among the Caucasian sub-group (OR 0.34, 95% CI 0.12, 0.98, p=.046), even though it did not reach significance in the sample as a whole

(OR=0.54, 95% CI 0.29, 1.00, *p*=.052) and was not associated with fatigue in the African-American sub-group (OR=0.71, 95% CI 0.27, 1.89, *p*=.492).

3.6 Analyses by gender sub-group

To ensure that the findings were not unduly influenced by the sample's unequal gender distribution, the regression models were repeated separately for male and female sub-groups, while still controlling for genomic ancestry and self-reported race. As shown in Table 5, several of the analyses indicate a significant genetic association among males (i.e. IL1B rs1071676 and IL4 rs2243274), but not among females. As with the analyses by racial/ethnic sub-group, the gender-specific analyses had limited statistical power, particularly for females. However, comparison of the odds ratios for males and females suggests that the magnitude of the genetic associations may differ by gender. Furthermore, the TNFA polymorphisms appeared to have markedly stronger associations with fatigue in the female sub-group than the male sub-group, despite the smaller sample of women.

4. Discussion

Five polymorphisms from three genes (IL1B, IL4, and TNFA) were significantly associated with fatigue after adjusting for genomic estimates of ancestry, self-reported race/ethnicity, and gender. Prior studies of adults with cancer have identified similar associations between fatigue and IL1B (Bower et al., 2013; Collado-Hidalgo et al., 2008) and TNFA polymorphisms (Aouizerat et al., 2009; Bower et al., 2013; Dhruva et al., in press; Jim et al., 2012), and IL4 polymorphisms have been previously associated with evening fatigue (Dhruva et al., in press), as well as a cluster of symptoms which included fatigue (Illi et al., 2012). A genetic association between IL6 and fatigue has been reported among adults with cancer (Bower et al., 2013; Miaskowski et al., 2010), but was not observed in this sample of adults with HIV. Although the polymorphisms associated with fatigue in non-HIV populations differed from associations we observed in our sample, replication of genetic associations between these three genes and fatigue severity in chronic illness populations suggests that these genes play an important role in the fatigue symptom experience.

In this study, adults with high levels of fatigue were found to have higher plasma levels of IL-6, but the differences were not significant after adjusting for genomic estimates of ancestry, self-reported race/ethnicity, and gender. Prior studies reported associations between pro-inflammatory IL-6 levels and fatigue (Schrepf et al., 2013), which may be mediated by sleep disturbance (Clevenger et al., 2012). Future studies should explore the role of other symptoms commonly associated with fatigue, such as sleep disturbance and depression.

Race/ethnicity and gender had strong influences on the associations between fatigue and the cytokine polymorphisms and plasma levels in this study. Because race/ethnicity and gender were confounded in this study (i.e., the majority of females were African-American and a majority of the Caucasians were male), we were not able to adequately distinguish the differential influence of gender and race/ethnicity in this sample. Nonetheless, even among the five polymorphisms associated with fatigue in adjusted analyses, exploratory analyses by racial and gender sub-groups suggest that the associations may vary by race and gender.

These findings highlight the importance of controlling for population substructure, as not doing so can substantially impact the results of a genetic association study and increase the likelihood of reporting false positive or false negative associations. Increasing our understanding of differential genetic associations in racial/ethnic and gender sub-groups may help guide both the diagnosis and treatment of problematic symptoms, such as fatigue. However, to our knowledge, race and gender have not been reported as significant moderators of the relationship between fatigue and cytokines in other studies. Given the present study's small samples sizes, limited racial diversity, unequal gender distribution, and confounding of race/ethnicity and gender, these findings warrant replication and further evaluation in larger studies.

Although gender seemed to have a strong effect on the genetic associations between cytokines and fatigue, gender itself was not strongly related to fatigue in this sample. This finding contradicts prior studies (Liu et al., 2013; Voss, 2005), in which women living with HIV were more likely than men to experience fatigue. One explanation for the discrepancy may be the confounding of gender and race/ethnicity in this sample The African-American sub-group generally had lower risk of fatigue than the Caucasian sub-group, and most of the women in this study were African-American. This explanation is supported by the fact that fatigue was much more common among the small number of Caucasian women (n=2/10, 83%) and women in the 'other' race/ethnicity group (n=6/9, 67%) than among the African-American women (n=8/34, 24%; p<0.001). This finding highlights the importance of addressing both gender and racial/ethnic differences in adjusted analyses.

Contrary to expectation, other demographic and clinical variables, such as age, HIV disease indicators, BMI, and hemoglobin level, were either not associated with fatigue or were not associated after controlling for the effect of race/ethnicity. Thus, these variables were not retained as covariates. These findings contradict prior reports of associations between fatigue and age (Bower et al., 2013), ART (Jong et al., 2010; Pence et al., 2009), and BMI (Bower et al., 2013). In addition to the potentially confounding influence of race/ethnicity and gender, other possible explanations for the divergent findings may pertain either to the way in which fatigue is measured or to the heterogeneity in study designs and sample characteristics across studies. Our selection of relatively high levels of fatigue in both the morning and evening may represent a more extreme type of fatigue that is associated with greater interference with daily function than fatigue limited to certain times of day (Lerdal et al., 2011). In addition, selecting a comparison group with relatively low levels of fatigue in both the morning and evening may have maximized some differences between the fatigue groups, while attenuating associations with other demographic and clinical variables. It is also possible that chronic illness and lifestyle factors (e.g., poor nutrition, low level of activity, poor sleep) of this sample had a greater influence on their level of fatigue than observed in general populations. In other words, any potential associations between fatigue and BMI or age may have been masked by their common chronic illness.

