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Cytokine Polymorphisms are Associated with Daytime Napping in Adults Living with HIV

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Abstract

Objective/Background—Daytime napping longer than one hour has been associated with an increased risk for all-cause mortality. Associations between cytokine polymorphisms and daytime napping in chronic illnesses such as HIV, however, have not been well described. The purpose of this study was to examine cytokine polymorphisms associated with long daytime napping in adults living with HIV.

Methods—A cross-sectional analysis was conducted using a convenience sample of 257 adults living with HIV. Daytime napping was assessed with wrist actigraphy data collected over three days. Participants categorized as long nappers (≥ 60 min) were compared to short nappers and non-nappers (< 60 min). Single nucleotide polymorphisms (SNPs) for 15 candidate genes involved in cytokine signaling were analyzed. Genes included: interferon-gamma (*IFNG*), *IFNG* receptor 1 (*IFNGR1*), interleukins (*IL1B, IL1R, IL1R2, IL2, IL4, IL6, IL8, IL10, IL13, IL17A*), nuclear factors of kappa light polypeptide gene enhancer in B cells (*NFKB1* and *NFKB2*), and tumor necrosis factor alpha (*TNFA*).

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Results—After adjusting for relevant demographic and clinical characteristics, long daytime napping was associated with 12 SNPs from seven genes: 1) *IFNG* rs2069728; 2) *IL1B* rs1143642, rs1143627, and rs16944; 3) *IL2* rs2069763; 4) *IL6* rs4719714, rs1554606, and rs2069845; 5) *IL17A* rs3819024 and rs8193036; 6) *NFKB1* rs4648110; and 7) *NFKB2* rs1056890.

Conclusions—Cytokine genetic variations may have a role in physiological regulation of daytime napping as well as nocturnal sleep. Cytokine polymorphisms associated with long daytime napping could help identify adults with HIV who may benefit from targeted therapeutic interventions.

Keywords

daytime napping; cytokine; inflammation; genetic; actigraphy; HIV

1. INTRODUCTION

Recent meta-analysis studies have suggested that daytime napping is an indicator of increased all-cause mortality [1, 2]. Although some beneficial effects of a short nap have been suggested [3], daytime napping of one hour per day or longer has been associated with an increased risk of cardiovascular disease [1] and all-cause mortality [1, 4]. Laboratory studies in healthy adults have shown that a short nap after experimental sleep deprivation can restore cognitive impairment and immune function [5–7]. However, long daytime naps that contain delta stage 3–4 non-rapid eye movement (NREM) can lead to impaired alertness after awakening [5, 8]. Even in healthy young adults, daytime napping has been associated with daytime sleepiness, depression, and problems with daytime functioning [9].

Adults living with human immunodeficiency virus (HIV) frequently report sleep problems [10], and up to 55% of adults living with HIV experience daytime sleepiness or drowsiness [11, 12]. In chronic illnesses like HIV, daytime sleepiness has been associated with poor medication adherence and cognitive impairment [13, 14]. Due to successful therapies for their HIV infection, these patients are living longer with multiple comorbidities [15]. Taking a long daytime nap may influence daily function or adherence to antiretroviral therapy and other complex medical regimens related to comorbidities. Studies in the HIV population have focused on nocturnal sleep duration, sleep maintenance or sleep quality, and daytime sleepiness [12, 16, 17]. Daytime sleep behavior has not been well studied in this chronic illness population and underlying mechanisms are unclear. One potential mechanism linked to daytime napping in HIV may involve the inflammatory process.

The HIV virus settles in perivascular macrophages and microglial cells in the brain, inducing an immune response that produces viral and inflammatory proteins [16, 18] and secretes cytokines such as interleukin-1 beta (IL-1β), tumor necrosis factor alpha (TNFα), and interferon-gamma (IFNγ) [19]. These cytokines are involved in induction of NREM sleep, and may change diurnal sleep-wake patterns by promoting long daytime napping [20, 21]. Inflammation and cytokine activity play an important role in sleep disturbance in chronic illness [22, 23]. Nuclear factor of kappa light polypeptide gene enhancer in B cells (NF-κB) and cytokines, such as IL-1β, IL-6, and TNFα are known to be involved in sleep regulation [21, 23]. We previously reported that polymorphisms of *IL1R2, IL2,* and *TNFA* were

associated with nocturnal sleep maintenance [17] and polymorphisms of *IL1B, IL6, IL13, NFKB1,* and *TNFA* were associated with sleep-onset insomnia in this sample of HIV-infected adults [24]. Elevated plasma levels of TNFα and IL-6 in individuals with sleep apnea, and elevated TNFα plasma levels in individuals with narcolepsy, have been reported if these individuals also experienced excessive daytime sleepiness [25].

Given these prior findings and the need to better understand the mechanisms underlying daytime napping behavior in chronic illness, the purpose of this study was to examine associations between cytokine-associated polymorphisms and daytime napping in adults living with HIV. Based on the findings of prior studies [1, 4], this initial association study focused on long daytime napping, defined as daytime sleep of 60 minutes or longer as objectively estimated by wrist actigraphy.

