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# Cytokine polymorphisms and plasma levels are associated with sleep onset insomnia in adults living with HIV/AIDS

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## Abstract

Sleep disturbance has been associated with inflammation and cytokine activity, and we previously described genetic associations between cytokine polymorphisms and sleep maintenance and duration among adults with HIV/AIDS. Although sleep onset insomnia (SOI) is also a commonly reported sleep problem, associations between cytokine biomarkers and SOI have not been adequately studied. The purpose of this study was to describe SOI in relation to cytokine plasma concentrations and gene polymorphisms in a convenience sample of 307 adults (212 men, 72 women, and 23 transgender) living with HIV/AIDS. Based on the Pittsburgh Sleep Quality Index item that asks the time it usually took to fall asleep in the past month, participants were categorized as either > 30 minutes to fall asleep (n=70, 23%) or 30 minutes or less to fall asleep (n=237). Plasma cytokines were analyzed, and genotyping was conducted for 15 candidate genes involved in cytokine signaling: interferon-gamma (IFNG), IFNG receptor 1 (IFNGR1), interleukins (IL1R2, IL2, IL4, IL6, IL8, IL10, IL13, IL17A), nuclear factor of kappa light polypeptide gene enhancer in B cells (NFKB1 and NFKB2), and tumor necrosis factor alpha (TNFA). Demographic and clinical variables were evaluated as potential covariates. After adjusting for genomic estimates of ancestry, self-reported race/ethnicity and viral load, SOI was

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associated with higher *IL-13* plasma levels and with six single nucleotide polymorphisms (SNPs): IL1B rs1143642 and rs1143623, IL6 rs4719714, IL13 rs1295686, NFKB1 rs4648110, and TNFA rs2857602. In addition, the IL1B rs1143642 polymorphism was associated with plasma levels of *IL-1β* in adjusted analyses. This study strengthens the evidence for an association between inflammation and sleep disturbance, particularly self-report of habitual SOI. In this chronic illness population, the cytokine polymorphisms associated with SOI provide direction for future personalized medicine intervention research.

### Keywords

sleep onset latency; insomnia; cytokine; inflammation; genetic; biomarker; HIV

## 1. Introduction

Cytokines play a role in both normal sleep and sleep disorders associated with infection and other chronic illnesses (Lorton et al., 2006; Zielinski and Krueger, 2011), and sleep disturbance has been associated with several cytokine polymorphisms in prior studies. More specifically, IL6 has been associated with poor sleep in cancer populations (Clevenger et al., 2012; Miaskowski et al., 2010), and among adults living with HIV/AIDS, tumor necrosis factor – alpha (TNFA), IL1R2, and IL2 have been associated with poor sleep maintenance and short sleep duration (Lee et al., 2014a), and IL1B and IL17A have been associated with symptoms of restless legs syndrome (Hennessy et al., 2014). Although difficulty falling asleep is also a commonly reported sleep problem among adults with HIV/AIDS (Lee et al., 2012) and among the general population (Roth, 2007), associations with cytokine genetic markers and plasma biomarkers have not been adequately studied. This is a particularly relevant issue for individuals with HIV who are known to have impaired cytokine function, which could contribute to their sleep problems (Zielinski and Krueger, 2011). Thus, the purpose of this study is to describe self-reported habitual sleep onset insomnia (SOI) in relation to cytokine plasma concentrations and gene polymorphisms. For this analysis, SOI is defined as self-reporting that it usually took longer than 30 minutes to fall asleep during the past month, regardless of daytime sleepiness or fatigue (American Psychiatric Association, 2013). Gene polymorphisms that were studied included cytokines previously known to be associated with sleep regulation (IL1B, TNFA, IL6, NKF-B)(Krueger, 2008; Zielinski and Krueger, 2011), daytime fatigue (IL1B, TNFA, IL4, IL6)(Aouizerat et al., 2009; Lee et al., 2014b), as well as with HIV infection (IL1, IL6, TNFa, IL10) (Breen, 2002). Given that cytokines are known to influence the function of other cytokines, a broad panel was selected for this initial association study.

## Methods

### 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 associations between cytokine-related biomarkers and prolonged sleep onset latency. The Committee on Human Research at the University of California, San Francisco (UCSF)

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approved the study protocol. A convenience sample of 350 adults with HIV was 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 sleep. 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, and HIV viral load values were obtained from the most recent laboratory report in the patient's medical record (no more than 3 months old) and were not obtained as part of the cytokine testing.

**2.2.2 Biomarkers—**Fasting blood samples were obtained from each participant during a morning 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 as a batch 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. C-reactive protein [*CRP*] plasma levels were also assayed using the same platform.

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)

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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 four 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, 18 SNPs with allele frequencies of less than 5% (n=10) or with less than 3 individuals homozygous for the rare allele (n=18) were excluded from analysis. As shown in Table 4, 82 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 Sleep onset insomnia (SOI)—**An item from the Pittsburgh Sleep Quality Index (Buysse et al., 1989) was used to assess the usual amount of time it took to fall asleep in the past month, based on the participant's self-report. Based on this item, participants were categorized into one of two groups: 1) individuals who usually took more than 30 minutes to fall asleep (the clinical definition of SOI) or 2) individuals who took 30 minutes or less to fall asleep.