A primary strength of this study is that it included both cytokine polymorphisms and plasma levels to better understand how they each may relate to fatigue among adults with HIV. The study also accounted for demographic and clinical variables, such as gender, race, and ART, that could potentially confound the observed associations between fatigue and cytokine

biomarkers. The racially and ethnically diverse sample is an additional strength of the study and allowed for preliminary evaluation of associations between fatigue and cytokine biomarkers in two racial/ethnic groups (i.e., African-American, Caucasian). Finally, the use of a fatigue phenotype that estimates severity of fatigue assessed prospectively twice each day is a strength of the study. Rather than retrospective reports about fatigue in the past week, we identified individuals with severe fatigue in both the morning and evening, rather than just morning fatigue (thus likely reflecting poor sleep) or evening fatigue (which may reflect a relatively normal circadian pattern).

The results of this study also need to be considered in light of its limitations. The main limitations are the modest sample size for a genetic association study, the relatively small number of women, and the small number of participants in some racial and ethnic groups. The number of participants with the discordant fatigue patterns was also too small for analysis of genetic association in these groups. Although the sample is representative of adults living with HIV in the San Francisco Bay Area, replication in other samples is needed. In addition, some associations may have been missed due to low minor allele frequencies, and the tagSNP approach may have failed to identify additional associations poorly captured by the tagSNP set selected for analysis. Finally, our use of a prospective measure that focuses on general fatigue severity in both the morning and evening makes it difficult to compare our findings with studies that do not address diurnal patterns of fatigue or that assess other dimensions of fatigue retrospectively across broad timeframes. Nevertheless, findings from this study in a chronic illness population do contribute to the growing evidence for an association between inflammatory pathways and fatigue, regardless of clinical characteristics. Further research is warranted to compare the therapeutic effects of anti-inflammatory medications to other pharmacologic interventions (e.g., modafinil) to reduce the impact of fatigue and improve quality of life in this patient population and other populations living with chronic illness and fatigue.

Acknowledgments

References

Aouizerat BE, Dodd M, Lee K, West C, Paul SM, Cooper BA, Wara W, Swift P, Dunn LB, Miaskowski C. Preliminary evidence of a genetic association between tumor necrosis factor alpha and the severity of sleep disturbance and morning fatigue. Biol Res. Nurs. 2009; 11:27–41. [PubMed: 19419979]

Barroso J, Voss JG. Fatigue in HIV and AIDS: an analysis of evidence. J. Assoc Nurses AIDS Care. 2013; 24:S5–S14. [PubMed: 23290377]

Bower JE. Cancer-related fatigue: links with inflammation in cancer patients and survivors. Brain. Behav. Immun. 2007; 21:863–871. [PubMed: 17543499]

Bower JE, Ganz PA, Irwin MR, Arevalo JM, Cole SW. Fatigue and gene expression in human leukocytes: increased NF-kappaB and decreased glucocorticoid signaling in breast cancer survivors with persistent fatigue. Brain. Behav. Immun. 2011; 25:147–150. [PubMed: 20854893]

Bower JE, Ganz PA, Irwin MR, Castellon S, Arevalo J, Cole SW. Cytokine genetic variations and fatigue among patients with breast cancer. J. Clin. Oncol. 2013; 31:1656–1661. [PubMed: 23530106]

Cameron BA, Bennett B, Li H, Boyle F, DeSouza P, Wilcken N, Friedlander M, Goldstein D, Lloyd AR. Post-cancer fatigue is not associated with immune activation or altered cytokine production. Ann. Oncol. 2012; 23:2890–2895. [PubMed: 22674147]

Clevenger L, Schrepf A, Christensen D, DeGeest K, Bender D, Ahmed A, Goodheart MJ, Penedo F, Lubaroff DM, Sood AK, Lutgendorf SK. Sleep disturbance, cytokines, and fatigue in women with ovarian cancer. Brain. Behav. Immun. 2012; 26:1037–1044. [PubMed: 22543257]

Collado-Hidalgo A, Bower JE, Ganz PA, Irwin MR, Cole SW. Cytokine gene polymorphisms and fatigue in breast cancer survivors: early findings. Brain. Behav. Immun. 2008; 22:1197–1200. [PubMed: 18617366]

daCosta DiBonaventura M, Gupta S, Cho M, Mrus J. The association of HIV/AIDS treatment side effects with health status, work productivity, and resource use. AIDS Care. 2012; 24:744–755. [PubMed: 22292729]

Dhruva A, Aouizerat BE, Cooper B, Paul SM, Dodd M, West C, MIaskowski C. Cytokine gene associations with self-report ratings of morning and evening fatigue in oncology patients and their family caregivers. Biological Research for Nursing. in press.

Dirksen SR, Kirschner KF, Belyea MJ. Association of Symptoms and Cytokines in Prostate Cancer Patients Receiving Radiation Treatment. Biol. Res. Nurs. 2013

Finter NB, Chapman S, Dowd P, Johnston JM, Manna V, Sarantis N, Sheron N, Scott G, Phua S, Tatum PB. The use of interferon-alpha in virus infections. Drugs. 1991; 42:749–765. [PubMed: 1723372]

Fung FY, Li M, Breunis H, Timilshina N, Minden MD, Alibhai SM. Correlation between cytokine levels and changes in fatigue and quality of life in patients with acute myeloid leukemia. Leuk. Res. 2013; 37:274–279. [PubMed: 23259987]

Gay CL, Lee KA, Lee SY. Sleep patterns and fatigue in new mothers and fathers. Biol Res Nurs. 2004; 5:311–318. [PubMed: 15068660]