2. METHODS

The Symptom and Genetic Study is a longitudinal study with a convenience sample of adults living with HIV/AIDS aimed at identifying biomarkers of symptom experience among HIV-infected adults [10]. This cross-sectional analysis focuses on potential cytokine-related genetic markers of daytime napping. The study was approved by the Committee on Human Research at the University of California, San Francisco (UCSF). Study participants were recruited using flyers at HIV clinics and community sites in the San Francisco Bay Area. All participants provided written informed consent and signed a Health Insurance Portability and Accountability Act release to access their protected medical information for this research.

Participants were included if they 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 with insomnia were not excluded, but those with other diagnosed sleep disorders, such as apnea and narcolepsy were excluded.

2.1 Sample Characteristics

Demographic characteristics of age, gender, race/ethnicity, education, employment, and income were collected using a demographic questionnaire. Years since HIV diagnosis and current medication regimen were obtained through self-report. The most recent CD4+ T-cell count and HIV viral load values, and hemoglobin values to determine anemia were obtained from patients' medical records. While most lab values were obtained within 45 days of the study visit, values up to six months old were included in the analysis. Medications were categorized as antiretroviral therapy (ART), sleep, anti-depressant, or opiate, based on the potential for such medications to impact daytime napping. Trained research staff obtained blood pressures and measures of weight in kilograms and height in meters to calculate body mass index (BMI; weight divided by squared height) during a Clinical Research Center

clinic appointment visit; these measures were evaluated as potential covariates because of their associations with daytime napping and cardiovascular disease.

2.2 Biomarkers

2.2.1 Fasting blood samples—Fasting blood samples were obtained from each participant during a morning Clinical Research Center visit. These samples were then delivered to the laboratory for short term storage at 4°C until processed. Processing typically occurred within 24 hours. Samples were processed for their plasma by ultracentrifugation and isolation of genomics DNA. Processed samples were then stored at −80°C. Plasma levels of six cytokine analytes associated with sleep regulation (i.e., IL-1β, IL-2, IL-6, IL-10, IL-13, TNFα) were assayed using the Luminex xMAP multiplex platform (BioMarker Services, EMD Millipore, St. Charles, MO). IL-4 was also included in the assay panel, but most values were below the lower limit of detection; thus IL-4 was excluded from analyses.

2.2.2 Gene selection and genotyping—Fifteen cytokine candidate genes involved in cytokine signaling were selected for analysis based on their known influence on inflammatory processes: interferon-gamma (*IFNG*), *IFNG* receptor 1 (*IFNGR1*), interleukins, (*IL1B, IL1R, IL1R2, IL2, IL4, IL6, IL8, IL10, IL13, IL17A*), *NFKB1, NFKB2,* and *TNFA*. Genomic DNA was extracted from peripheral blood mononuclear cells and maintained by the UCSF Genomic Markers of Symptoms Tissue Bank [26, 27] using the PUREGene DNA Isolation System (Invitrogen, Carlsbad, CA). Of the 350 participants recruited, DNA could be isolated from 348.

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 the 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 resolved by a third reviewer.

2.2.3 Selection of single nucleotide polymorphisms (SNPs)—A broad panel of SNPs involved in cytokine signaling was selected for this initial study based on their known influence on inflammatory processes. The panel included a combination of tagging SNPs and literature driven SNPs (i.e., SNPs reported as being associated with altered function) [17, 24, 26–29]. Tagging SNPs were required to be common (defined as having a minor allele frequency ≥ 0.05) in public databases (eg, HapMap). In order to ensure robust genetic association analyses, quality control filtering of SNPs was performed. All SNPs had call rates of > 95% and five 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, SNPs with allele frequencies of less than five percent (n=10) or with < 3 individuals homozygous for the rare allele (n=19) were also excluded from analysis. In order to control for potential confounding due to population substructure (eg, race, ethnicity), 106 ancestry informative

marker (AIM) SNPs were genotyped. As shown in the supplemental Table, 82 SNPs among the 15 candidate cytokine genes passed all quality control filters and were included in the genetic association analyses.

2.3 Actigraphy Daytime Napping and Sleep Diary

Daytime napping was objectively estimated with a noninvasive battery-operated wrist actigraph microprocessor with a piezoelectric beam that detects movement and acceleration (Mini Motionlogger Actigraph, AAM-32 Ambulatory Monitoring, Inc. Ardsley, NY). Based on prior studies focusing on poor health outcomes associated with long daytime napping [1, 4], the sample was categorized into one of two groups based on their actigraphy data: participants who napped < 60 min and participants who napped ≥ 60 min between 09:00 and 21:00. For sensitivity analyses, participants were further categorized as long nappers (≥ 60 min), short nappers (15–59 min), and non-nappers (<15 min).