### 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. CD4+ T-cell count and viral load were analyzed in clinically meaningful categories. Demographic and clinical differences between the sleep groups were evaluated using analysis of variance 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 levels of plasma cytokines between the two sleep groups were assessed using Mann-Whitney U Test due to non-normal distributions that could not be adequately corrected through transformation. Adjusted associations were evaluated using logistic regression models predicting membership in the SOI group (sleep onset latency > 30 minutes) 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 SOI group when adjusting for relevant covariates. All adjusted regression models controlled for genomic estimates of ancestry (described below) and self-reported race/ethnicity (i.e., Caucasian, African-American, other), given the potential of these variables to confound

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genetic associations and therefore downstream biomarkers (i.e., protein product of a gene). In addition, all demographic and clinical variables associated with sleep 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 SOI group. Three genetic models were tested (i.e., additive, dominant, recessive), and the model that best fit the data (barring improvements of delta < 10%) was reported for each SNP. Adjustments were not made for multiple testing (Perneger, 1998; Rothman, 1990).

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 SOI group. A model was fit for each genetic marker to estimate its unique contribution to sleep group when adjusting for relevant covariates. The same covariates used for the adjusted plasma cytokine models were included in the adjusted genetic association models. Each polymorphism associated with sleep onset group in adjusted analyses was evaluated with respect to its impact on cytokine plasma levels using linear regression models, 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.

## 2. Results

### 3.1 Sample characteristics

Prior to analysis, 43 of the 350 enrolled participants were excluded for screening positive for illicit drugs (n=31), being unable to submit a urine or blood sample (n=2), or not answering the question about how long it usually took to fall asleep in the past month (n =10). Of the remaining 307 participants, 70 (22.8%) reported usually taking more than 30 minutes to fall asleep in the past month, and the remaining 237 (77.2%) reported usually taking 30 minutes or less to fall asleep. Demographic and clinical characteristics for the 307 participants included in this 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

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been living with HIV for an average of 12.1 ± 7.1 years; 53% had been diagnosed with AIDS, but only 28% of those with an AIDS diagnosis had current CD4+ T-cell counts below 200 cells/mm$^3$. Most (75%) were receiving medical disability assistance, 72% were currently receiving ART, and they were taking an average of 6.0 ± 4.0 medications (median 6, range 0–22).

### 3.2 Sleep onset characteristics

The self-reported mean time to fall asleep was 26.3 ± 23.1 minutes, but for the 70 participants who reported usually taking more than 30 minutes to fall asleep (SOI) in the last month, the mean time to fall asleep was 58.3 ± 26.8 minutes. For participants who reported taking 30 minutes or less to fall asleep, their mean time was 16.9 ± 9.4 minutes. As shown in Table 1, income and viral load differed by sleep group. For males, there was a non-significant difference in BMI by sleep group, but this trend was reversed for females and absent among transgender adults. The sleep groups did not differ with respect to any of the medication classes examined (ART, sleep medication, anxiolytics, antidepressants, and opiates).

As shown in Table 2, the two sleep groups did not differ with respect to any of the cytokine plasma levels. To estimate the association between plasma cytokines and SOI when adjusting for relevant covariates, multiple linear regression models were fit predicting each cytokine plasma level. Genomic estimates of ancestry and self-reported race/ethnicity were forced into all models to control for population substructure. Income, viral load, and BMI were evaluated as potential covariates, but only viral load met the criterion for retention ($p <$ 0.05) in the final models. After adjusting for genomic estimates of ancestry, self-reported race/ethnicity, and viral load in the multivariable analyses, plasma level of *IL-13* was significantly associated with sleep group (OR = 1.34 for a 1 SD unit change in *IL-13*, 95% CI: 1.04, 1.72, p=.024). Although not statistically significant, the SOI group also had slightly higher *IL-10* plasma levels (OR = 1.27 for a 1 SD unit change in *IL-10*, 95% CI: 0.98, 1.65, p=.068).

### 3.3 Genetic associations

Of the 82 SNPs included in the analysis, 6 SNPs mapping to 5 of the 15 evaluated candidate genes were significantly associated with sleep group in bivariate analyses (Table 3). To estimate genotype's effect on sleep group when adjusting for relevant covariates, multiple logistic regression models were fit using the same approach and covariates as in the plasma level models described above. Of the 6 SNPs associated with sleep group in bivariate analyses, 4 remained associated with sleep group after adjusting for genomic estimates of ancestry, self-reported race/ethnicity, and viral load: IL1B rs1143642, IL6 rs4719714, NFKB1 rs4648110, TNFA rs2857602 (Table 4). In addition, two SNPs (IL1B rs1143623 and IL13 rs1295686) not associated with sleep group in bivariate analyses were significantly associated with sleep group after controlling for the confounding effects of genomic ancestry, self-reported race/ethnicity, and viral load.