Geinitz H, Zimmermann FB, Thamm R, Keller M, Busch R, Molls M. Fatigue in patients with adjuvant radiation therapy for breast cancer: long-term follow-up. J. Cancer Res. Clin. Oncol. 2004; 130:327–333. [PubMed: 15007642]

Grady C, Anderson R, Chase GA. Fatigue in HIV-infected men receiving investigational interleukin-2. Nurs. Res. 1998; 47:227–234. [PubMed: 9683118]

Halder I, Shriver M, Thomas M, Fernandez JR, Frudakis T. A panel of ancestry informative markers for estimating individual biogeographical ancestry and admixture from four continents: utility and applications. Hum. Mutat. 2008; 29:648–658. [PubMed: 18286470]

Hamre H, Zeller B, Kanellopoulos A, Ruud E, Fossa SD, Loge JH, Aukrust P, Halvorsen B, Mollnes TE, Kiserud CE. Serum cytokines and chronic fatigue in adults surviving after childhood leukemia and lymphoma. Brain. Behav. Immun. 2013; 30:80–87. [PubMed: 23333795]

Harrington ME. Neurobiological studies of fatigue. Prog. Neurobiol. 2012; 99:93–105. [PubMed: 22841649]

Hoggart CJ, Parra EJ, Shriver MD, Bonilla C, Kittles RA, Clayton DG, McKeigue PM. Control of confounding of genetic associations in stratified populations. Am. J. Hum. Genet. 2003; 72:1492–1504. [PubMed: 12817591]

Illi J, Miaskowski C, Cooper B, Levine JD, Dunn L, West C, Dodd M, Dhruva A, Paul SM, Baggott C, Cataldo J, Langford D, Schmidt B, Aouizerat BE. Association between pro- and anti-inflammatory cytokine genes and a symptom cluster of pain, fatigue, sleep disturbance, and depression. Cytokine. 2012; 58:437–447. [PubMed: 22450224]

Inagaki M, Akechi T, Okuyama T, Sugawara Y, Kinoshita H, Shima Y, Terao K, Mitsunaga S, Ochiai A, Uchitomi Y. Associations of interleukin-6 with vegetative but not affective depressive symptoms in terminally ill cancer patients. Support. Care Cancer. 2013; 21:2097–2106. [PubMed: 23446881]

Jim HS, Park JY, Permuth-Wey J, Rincon MA, Phillips KM, Small BJ, Jacobsen PB. Genetic predictors of fatigue in prostate cancer patients treated with androgen deprivation therapy: preliminary findings. Brain. Behav. Immun. 2012; 26:1030–1036. [PubMed: 22475653]

Jong E, Oudhoff LA, Epskamp C, Wagener MN, van Duijn M, Fischer S, van Gorp EC. Predictors and treatment strategies of HIV-related fatigue in the combined antiretroviral therapy era. AIDS. 2010; 24:1387–1405. [PubMed: 20523204]

Klimas NG, Broderick G, Fletcher MA. Biomarkers for chronic fatigue. Brain. Behav. Immun. 2012; 26:1202–1210. [PubMed: 22732129]

Lee KA, Gay C, Portillo CJ, Coggins T, Davis H, Pullinger CR, Aouizerat BE. Symptom experience in HIV-infected adults: a function of demographic and clinical characteristics. J. Pain Symptom Manage. 2009; 38:882–893. [PubMed: 19811886]

Lee KA, Hicks G, Nino-Murcia G. Validity and reliability of a scale to assess fatigue. Psychiatry Res. 1991; 36:291–298. [PubMed: 2062970]

Lee KA, Portillo CJ, Miramontes H. The fatigue experience for women with human immunodeficiency virus. J. Obstet. Gynecol. Neonatal Nurs. 1999; 28:193–200.

Lerdal A, Gay CL, Aouizerat BE, Portillo CJ, Lee KA. Patterns of morning and evening fatigue among adults with HIV/AIDS. J. Clin. Nurs. 2011; 20:2204–2216. [PubMed: 21752119]

Liu L, Mills PJ, Rissling M, Fiorentino L, Natarajan L, Dimsdale JE, Sadler GR, Parker BA, Ancoli-Israel S. Fatigue and sleep quality are associated with changes in inflammatory markers in breast cancer patients undergoing chemotherapy. Brain. Behav. Immun. 2012; 26:706–713. [PubMed: 22406004]

Liu Z, Yang J, Liu H, Jin Y. Factors associated with fatigue in acquired immunodeficiency syndrome patients with antiretroviral drug adverse reactions: a retrospective study. J. Tradit. Chin. Med. 2013; 33:316–321. [PubMed: 24024326]

Miaskowski C, Dodd M, Lee K, West C, Paul SM, Cooper BA, Wara W, Swift PS, Dunn LB, Aouizerat BE. Preliminary evidence of an association between a functional interleukin-6 polymorphism and fatigue and sleep disturbance in oncology patients and their family caregivers. J. Pain Symptom Manage. 2010; 40:531–544. [PubMed: 20570482]

Miaskowski C, Paul SM, Cooper BA, Lee K, Dodd M, West C, Aouizerat BE, Swift PS, Wara W. Trajectories of fatigue in men with prostate cancer before, during, and after radiation therapy. J. Pain Symptom Manage. 2008; 35:632–643. [PubMed: 18358683]

Millikin CP, Rourke SB, Halman MH, Power C. Fatigue in HIV/AIDS is associated with depression and subjective neurocognitive complaints but not neuropsychological functioning. J. Clin. Exp. Neuropsychol. 2003; 25:201–215. [PubMed: 12754678]

Pence BW, Barroso J, Harmon JL, Leserman J, Salahuddin N, Hammill BG. Chronicity and remission of fatigue in patients with established HIV infection. AIDS Patient Care STDS. 2009; 23:239–244. [PubMed: 19281346]

Piraino B, Vollmer-Conna U, Lloyd AR. Genetic associations of fatigue and other symptom domains of the acute sickness response to infection. Brain. Behav. Immun. 2012; 26:552–558. [PubMed: 22227623]

Price AL, Patterson NJ, Plenge RM, Weinblatt ME, Shadick NA, Reich D. Principal components analysis corrects for stratification in genome-wide association studies. Nat. Genet. 2006; 38:904–909. [PubMed: 16862161]

Rohleder N, Aringer M, Boentert M. Role of interleukin-6 in stress, sleep, and fatigue. Ann. N. Y. Acad. Sci. 2012; 1261:88–96. [PubMed: 22823398]

Saligan LN, Kim HS. A systematic review of the association between immunogenomic markers and cancer-related fatigue. Brain. Behav. Immun. 2012; 26:830–848. [PubMed: 22595751]

Schrepf A, Clevenger L, Christensen D, DeGeest K, Bender D, Ahmed A, Goodheart MJ, Dahmoush L, Penedo F, Lucci JA 3rd, Ganjei-Azar P, Mendez L, Markon K, Lubaroff DM, Thaker PH, Slavich GM, Sood AK, Lutgendorf SK. Cortisol and inflammatory processes in ovarian cancer patients following primary treatment: relationships with depression, fatigue, and disability. Brain. Behav. Immun. 2013; 30(Suppl):S126–S134. [PubMed: 22884960]

Schubert C, Hong S, Natarajan L, Mills PJ, Dimsdale JE. The association between fatigue and inflammatory marker levels in cancer patients: a quantitative review. Brain. Behav. Immun. 2007; 21:413–427. [PubMed: 17178209]

Starkweather A. Psychologic and biologic factors associated with fatigue in patients with persistent radiculopathy. Pain Manag. Nurs. 2013; 14:41–49. [PubMed: 23452526]

Sullivan PS, Dworkin MS. Prevalence and correlates of fatigue among persons with HIV infection. J. Pain Symptom Manage. 2003; 25:329–333. [PubMed: 12691684]

Tian C, Gregersen PK, Seldin MF. Accounting for ancestry: population substructure and genome-wide association studies. Hum. Mol. Genet. 2008; 17:R143–R150. [PubMed: 18852203]

van Zuiden M, Kavelaars A, Amarouchi K, Maas M, Vermetten E, Geuze E, Heijnen CJ. IL-1beta reactivity and the development of severe fatigue after military deployment: a longitudinal study. J. Neuroinflammation. 2012; 9:205. [PubMed: 22908999]

Voss JG. Predictors and Correlates of Fatigue in HIV/AIDS. J. Pain Symptom Manage. 2005; 29:173–184. [PubMed: 15733809]

Highlights

- Fatigue is associated with IL1B, IL4, and TNFA polymorphisms in adults with HIV.

- Genetic associations between fatigue and cytokines may differ by race.

- Genetic associations between fatigue and cytokines may differ by gender.

- Adjusting for race and gender may control false positive/negative associations.

Table 1

Demographic and clinical characteristics by fatigue group (n=224)

	Total Sample (n=224)	Fatigue Level		Statistics
		Low (n=110)	High (n=114)	
Demographics				
Age, years, mean (SD) (range 22 – 77)	45.4 (8.3)	45.5 (8.3)	45.3 (8.3)	$F(1,222)=0.06$, $p=.808$
Gender				$\chi^2(2,224)=4.73$, ***p=.094***
Male	151 (67%)	67 (61%)	84 (74%)	
Female	55 (25%)	31 (28%)	24 (21%)	
Transgender	18 (8%)	12 (11%)	6 (5%)	
Race/ethnicity				$\chi^2(2,224)=29.5$, ***p<.001***
Caucasian	92 (41%)	30 (27%)	62 (54%)	AA<CA, $p<.001$
African-American	90 (40%)	64 (58%)	26 (23%)	AA<O, $p=.001$
Other	42 (19%)	16 (15%)	26 (23%)	
Education				$\chi^2(2,224)=8.33$, ***p=.016***
High school or less	104 (46%)	61 (55%)	43 (38%)	HS<CD, $p=.006$
Vocational or some college	71 (32%)	32 (29%)	39 (34%)	
College degree	49 (22%)	17 (16%)	32 (28%)	
Employment, n (%)				$\chi^2(2,224)=1.75$, $p=.416$
Employed/student	36 (16%)	83 (75%)	85 (75%)	
Unemployed	20 (9%)	15 (14%)	21 (18%)	
Disability	168 (75%)	12 (11%)	8 (7%)	
Monthly Income				$\chi^2(2,224)=8.33$, ***p=.014***
< $1,000	158 (71%)	86 (78%)	72 (63%)	
$1,000	66 (29%)	24 (22%)	42 (37%)	
Clinical Characteristics				
CD4+ T-cell count (cells/mm^3)	n=215[a]	n=107[a]	n=108[a]	$\chi^2(2,215)=0.00$, $p=.975$
<200	38 (18%)	19 (18%)	19 (18%)	
200	177 (82%)	88 (82%)	89 (82%)	
Viral Load (log$_{10}$ copies/mL)	n=210[a]	n=106[a]	n=104[a]	$\chi^2(2,210)=1.55$, $p=.214$
Undetectable	106 (50%)	49 (46%)	57 (55%)	
Detectable	104 (50%)	57 (54%)	47 (45%)	
Years since HIV diagnosis				$F(1,222)=2.22$, $p=.138$
Mean (SD), range 0.2 –26	12.4 (6.8)	11.7 (6.3)	13.0 (7.2)	
Anti-retroviral therapy				$\chi^2(2,224)=4.24$, ***p=.039***
Not on treatment	69 (31%)	41 (37%)	28 (25%)	
On treatment	155 (69%)	69 (63%)	86 (75%)	
AIDS Diagnosis				$\chi^2(2,224)=4.55$, ***p=.033***
No	110 (49%)	62 (56%)	48 (42%)	