One of the IL1B polymorphisms (rs1143642) in Table 4 was associated with plasma levels of *IL-1β* in regression analyses adjusting for genomic estimates of ancestry, self-reported

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race/ethnicity, and viral load ($p$=.001). Because *NFKB-1* levels cannot be measured from plasma, their association with the NFKB1 polymorphism (rs4648110) could not be examined.

## 3. Discussion

Almost one in four (23%) participants in this sample of HIV-infected adults met criterion for SOI (>30 minutes to fall asleep) using a self-report measure that reflected the past month. In the group with SOI, the mean time to sleep onset was close to one hour, while the comparison group averaged about 15 minutes to fall asleep. The incidence of SOI was higher for participants with lower income and for participants with higher viral load, although income was no longer associated with SOI after controlling for genomic estimates of ancestry and self-reported race/ethnicity. There were no significant sex differences or differences based on race/ethnicity, education, or employment. In bivariate analyses, there were no group differences in plasma cytokine levels.

Although there were no differences in SOI based on self-reported race/ethnicity, final models were adjusted for both genomic estimates of ancestry and self-reported race/ethnicity given their potential to influence genetic associations (Lee et al., 2014b). After adjusting for these ancestry variables as well as viral load, single polymorphisms from four genes (IL6, IL13, NFKB1, and TNFA), and two polymorphisms from IL1B were significantly associated with SOI. Four of these polymorphisms were associated with SOI prior to adjusting for ancestry, self-reported race/ethnicity and viral load (IL1B rs1143642, IL6 rs4719714, NFKB1 rs4648110, TNFA rs2857602). Of the six polymorphisms associated with sleep onset insomnia, four were positively associated with longer sleep onset times and two were negatively associated with difficulty initiating sleep (i.e., were associated with shorter sleep onset times). One IL1B SNP (rs1143642) was associated with an increased risk of SOI in a dominant mode; the IL6 SNP (rs4719714) was associated with increased risk of SOI in an additive pattern (the more copies of the minor allele of the gene, the greater the likelihood of having SOI), and the SNPs from NFKB1 (rs4648110) and TNFA (rs2857602) were associated with SOI in a recessive mode. Two polymorphisms, both in the additive mode, were negatively associated with SOI: one IL1B SNP (rs1143623) and one IL13 SNP (rs1295686). These associations are intriguing, as three of these genes (IL1B, NFKB1, TNFA) are related to sleep-regulating substances (Krueger and Majde, 2006; Zielinski and Krueger, 2011) and two genes (IL1B, TNFA), although different SNPs, have been associated with fatigue in the HIV population (Lee et al., 2014b). One gene (IL6) has been previously shown to be associated with poor sleep and fatigue (Miaskowski et al., 2012; Miaskowski et al., 2010; Schrepf et al., 2013; Vgontzas and Chrousos, 2002). *IL1-$\beta$* and *TNF$\alpha$* have sleep regulatory properties and may increase non-REM sleep (Krueger, 2008; Zielinski et al., 2013). *IL-6* and *TNF$\alpha$* levels are increased during the day in subjects with insomnia and increased daytime levels of *IL-6* are associated with either hypersomnolence or fatigue (Vgontzas and Chrousos, 2002). Elevated levels of *IL-1*, *IL-6*, and *TNF-alpha* are seen in HIV infection (Breen, 2002). Given their roles in sleep regulation and daytime fatigue, it is not surprising to see specific polymorphisms of those cytokines contribute to the individual variability in sleep onset latency. Although *IL-13* has not been proven to be a sleep regulatory substance in humans, it does inhibit non-REM sleep in rabbits (Kubota et

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al., 2000). In the present study, adults with SOI also had higher plasma levels of *IL-13* after controlling for viral load. Thus, these findings support a possible role for *IL-13* in human sleep regulatory functioning.

A primary strength of this study is that it included both cytokine polymorphisms and plasma levels of cytokines to better understand how they each may relate to SOI 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 insomnia and cytokine biomarkers. Finally, the use of an insomnia phenotype that estimates habitual sleep onset latency by self-report is a strength of the study. In our sample, this SOI measure was correlated with 3-day diary recordings (rho=0.59, $p<.001$) and to a lesser extent with actigraphy measures (rho=0.16, p=.007) of sleep onset latency, but a habitual self-report over the past month spans a broader time frame, and thus is more suitable for a genetic association study.

The results of this study also need to be considered in light of its limitations. The main limitations for a genetic association study are the modest sample size with relatively fewer women and participants in some racial and ethnic groups. The number of participants with SOI was reflective of the general population, but also relatively small for analysis of genetic association in these groups. Given the small sample size, these findings require replication in other samples. In addition, the medication classes evaluated in this study were not associated with sleep onset latency, and were not included in subsequent analyses. However, the relatively small sample precluded analysis of specific medications which may be relevant to inflammatory activity, and larger studies are needed to evaluate their potential impact on the observed associations. Furthermore, the CD4 and viral load measures may have been up to 3 months older than the cytokine plasma values, which may have limited their association. Although the sample is representative of adults living with HIV in the San Francisco Bay Area, replication in other types of chronic illness 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. Although fasting blood samples were collected in the morning, variations in the timing of collection may have limited associations between plasma cytokines and both sleep onset latency and cytokine polymorphisms. Moreover, morning nadir cytokine levels may be less associated with sleep onset latency than evening, which should be investigated in future studies. Finally, sleep onset latency was assessed using a single retrospective item from the Pittsburgh Sleep Quality Index, and the observed associations could presumably be stronger with the use of a diagnostic interview specifically focused on initiation insomnia.