	Total Sample (n=224)	Fatigue Level		Statistics
		Low (n=110)	High (n=114)	
Yes	114 (51%)	48 (44%)	66 (58%)	
Body mass index, mean (SD)				
Male	25.7 (3.9)	25.6 (3.9)	25.7 (3.9)	$F(1,149)=0.03$, $p=.858$
Female	28.5 (6.4)	28.9 (5.8)	28.1 (7.3)	$F(1,53)=0.20$, $p=.658$
Transgender	29.2 (7.1)	29.7 (7.8)	28.3 (6.1)	$F(1,16)=0.15$, $p=.708$
Hemoglobin, g/dL mean (SD)	n=134[a]	n=66[a]	n=68[a]	
Male	14.5 (1.7)	14.4 (1.7)	14.6 (1.6)	$F(1,92)=0.69$, $p=.408$
Female	12.5 (1.6)	12.2 (1.4)	12.9 (1.7)	$F(1,26)=1.12$, $p=.300$
Transgender	12.8 (1.0)	12.9 (1.1)	12.4 (1.0)	$F(1,10)=0.60$, $p=.456$

Note: AA = African-American; CA = Caucasian; CD = college degree; HS = high school or less SD = standard deviation; **Bolded** variables are associated ($p < 0.10$) with fatigue group and were evaluated as potential covariates in adjusted regression models.

[a]Sample sizes were lower due to missing data from medical records

Table 2

Spearman correlation matrix of potential covariates

		1	2	3	4	5	6	7	8	9	10	11
1	High fatigue											
2	PC1 [a]	−0.26										
3	PC2 [a]	0.21	−0.63									
4	PC3 [a]	0.12	−0.12	0.01								
5	African-American [b]	−0.37	0.82	−0.72	−0.16							
6	Caucasian [b]	0.28	−0.78	0.40	0.13	−0.68						
7	Male	0.14	−0.28	0.29	−0.03	−0.32	0.29					
8	Female	−0.09	0.20	−0.18	0.04	0.25	−0.22	−0.82				
9	Education	0.19	−0.21	0.07	0.13	−0.23	0.24	0.28	−0.24			
10	Income	0.21	−0.17	0.16	−0.02	−0.17	0.21	0.11	−0.09	0.21		
11	ART	0.18	0.00	−0.02	0.04	−0.01	0.09	0.16	−0.14	0.18	0.23	
12	AIDS	0.10	−0.18	0.14	0.07	−0.16	0.15	0.20	−0.11	0.10	0.09	0.48

Note. Although genomic estimates of ancestry (PC1, PC2, and PC3) and self-reported race/ethnicity are highly correlated, all were included in adjusted analyses as a conservative approach to minimizing bias in plasma and genetic associations due to population substructure.

[a] PC1, PC2, PC3: genomic estimates of ancestry corresponding to the first three principal components derived from 106 ancestry informative markers

[b] Self-reported race/ethnicity

Table 3

Plasma cytokine and CRP levels by fatigue group (n=222)

	Total Sample (n=222)	Low Fatigue Group (n=108)	High Fatigue Group (n=114)	Statistics
IL-1β (pg/mL)	4.11 (3.57)	4.00 (3.33)	4.22 (3.80)	MWU p=.814
IL-2 (pg/mL)	8.58 (14.4)	8.75 (15.0)	8.42 (13.8)	MWU p=.145
IL-6 (pg/mL)	23.6 (37.6)	21.9 (40.3)	25.2 (34.9)	**MWU p=.003**
IL-10 (pg/mL)	26.5 (55.2)	19.7 (46.4)	33.0 (61.9)	MWU p=.021
IL-13 (pg/mL)	6.40 (11.3)	5.94 (10.2)	6.84 (12.3)	MWU p=.075
TNFα (pg/mL)	12.5 (11.5)	12.2 (11.7)	12.7 (11.4)	MWU p=.499
CRP (ng/mL)	12.0 (18.6)	11.2 (16.7)	12.8 (20.2)	MWU p=.629

Note: CRP = C-reactive protein; IL = interleukin; MWU = Mann Whitney U Test; ng/mL = nanograms per milliliter; pg/mL = picograms per milliliter; TNFα = tumor necrosis factor alpha. **Bolded** variable differed by fatigue group using a Bonferroni-corrected $p < 0.007$. None of the plasma cytokine levels were associated with fatigue group in adjusted analyses.

Table 4

Unadjusted associations between 80 cytokine polymorphisms and fatigue group (n=224)

Gene	SNP	HGVS. Description	HuRef Position	Chr	MAF	OR	95% CI	p	Model
IFNG	**rs2069728**	**(3' of gene, G>A)**	**65598422**	**12**	**.155**	**0.43**	**0.24, 0.78**	**.006**	**D**
	rs2069727	**(3' of gene, A>G)**	**65598861**	**12**	**.321**	**2.02**	**1.19, 3.45**	**.010**	**D**
	rs2069718	c.367−895T>C	65600800	12	.493	1.36	0.94, 1.99	.106	A
	rs1861493	c.366−497C>T	65601835	12	.217	2.97	0.59, 15.1	.188	R
	rs1861494	c.366−284G>A	65602048	12	.226	2.97	0.59, 15.1	.188	R
	rs2069709*a,b*	(5' of gene, G>T)	65604339	12	.013				
IFNGR1	rs9376268	c.86−4537C>T	135094927	6	.183	2.55	0.77, 8.38	.124	R
IL1B	**rs1071676**	**c.*505G>C**	**106042060**	**2**	**.168**	**2.00**	**1.16, 3.43**	**.012**	**A**
	rs1143643	**c.598−152G>A**	**106042929**	**2**	**.252**	**1.85**	**1.08, 3.18**	**.025**	**D**
	rs1143642*b*	**c.597+316T>C**	106043180	2	.139				
	rs1143634	**c.315C>T**	**106045017**	**2**	**.164**	**2.02**	**1.17, 3.49**	**.012**	**A**
	rs1143633	c.302−64G>A	106045094	2	.275	1.55	0.91, 2.64	.103	D
	rs1143630	c.100−503A>C	106046282	2	.146	0.18	0.02, 1.60	.125	R
	rs3917356	c.99+780G>A	106046990	2	.377	0.69	0.35, 1.39	.302	R
	rs1143629	c.47+242C>T	106048145	2	.400	0.53	0.27, 1.05	.068	R
	rs1143627	**(5' of gene, T>C)**	**106049014**	**2**	**.491**	**0.43**	**0.23, 0.79**	**.007**	**R**
	rs16944	**(5' of gene, G>A)**	**106049494**	**2**	**.455**	**0.44**	**0.22, 0.84**	**.014**	**R**
	rs1143623	**(5' of gene, G>C)**	**106050452**	**2**	**.217**	**2.02**	**1.16, 3.51**	**.013**	**D**
	rs13032029	(5' of gene, C>T)	106055022	2	.357	0.73	0.36, 1.48	.388	R
IL1R	rs949963	(5' of gene, G>A)	96533648	2	.209	0.67	0.42, 1.06	.090	A
	rs22281394*a,b*	c.371C>G	96545511	2	.029				
	rs3917320*b*	c.1366A>C	96556738	2	.078				
	rs2110726	**c.*1063G>A**	**96558145**	**2**	**.261**	**2.10**	**1.23, 3.58**	**.007**	**D**
	rs3917332*b*	(3' of gene, T>A)	96560387	2	.261				
IL1R2	**rs4141134**	**(5' of gene, T>C)**	**96370336**	**2**	**.293**	**2.03**	**1.18, 3.47**	**.010**	**D**

Gene	SNP	HGVS. Description	HuRef Position	Chr	MAF	OR	95% CI	p	Model
	rs11674595	**c.-62+2519T>C**	**96374804**	**2**	**.199**	**1.90**	**1.10, 3.31**	**.023**	**D**
	rs7570441	c.-62+1417G>A	96380807	2	.471	0.72	0.38, 1.38	.321	R
IL2	**rs1479923**	**(3' of gene, C>T)**	**119096993**	**4**	**.212**	**1.82**	**1.15, 2.88**	**.011**	**A**
	rs2069776	(3' of gene, T>C)	119098582	4	.182	0.47	0.04, 5.30	.544	R
	rs2069772	**c.352−116A>G**	**119099739**	**4**	**.185**	**8.23**	**1.01, 66.9**	**.049**	**R**
	rs2069777[b]	c.207+862C>T	119103043	4	.056					
	rs2069763[c]	c.114G>T	119104088	4	.306					
IL4	rs2243248	(5' of gene, T>G)	127200946	5	.123	0.31	0.03, 3.05	.317	R
	rs2243250	**(5' of gene, C>T)**	**127201455**	**5**	**.359**	**0.34**	**0.20, 0.59**	**<.001**	**D**
	rs2070874	**c.-33C>T**	**127202011**	**5**	**.254**	**0.48**	**0.28, 0.83**	**.008**	**D**
	rs2227284	**c.183−2527T>G**	**127205027**	**5**	**.467**	**1.89**	**1.32, 2.70**	**<.001**	**A**
	rs2227282[c]	c.184−2227C>G	127205481	5	.455					
	rs2243263	c.184−2107C>G	127205601	5	.158	2.95	0.30, 28.8	.353	R
	rs2243266	**c.184−1617G>A**	**127206091**	**5**	**.243**	**0.46**	**0.27, 0.79**	**.005**	**D**
	rs2243267	**c.184−1520G>C**	**127206188**	**5**	**.243**	**0.46**	**0.27, 0.79**	**.005**	**D**
	rs2243274	**c.184−574G>A**	**127207134**	**5**	**.362**	**0.30**	**0.17, 0.52**	**<.001**	**D**
IL6	rs4719714	(5' of gene, A>T)	22643793	7	.208	1.10	0.69, 1.76	.691	A
	rs2069827[b]	(5' of gene, G>T)	22648536	7	.054					
	rs1800796	(5' of gene, G>C)	22649326	7	.094	0.23	0.03, 2.11	.195	R
	rs1800795	**(5' of gene, C>G)**	**22649725**	**7**	**.228**	**1.94**	**1.21, 3.12**	**.006**	**A**
	rs2069835[b]	c.211−441T>C	22650951	7	.078					
	rs2066992	c.211−63G>T	22651329	7	.092	0.31	0.03, 3.05	.317	R
	rs2069840	c.324−147C>G	22651652	7	.259	1.48	0.95, 2.31	.080	A
	rs1554606	c.324−282T>G	22651787	7	.390	0.91	0.63, 1.56	.731	D
	rs2069845	c.471+870G>A	22653229	7	.386	1.18	0.56, 2.47	.670	R
	rs2069849[b]	c.603C>T	22654236	7	.105					
	rs2069861[a,b]	(3' of gene, C>T)	22654734	7	.036					
	rs35610689	(3' of gene, A>G)	22656903	7	.225	1.37	0.86, 2.19	.187	A