The present study's findings of significant associations with cytokine gene polymorphisms, but little in relation to the plasma cytokines, may reflect the challenges of measuring clinically meaningful cytokine levels (e.g., central versus peripheral levels, circadian fluctuations). In addition, future studies should explore the role of race and ethnicity as well as other symptoms that commonly co-occur with insomnia, such as anxiety and depression.

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Findings from this study in a chronic illness population do contribute to the growing evidence for an association between inflammatory pathways and sleep onset insomnia, regardless of clinical characteristics. However, the mechanism by which cytokines influence sleep onset latency remains unclear, and further investigation into potential mechanisms for these associations is warranted. It is also unclear from the results of this study whether or how these associations might impact HIV progression and further research in this area is also needed. Nonetheless, the association between cytokine polymorphisms and initiation insomnia suggests that anti-inflammatory medication could have therapeutic potential for decreasing sleep onset latency among adults living with HIV. Further research is warranted to compare the therapeutic effects of anti-inflammatory medications to other pharmacologic interventions for this type of insomnia.

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## Highlights

- Self-report of habitual sleep onset insomnia (taking more than 30 minutes to fall asleep) is associated with:
  - higher IL-13 plasma levels.
  - IL1B, IL6, and IL13 polymorphisms.
  - NFKB1 and TNFA polymorphisms.
- Adjusting for race may control false positive/negative associations.

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**Table 1**

Demographic and clinical characteristics by sleep onset latency group (n=307)

| | Total Sample (n=307) | Sleep Onset Latency Group | | Statistics |
|---|---|---|---|---|
| | | 30 minutes (n=237) | >30 minutes (n=70) | |
| **Demographics** | | | | |
| Age, years, mean (SD) (range 22 – 77) | 44.9 (8.3) | 44.9 (8.4) | 44.9 (8.3) | $F(1,305)=0.00$, $p=.999$ |
| Gender | | | | $\chi^2(2,307)=0.68$, $p=.713$ |
| Male | 212 (69%) | 162 (68%) | 50 (71%) | |
| Female | 72 (23%) | 58 (25%) | 14 (20%) | |
| Transgender | 23 (8%) | 17 (7%) | 6 (9%) | |
| Race/ethnicity | | | | $\chi^2(2,307)=4.57$, $p=.102$ |
| Caucasian | 129 (42%) | 101 (43%) | 28 (40%) | |
| African-American | 113 (37%) | 92 (39%) | 21 (30%) | |
| Other | 65 (21%) | 44 (18%) | 21 (30%) | |
| Education | | | | $\chi^2(2,307)=0.60$, $p=.741$ |
| High school or less | 132 (43%) | 103 (43%) | 29 (41%) | |
| Vocational or some college | 107 (35%) | 80 (34%) | 27 (39%) | |
| College degree | 68 (22%) | 54 (23%) | 14 (20%) | |
| Employment, n (%) | | | | $\chi^2(2,307)=0.81$, $p=.666$ |
| Employed/student | 49 (16%) | 40 (17%) | 9 (13%) | |
| Unemployed | 18 (9%) | 23 (10%) | 6 (9%) | |
| Disability | 229 (75%) | 174 (73%) | 55 (78%) | |
| **Monthly Income** | | | | $\chi^2(2,307)=5.36$, $\mathbf{p=.021}$ |
| < $1,000 | 211 (69%) | 155 (65%) | 56 (80%) | |
| $1,000 | 96 (31%) | 82 (35%) | 14 (20%) | |
| **Clinical Characteristics** | | | | |
| CD4+ T-cell count (cells/mm$^3$) | n=293[a] | n=230[a] | n=63[a] | $\chi^2(2,293)=0.01$, $p=.925$ |
| <200 | 50 (17%) | 39 (17%) | 11 (17%) | |
| 200 | 243 (83%) | 191 (83%) | 52 (83%) | |
| **Viral Load ($\mathbf{log_{10}}$ copies/mL)** | n=287[a] | n=226[a] | n=61[a] | $\chi^2(2,287)=4.12$, $\mathbf{p=.042}$ |
| Undetectable | 146 (51%) | 122 (54%) | 24 (39%) | |
| Detectable | 141 (49%) | 104 (46%) | 37 (61%) | |
| Years since HIV diagnosis | | | | $F(1,305)=0.00$, $p=.973$ |
| Mean (SD), range 0.2 - 26 | 12.1 (7.0) | 12.1 (7.0) | 12.1 (7.0) | |
| Anti-retroviral therapy | | | | $\chi^2(2,307)=0.12$, $p=.726$ |
| Not on treatment | 87 (28%) | 66 (28%) | 21 (30%) | |
| On treatment | 220 (72%) | 171 (72%) | 49 (70%) | |
| AIDS Diagnosis | | | | $\chi^2(2,307)=0.08$, $p=.772$ |
| No | 145 (47%) | 113 (48%) | 32 (46%) | |
| Yes | 162 (53%) | 124 (52%) | 38 (54%) | |