Gene	SNP	HGVS. Description	Chr	HuRef Position	MAF	OR	95% CI	p	Model
IL8	**rs4073**	**(5' of gene, T>A)**	**4**	**70417508**	**.426**	**2.24**	**1.13, 4.44**	**.021**	**R**
	rs2227306	**c.65−204C>T**	**4**	**70418539**	**.279**	**1.65**	**1.10, 2.48**	**.015**	**A**
	rs2227543	**c.284+161C>T**	**4**	**70419394**	**.279**	**1.65**	**1.10, 2.48**	**.015**	**A**
IL10	rs3024505b	(3' of gene, C>T)	1	177638230	.121				
	rs3024498	c.*452A>G	1	177639855	.173	1.63	0.98, 2.69	.059	A
	rs3024496	c.*117T>C	1	177640190	.437	1.21	0.83, 1.76	.313	A
	rs1878672	**c.379−474C>G**	**1**	**177642039**	**.379**	**1.60**	**1.09, 2.36**	**.017**	**A**
	rs3024492	**c.378+140A>T**	**1**	**177642438**	**.148**	**2.27**	**1.30, 3.98**	**.004**	**A**
	rs1518111	c.225−56A>G	1	177642971	.309	0.79	0.53, 1.19	.260	A
	rs1518110	c.166−101T>G	1	177643187	.307	0.73	0.43, 1.24	.249	D
	rs3024491	**c.166−286G>T**	**1**	**177643372**	**.377**	**1.57**	**1.07, 2.31**	**.022**	**A**
IL13	rs1881457	(5' of gene, A>C)	5	127184713	.217	0.86	0.50, 1.48	.591	D
	rs1800925	**(5' of gene, C>T)**	**5**	**127185113**	**.280**	**0.51**	**0.30, 0.87**	**.014**	**D**
	rs2069743	(5' of gene, A>G)	5	127185579	.092	0.50	0.24, 1.03	.060	D
	rs1295686	**c.334−24T>C**	**5**	**127188147**	**.431**	**0.57**	**0.40, 0.82**	**.003**	**A**
	rs20541	c.431A>G	5	127188268	.219	1.37	0.42, 4.47	.597	R
IL17A	**rs4711998**	**(5' of gene, G>A)**	**6**	**51881422**	**.400**	**0.67**	**0.47, 0.96**	**.031**	**A**
	rs8193036	(5' of gene, T>C)	6	51881562	.249	0.92	0.59, 1.42	.700	A
	rs3819024	(5' of gene, A>G)	6	51881855	.289	0.76	0.45, 1.29	.316	D
	rs2275913	(5' of gene, G>A)	6	51882102	.194	2.55	0.77, 8.38	.124	R
	rs3805513a,b	c.230+594A>T	6	51884266	.022				
	rs7747909	c.*159G>A	6	51885318	.157	1.63	0.90, 2.98	.109	D
NFKB1	rs3774933	c.-8+3394T>C	4	99162722	.355	1.93	0.78, 4.76	.152	R
	rs170731	c.40−2090T>A	4	99185284	.252	1.23	0.81, 1.87	.339	A
	rs17032779b	c.255+7137T>C	4	99202630	.063				
	rs230510	c.256−11978T>A	4	99212552	.336	1.40	0.83, 2.38	.210	D
	rs230494	c.256−1175G>A	4	99223356	.336	0.95	0.65, 1.38	.778	A
	rs4648016a,b	c.404+1378C>T	4	99226057	.036				

Gene	SNP	HGVS. Description	HuRef Position	Chr	MAF	OR	95% CI	p	Model
	rs4648018[b]	c.404+1908G>C	99226587	4	.052				
	rs3774956	c.1063+2549C>T	99244914	4	.375	0.98	0.67, 1.44	.923	A
	rs10489114[b]	c.1064-3194T>C	99247604	4	.052				
	rs4648068	c.1493-372A>G	99254521	4	.290	0.88	0.60, 1.30	.533	A
	rs4648095[a,b]	c.1951+22T>C	99264093	4	.047				
	rs4648110	c.2589+58T>A	99270046	4	.272	1.29	0.54, 3.07	.569	R
	rs4648135[b]	c.2747-921A>G	99272895	4	.080				
	rs4648141	**c.2747-690G>A**	**99273126**	**4**	**.335**	**1.86**	**1.09, 3.16**	**.022**	**R**
	rs1609798	c.2747-149C>T	99273667	4	.238	1.41	0.82, 2.42	.215	D
NFKB2	rs12772374	c.395-99A>G	97790120	10	.123	1.50	0.84, 2.65	.168	A
	rs7897947	c.662-27T>G	97790920	10	.287	0.64	0.38, 1.08	.097	D
	rs11574849[b]	c.1470-141G>A	97792905	10	.063				
	rs1056890	**c.*187C>T**	**97795944**	**10**	**.260**	**1.94**	**1.13, 3.32**	**.016**	**D**
TNFA	rs2857602	g.31473378G>A	31533378	6	.310	1.26	0.55, 2.91	.588	R
	rs1800683	c.-18G>A	31540071	6	.455	1.12	0.78, 1.62	.541	A
	rs2239704[d]	c.-92A>C	31540141	6	.308	1.39	0.59, 3.27	.457	R
	rs2229094[d]	c.37T>C	31540556	6	.252	0.74	0.49, 1.13	.165	A
	rs1041981[d]	c.179C>A	31540784	6	.455	1.30	0.73, 2.30	.370	D
	rs1799964	g.31482308T>C	31542308	6	.179	0.75	0.47, 1.22	.252	A
	rs1800750[a,b]	g.31482963G>A	31542963	6	.013				
	rs1800629[b]	c.-308G>A	31543031	6	.132				
	rs1800610	c.186+123G>A	31543827	6	.071	0.64	0.29, 1.42	.275	D
	rs3093662[b]	c.187-122A>G	31544189	6	.070				