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| | Total Sample (n=307) | Sleep Onset Latency Group | | Statistics |
|---|---|---|---|---|
| | | 30 minutes (n=237) | >30 minutes (n=70) | |
| **Body mass index, mean (SD)** | | | | |
| Male | 26.1 (4.8) | 25.7 (4.2) | 27.2 (6.5) | $F(1,210)=3.54$, $\boldsymbol{p=.061}$ |
| Female | 28.9 (6.4) | 29.5 (6.5) | 26.8 (5.5) | $F(1,70)=1.98$, $p=.164$ |
| Transgender | 28.9 (6.7) | 29.1 (6.7) | 28.6 (7.7) | $F(1,21)=0.02$, $p=.895$ |

*Note*: SD = standard deviation; **Bolded** variables are associated ($p < 0.10$) with sleep group and were evaluated as potential covariates in adjusted regression models. Use of sleep medication, anxiolytics, antidepressants, or opiates was not associated with sleep group.

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

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## Table 2

Plasma cytokine and CRP levels by sleep onset latency group (n=305)

| | Total Sample (n=305) | Sleep Onset Latency Group | | Statistics |
|---|---|---|---|---|
| | | 30 minutes (n=236) | >30 minutes (n=69) | |
| *IL-1β* (pg/mL) | 4.11 (3.53) | 4.07 (3.39) | 4.29 (4.00) | MWU *p*=.821 |
| *IL-2* (pg/mL) | 8.65 (13.9) | 8.40 (13.3) | 9.52 (15.7) | MWU *p*=.937 |
| *IL-6* (pg/mL) | 21.2 (34.8) | 20.7 (34.3) | 22.8 (36.5) | MWU *p*=.547 |
| *IL-10* (pg/mL) | 24.3 (49.3) | 21.7 (41.9) | 33.2 (68.6) | MWU *p*=.449 |
| *IL-13* (pg/mL)[a] | 6.04 (10.1) | 5.43 (7.23) | 8.12 (16.3) | MWU *p*=.331 |
| *TNFα* (pg/mL) | 12.3 (11.6) | 12.7 (12.1) | 11.0 (9.7) | MWU *p*=.421 |
| *CRP* (ng/mL) | 13.0 (19.6) | 13.5 (20.8) | 11.0 (14.5) | MWU *p*=.786 |

*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.

[a]*IL-13* plasma levels were associated with sleep group in analyses adjusting for genomic estimates of ancestry, self-reported race/ethnicity, and viral load (OR=1.34 for a 1 SD unit change in *IL-13*, 95% CI: 1.04, 1.72, p=.024).

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**Table 3**

Unadjusted associations between 82 cytokine polymorphisms and sleep onset latency group (n=307)

| Gene | SNP | HGVS. Description | HuRef Position | Chr | MAF | OR | 95% CI | *p* | Model |
|---|---|---|---|---|---|---|---|---|---|
| IFNG | rs2069728 | (3′ of gene, G>A) | 65598422 | 12 | .147 | 1.15 | 0.64, 2.09 | .639 | D |
| | rs2069727 | (3′ of gene, A>G) | 65598861 | 12 | .325 | 1.59 | 0.92, 2.76 | .095 | D |
| | rs2069718 | c.367-895T>C | 65600800 | 12 | .489 | 1.61 | 0.82, 3.13 | .165 | D |
| | rs1861493 | c.366+497C>T | 65601835 | 12 | .224 | 0.32 | 0.04, 2.58 | .288 | R |
| | rs1861494 | c.366+284G>A | 65602048 | 12 | .236 | 0.33 | 0.04, 2.59 | .289 | R |
| | rs2069709[a,b] | (5′ of gene, G>T) | 65604339 | 12 | .011 | | | | |
| IFNGR1 | rs9376268 | c.86-4537C>T | 135094927 | 6 | .193 | 1.09 | 0.62, 1.92 | .761 | D |
| IL1B | rs1071676 | c.*505G>C | 106042060 | 2 | .165 | 1.09 | 0.62, 1.93 | .761 | D |
| | rs1143643 | c.598-152G>A | 106042929 | 2 | .263 | 1.38 | 0.58, 3.29 | .466 | R |
| | **rs1143642** | **c.597+316T>C** | **106043180** | **2** | **.141** | **1.89** | **1.07, 3.34** | **.029** | **D** |
| | rs1143634 | c.315C>T | 106045017 | 2 | .162 | 1.13 | 0.64, 2.00 | .674 | D |
| | rs1143633 | c.302-64G>A | 106045094 | 2 | .278 | 1.51 | 0.66, 3.47 | .331 | R |
| | rs1143630 | c.100-503A>C | 106046282 | 2 | .149 | 1.66 | 0.94, 2.93 | .083 | D |
| | rs3917356 | c.99+780G>A | 106046990 | 2 | .373 | 1.14 | 0.79, 1.64 | .475 | A |
| | rs1143629 | c.47+242C>T | 106048145 | 2 | .417 | 0.56 | 0.26, 1.21 | .142 | R |
| | rs1143627 | (5′ of gene, T>C) | 106049014 | 2 | .492 | 0.82 | 0.57, 1.18 | .278 | A |
| | rs16944 | (5′ of gene, G>A) | 106049494 | 2 | .469 | 0.76 | 0.52, 1.11 | .149 | A |
| | rs1143623 | (5′ of gene, G>C) | 106050452 | 2 | .228 | 0.70 | 0.44, 1.11 | .132 | A |
| | rs13032029 | (5′ of gene, C>T) | 106055022 | 2 | .354 | 1.20 | 0.84, 1.73 | .313 | A |
| IL1R | rs949963 | (5′ of gene, G>A) | 96533648 | 2 | .234 | 2.24 | 0.83, 6.02 | .109 | R |
| | rs2228139[a,b] | c.371C>G | 96545511 | 2 | .036 | | | | |
| | rs3917320[b] | c.1366A>C | 96556738 | 2 | .067 | | | | |
| | rs2110726 | c.*1063G>A | 96558145 | 2 | .244 | 2.18 | 0.91, 5.23 | .080 | R |
| | **rs3917332** | **(3′ of gene, T>A)** | **96560387** | **2** | **.157** | **10.5** | **1.07, 102.4** | **.043** | **R** |
| IL1R2 | rs4141134 | (5′ of gene, T>C) | 96370336 | 2 | .302 | 1.99 | 0.95, 4.15 | .068 | R |
| | rs11674595 | c.-62+2519T>C | 96374804 | 2 | .202 | 0.33 | 0.04, 2.62 | .293 | R |

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| Gene | SNP | HGVS. Description | HuRef Position | Chr | MAF | OR | 95% CI | *p* | Model |
|---|---|---|---|---|---|---|---|---|---|
| | rs7570441 | c.-62+1417G>A | 96380807 | 2 | .454 | 0.93 | 0.52, 1.67 | .809 | D |
| IL2 | rs1479923 | (3′ of gene, C>T) | 119096993 | 4 | .215 | 2.28 | 0.85, 6.13 | .102 | R |
| | rs2069776 | (3′ of gene, T>C) | 119098582 | 4 | .191 | 1.15 | 0.71, 1.87 | .571 | A |
| | rs2069772 | c.352-116A>G | 119099739 | 4 | .193 | 1.94 | 0.63, 5.99 | .249 | R |
| | rs2069777[a,b] | c.207+862C>T | 119103043 | 4 | .048 | | | | |
| | rs2069763[c] | c.114G>T | 119104088 | 4 | .299 | | | | |
| IL4 | rs2243248 | (5′ of gene, T>G) | 127200946 | 5 | .114 | 0.74 | 0.39, 1.41 | .364 | A |
| | rs2243250[c] | (5′ of gene, C>T) | 127201455 | 5 | .377 | | | | |
| | rs2070874 | c.-33C>T | 127202011 | 5 | .278 | 0.70 | 0.40, 1.20 | .191 | D |
| | rs2227284[c] | c.183+2527T>G | 127205027 | 5 | .464 | | | | |
| | rs2227282[c] | c.184-2227C>G | 127205481 | 5 | .454 | | | | |
| | rs2243263 | c.184-2107C>G | 127205601 | 5 | .145 | 3.44 | 0.48, 24.9 | .221 | R |
| | rs2243266 | c.184-1617G>A | 127206091 | 5 | .263 | 1.90 | 0.81, 4.48 | .142 | R |
| | rs2243267 | c.184-1520G>C | 127206188 | 5 | .262 | 1.91 | 0.81, 4.50 | .139 | R |
| | rs2243274 | c.184-574G>A | 127207134 | 5 | .374 | 0.70 | 0.41, 1.19 | .188 | D |
| IL6 | **rs4719714** | **(5′ of gene, A>T)** | **22643793** | **7** | **.211** | **1.92** | **1.12, 3.29** | **.018** | **D** |
| | rs2069827[b] | (5′ of gene, G>T) | 22648536 | 7 | .051 | | | | |
| | rs1800796 | (5′ of gene, G>C) | 22649326 | 7 | .101 | 2.07 | 0.48, 8.88 | .328 | R |
| | rs1800795 | (5′ of gene, C>G) | 22649725 | 7 | .247 | 0.84 | 0.54, 1.31 | .443 | A |
| | rs2069835[b] | c.211-441T>C | 22650951 | 7 | .075 | | | | |
| | rs2066992 | c.211-63G>T | 22651329 | 7 | .100 | 1.18 | 0.67, 2.08 | .559 | A |
| | rs2069840 | c.324+147C>G | 22651652 | 7 | .265 | 1.65 | 0.97, 2.83 | .067 | D |
| | rs1554606 | c.324+282T>G | 22651787 | 7 | .385 | 0.75 | 0.51, 1.10 | .135 | A |
| | rs2069845 | c.471+870G>A | 22653229 | 7 | .38 | 0.76 | 0.51, 1.12 | .163 | A |
| | rs2069849[b] | c.603C>T | 22654236 | 7 | .088 | | | | |
| | rs2069861[a,b] | (3′ of gene, C>T) | 22654734 | 7 | .046 | | | | |
| | rs35610689 | (3′ of gene, A>G) | 22656903 | 7 | .234 | 1.07 | 0.68, 1.70 | .762 | A |
| IL8 | rs4073 | (5′ of gene, T>A) | 70417508 | 4 | .459 | 1.31 | 0.71, 2.41 | .384 | R |