Notes. Models predict membership in the high fatigue group, with the low fatigue group serving as the reference. A = additive model; Chr = chromosome; CI = confidence interval; D = dominant model; HGVS. = human genome variation society; HuRef = human reference sequence; IL = interleukin; IL1R2 = interleukin 1 receptor 2; INFG = interferon-gamma; IFNGR1 = interferon-gamma receptor 1; MAF = minor allele frequency; NFKB = nuclear factor of kappa light polypeptide gene enhancer in B cells; OR = odds ratio based on dose of the minor allele (OR<1 indicates that the minor allele reduces risk of high fatigue, while OR>1 increases risk of high fatigue); R = recessive model; SNP = single nucleotide polymorphism; TNFA = tumor necrosis factor alpha. **Bold** SNPs have p < 0.05.

[a] SNP excluded from analysis because MAF < 0.05 (n=7 SNPs)

[b] SNP excluded from analysis because one of the genotypes had a frequency <3 (n=22 SNPs)

[c] SNP excluded from analysis because distribution violated Hardy-Weinberg equilibrium (n=2 SNPs)

[d] This TNFA SNP uses NM_000595.2

Table 5

Significant adjusted associations between cytokine genotype and fatigue group (n=223)

Gene	SNP	Model	OR	95% CI	p	R^2	Total R^2	Full models
IL1B	rs1071676	A	1.82	1.01, 3.30	.048	.013	.126	χ^2=39.0, *p*<.001
	African-Americans:		1.68	0.58, 4.90	.339	.008	.091	χ^2=9.84, *p*=.132
	Caucasians:		1.60	0.64, 4.02	.318	.009	.030	χ^2=3.36, *p*=.644
	Females:		1.39	0.34, 5.67	.649	.003	.282	χ^2=21.3, *p*=.002
	Males:		2.14	1.01, 4.54	.048	.020	.074	χ^2=15.2, *p*=.019
IL1B	rs1143627	R	0.49	0.25, 0.99	.048	.013	.126	χ^2=39.0, *p*<.001
	African-Americans:		0.48	0.16, 1.46	.196	.016	.099	χ^2=10.7, *p*=.099
	Caucasians:		0.73	0.20, 2.63	.631	.002	.023	χ^2=2.57, *p*=.767
	Females:		0.32	0.05, 2.24	.250	.020	.299	χ^2=22.6, *p*=.001
	Males:		0.65	0.29, 1.45	.295	.005	.059	χ^2=12.2, *p*=.058
IL4	rs2243274	D	0.43	0.22, 0.87	.018	.018	.131	χ^2=40.5, *p*<.001
	African-Americans:		0.34	0.09, 1.26	.107	.024	.107	χ^2=11.5, *p*=.073
	Caucasians:		0.42	0.14, 1.22	.110	.023	.044	χ^2=4.90, *p*=.428
	Females:		0.54	0.10, 2.81	.461	.007	.287	χ^2=21.6, *p*=.001
	Males:		0.34	0.15, 0.79	.012	.032	.085	χ^2=17.6, *p*=.007
TNFA	rs1800683	A	1.55	1.01, 2.40	.047	.013	.125	χ^2=38.3, *p*<.001
	African-Americans:		2.16	1.01, 4.62	.048	.040	.124	χ^2=13.3, *p*=.039
	Caucasians:		1.40	0.68, 2.87	.361	.008	.029	χ^2=3.19, *p*=.671
	Females:		2.88	0.94, 8.85	.065	.053	.333	χ^2=25.1, *p*<.001
	Males:		1.14	0.67, 1.91	.633	.001	.052	χ^2=10.5, *p*=.104
TNFA	rs1041981	D	1.96	1.02, 3.77	.043	.014	.127	χ^2=387, *p*<.001
	African-Americans:		3.94	0.81, 19.2	.090	.034	.121	χ^2=13.0, *p*=.044
	Caucasians:		1.61	0.63, 4.11	.320	.009	.029	χ^2=3.18, *p*=.673
	Females:		5.09	0.70, 36.8	.107	.044	.323	χ^2=24.4, *p*<.001
	Males:		1.42	0.67, 3.01	.365	.004	.055	χ^2=10.9, *p*=.090

Notes. Models predict membership in the high fatigue group, with the low fatigue group serving as the reference. All models adjusted for genomic estimates of ancestry. In addition, the overall models also adjusted for self-reported race/ethnicity and gender, while the race-specific models also adjusted for gender, and the gender-specific models also adjusted for self-reported race/ethnicity; n=90 for analysis of the African American sub-group and n=91 for the Caucasian sub-group; n=150 for the male sub-group and n=55 for the female sub-group. A = additive model; CI = confidence interval; D = dominant model; IL = interleukin; OR = odds ratio based on dose of minor allele; R = recessive model; R^2 = proportion of variance in fatigue group explained by the full model; R^2 = proportion of variance in fatigue group accounted for by genotype when adjusting for covariates; TNFA = tumor necrosis factor alpha.