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| Gene | SNP | HGVS. Description | HuRef Position | Chr | MAF | OR | 95% CI | *p* | Model |
|---|---|---|---|---|---|---|---|---|---|
| | rs2227306 | c.65-204C>T | 70418539 | 4 | .273 | 0.81 | 0.47, 1.39 | .445 | D |
| | rs2227543 | c.284+161C>T | 70419394 | 4 | .272 | 0.82 | 0.48, 1.40 | .462 | D |
| IL10 | rs3024505 | (3′ of gene, C>T) | 177638230 | 1 | .125 | 0.88 | 0.49, 1.59 | .671 | A |
| | rs3024498 | c.*452A>G | 177639855 | 1 | .182 | 1.46 | 0.37, 5.80 | .592 | R |
| | rs3024496 | c.*117T>C | 177640190 | 1 | .453 | 0.95 | 0.65, 1.39 | .791 | A |
| | rs1878672 | c.379-474C>G | 177642039 | 1 | .395 | 0.79 | 0.46, 1.36 | .388 | D |
| | rs3024492 | c.378+140A>T | 177642438 | 1 | .152 | 1.19 | 0.73, 1.96 | .488 | A |
| | rs1518111 | c.225+56A>G | 177642971 | 1 | .300 | 0.86 | 0.34, 2.21 | .761 | R |
| | rs1518110 | c.166-101T>G | 177643187 | 1 | .298 | 0.91 | 0.35, 2.34 | .841 | R |
| | rs3024491 | c.166-286G>T | 177643372 | 1 | .392 | 0.81 | 0.47, 1.40 | .450 | D |
| IL13 | rs1881457 | (5′ of gene, A>C) | 127184713 | 5 | .224 | 1.71 | 1.00, 2.93 | .050 | D |
| | rs1800925 | (5′ of gene, C>T) | 127185113 | 5 | .289 | 1.36 | 0.89, 2.07 | .150 | A |
| | rs2069743 | (5′ of gene, A>G) | 127185579 | 5 | .078 | 0.67 | 0.31, 1.44 | .301 | A |
| | rs1295686 | c.334-24T>C | 127188147 | 5 | .438 | 0.51 | 0.25, 1.06 | .073 | R |
| | rs20541 | c.431A>G | 127188268 | 5 | .232 | 0.40 | 0.09, 1.80 | .235 | R |
| IL17A | rs4711998 | (5′ of gene, G>A) | 51881422 | 6 | .366 | 0.66 | 0.39, 1.13 | .127 | D |
| | rs8193036 | (5′ of gene, T>C) | 51881562 | 6 | .245 | 1.14 | 0.74, 1.75 | .550 | A |
| | rs3819024 | (5′ of gene, A>G) | 51881855 | 6 | .293 | 1.39 | 0.58, 3.30 | .460 | R |
| | rs2275913 | (5′ of gene, G>A) | 51882102 | 6 | .209 | 1.61 | 0.59, 4.40 | .355 | R |
| | rs3804513[a,b] | c.230+594A>T | 51884266 | 6 | .023 | | | | |
| | rs7747909 | c.*159G>A | 51885318 | 6 | .172 | 0.89 | 0.54, 1.44 | .626 | A |
| NFKB1 | rs3774933 | c.-8+3394T>C | 99162722 | 4 | .369 | 0.93 | 0.62, 1.40 | .724 | A |
| | rs170731 | c.40-2090T>A | 99185284 | 4 | .271 | 1.43 | 0.57, 3.61 | .447 | R |
| | rs17032779[b] | c.255+7137T>C | 99202630 | 4 | .054 | | | | |
| | rs230510 | c.256-11978T>A | 99212552 | 4 | .343 | 0.69 | 0.31, 1.56 | .374 | R |
| | rs230494 | c.256-1175G>A | 99223356 | 4 | .377 | 1.05 | 0.71, 1.55 | .818 | A |
| | rs4648016[a,b] | c.404+1378C>T | 99226057 | 4 | .029 | | | | |
| | rs4648018[a,b] | c.404+1908G>C | 99226587 | 4 | .046 | | | | |
| | rs3774956 | c.1063+2549C>T | 99244914 | 4 | .392 | 1.00 | 0.68, 1.49 | .984 | A |

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| Gene | SNP | HGVS. Description | HuRef Position | Chr | MAF | OR | 95% CI | *p* | Model |
|---|---|---|---|---|---|---|---|---|---|
| | rs10489114[a,b] | c.1064-3194T>C | 99247604 | 4 | .046 | | | | |
| | rs4648068 | c.1493-372A>G | 99254521 | 4 | .306 | 0.97 | 0.64, 1.45 | .872 | A |
| | rs4648095[a,b] | c.1951+22T>C | 99264093 | 4 | .044 | | | | |
| | **rs4648110** | **c.2589+58T>A** | **99270046** | **4** | **.259** | **2.86** | **1.28, 6.38** | **.010** | **R** |
| | rs4648135[b] | c.2747-921A>G | 99272895 | 4 | .075 | | | | |
| | rs4648141 | c.2747-690G>A | 99273126 | 4 | .319 | 1.69 | 0.82, 3.48 | .151 | R |
| | rs1609798 | c.2747-149C>T | 99273667 | 4 | .257 | 0.92 | 0.61, 1.38 | .677 | A |
| NFKB2 | rs12772374 | c.395+99A>G | 97790120 | 10 | .128 | 1.37 | 0.81, 2.31 | .246 | A |
| | rs7897947 | c.662-27T>G | 97790920 | 10 | .287 | 0.91 | 0.59, 1.38 | .645 | A |
| | rs11574849[b] | c.1470-141G>A | 97792905 | 10 | .068 | | | | |
| | rs1056890 | c.*187C>T | 97795944 | 10 | .263 | 0.96 | 0.64, 1.45 | .863 | A |
| TNFA | **rs2857602** | **g.31473378G>A** | **31533378** | **6** | **.315** | **3.11** | **1.49, 6.52** | **.003** | **R** |
| | rs1800683 | c.-18G>A | 31540071 | 6 | .434 | 0.67 | 0.39, 1.17 | .157 | D |
| | **rs2239704**[d] | **c.-92A>C** | **31540141** | **6** | **.310** | **2.98** | **1.40, 6.37** | **.005** | **R** |
| | rs2229094[d] | c.37T>C | 31540556 | 6 | .265 | 0.79 | 0.46, 1.36 | .398 | D |
| | rs1041981[d] | c.179C>A | 31540784 | 6 | .436 | 0.67 | 0.39, 1.17 | .157 | D |
| | rs1799964 | g.31482308T>C | 31542308 | 6 | .184 | 0.80 | 0.49, 1.31 | .374 | A |
| | rs1800750[a,b] | g.31482963G>A | 31542963 | 6 | .018 | | | | |
| | rs1800629 | c.-308G>A | 31543031 | 6 | .134 | 6.91 | 0.62, 77.4 | .117 | R |
| | rs1800610 | c.186+123G>A | 31543827 | 6 | .079 | 1.55 | 0.83, 2.91 | .169 | A |
| | rs3093662[b] | c.187-122A>G | 31544189 | 6 | .085 | | | | |

Notes. Models predict membership in the group with sleep onset latency >30 minutes, and the group with sleep onset latency  30 minutes serves 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 the risk of sleep onset latency >30 minutes, while OR>1 increases risk of sleep onset latency > 30 minutes); 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=10 SNPs)

[b]SNP excluded from analysis because one of the genotypes had a frequency <3 (n=18 SNPs)

[c]SNP excluded from analysis because distribution violated Hardy-Weinberg equilibrium (n=4 SNPs)

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[d] This TNFA SNP uses NM_000595.2

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**Table 4**

Significant adjusted associations between cytokine genotype and sleep onset latency group (n=285)

| Gene | SNP | Model | OR | 95% CI | p | Full models |
|---|---|---|---|---|---|---|
| ILIB | rs1143642 | D | 2.37 | 1.21, 4.64 | .012 | $\chi^2$=16.5, $p$=.021 |
| ILIB | rs1143623 | A | **0.54** | 0.31, 0.95 | .034 | $\chi^2$=15.2, $p$=.034 |
| IL6 | rs4719714 | A | 1.71 | 1.03, 2.85 | .039 | $\chi^2$=14.5, $p$=.044 |
| IL13 | rs1295686 | A | **0.59** | 0.36, 0.97 | .037 | $\chi^2$=14.8, $p$=.039 |
| NFKB1 | rs4648110 | R | 4.28 | 1.76, 10.44 | .001 | $\chi^2$=20.0, $p$=.006 |
| TNFA | rs2857602 | R | 2.57 | 1.09, 6.06 | .030 | $\chi^2$=14.7, $p$=.040 |

Notes. Models predict membership in the group with sleep onset latency >30 minutes, and the group with sleep onset latency 30 minutes serves as the reference. All models adjusted for genomic estimates of ancestry, self-reported race/ethnicity, and viral load. The sample size for adjusted analyses was 285 due to missing viral load values for 22 participants. A = additive model; CI = confidence interval; D = dominant model; IL = interleukin; NKFKB = nuclear factor of kappa light polypeptide gene enhancer in B cells; OR = odds ratio based on dose of minor allele; R = recessive model; TNFA = tumor necrosis factor alpha.