Actigraphy provides continuous movement counts, and data were sampled in 30-second epochs using zero-crossing mode. Participants wore the actigraphy monitor continuously on the non-dominant wrist for 72 hours on three consecutive weekdays between Monday and Friday to control for potential weekend variability and to reduce subject burden in this chronic-illness patient population. Wrist actigraphy has been validated with polysomnography measures of sleep and wake time for healthy and disturbed sleepers [30–32].

Participants also documented their self-reported sleep in a three-day diary and these data were used to validate daytime napping start and end times for wrist actigraphy assessments. A daytime sleep episode or nap was determined by one of two approaches: a) participant pressing the event marker on the actigraph to indicate beginning and ending of a nap, or b) the daily sleep diary entry of clock time was used if it matched with a 50% change in movement during the same 10-minute block of time on actigraphy. Minutes of sleep and wake were determined using the Cole–Kripke algorithm from an automatic sleep-scoring program (Action4® Software Program, Ambulatory Monitoring Inc., Ardsley, NY) to reduce researcher scoring bias. The intra-class correlation coefficient across the three days was 0.68. The napping groups used in the analyses were based on the 3-day mean for daytime sleep minutes.

2.4 Other Correlates of Daytime Napping

2.4.1 Perceived Problems with Cognitive Function—Self-reported problems with cognitive function were assessed with the Medical Outcome Study (MOS) Cognitive Functioning Scale [33]. The scale contains six items that assess the frequency during the past month in which one has experienced problems in six cognitive domains: reasoning, concentration and thinking, confusion, memory, attention, and psychomotor. Each item is rated from one (all of the time) to six (none of the time). The six items are summed to yield a score from 6 to 36, and the score is then converted to a 0–100 point scale, with higher scores indicating better perceived cognitive function. The construct and discriminant validity of the scale have been demonstrated, internal consistency reliability (Cronbach alpha coefficient) of the scale was 0.93 in individuals with HIV [34]. In the current HIV sample,

the Cronbach alpha coefficient for the MOS Cognitive Functioning Scale was 0.91. The relationship between nocturnal sleep and perceived problems with cognitive function in this sample was previously reported [35].

2.4.2 Depressive symptoms—The Center for Epidemiological Studies-Depression Scale (CES-D) was used to assess frequency of depressive symptoms in the past week [36]. The CES-D consists of 20 items that represent major symptoms in the clinical syndrome of depression. Scores can range from 0 to 60 with scores of ≥ 16 indicating the need for clinical evaluation for major depression. The CES-D has well-established concurrent and constructs validity [36]. In our current HIV sample, the Cronbach alpha coefficient was 0.88.

2.4.3 Pain—The Memorial Symptom Assessment Scale (MSAS) was used to assess pain and 31 other symptoms [37]. Convergent and discriminant validity of the MSAS have been established [37], and Cronbach alpha coefficients for the validated subscales in other chronic illness samples ranged from 0.82 to 0.95 [38]. In our study, the MSAS item on pain occurrence in the past week (yes or no) was used. Findings on the pain experience in this sample were previously reported [39].

2.4.4 Sleep Quality—The Pittsburgh Sleep Quality Index (PSQI) was used to measure subjective sleep quality and types of sleep disturbances over the previous month [40]. The scale includes 19 items that yield seven component scores: subjective sleep quality, sleep latency, sleep duration, habitual sleep efficiency, sleep disturbances, use of sleeping medication, and daytime dysfunction. The global sleep quality score is a sum of the seven component scores and can range from 0 to 21, with higher scores indicating poorer sleep quality. A global PSQI score of greater than five is a sensitive and specific measure of poor sleep quality. The scale demonstrates adequate reliability and validity across many patient populations [40].

2.6 Nocturnal Sleep

Wake after sleep onset (WASO), and total sleep time (TST) in minutes were assessed using wrist actigraphy over three consecutive nights. WASO was standardized as a percentage of the person's TST to control for varying sleep durations. The intra-class correlation coefficient across the three nights was 0.83 for WASO and 0.76 for TST. The three-night means for WASO and TST were used for analyses.

2.7 Statistical Analysis

All analyses were conducted using SPSS (version 22, IBM Corporation, Armonk, NY) and Stata (version 13, StataCorp, College Station, TX). Descriptive statistics were used to summarize demographic, clinical, and biomarker characteristics. CD4+ T-cell count and HIV viral load were analyzed in clinically meaningful categories [41]. Demographic and clinical associations with long daytime napping were evaluated using independent sample *t*-tests or chi-square tests of independence. Allele and genotype frequencies were determined by gene counting. Hardy–Weinberg equilibrium was assessed by the chi-square exact test. Measures of linkage disequilibrium (i.e., D' and r^2) were computed from participants'

genotypes with Haploview 4.1. Differences in cytokine plasma levels in the daytime napping group were assessed using Mann–Whitney *U* Tests, due to non-normal distributions.

Unadjusted genetic associations with long daytime napping (≥ 60 minutes) were determined using logistic regression. Three genetic models (additive, dominant, and recessive) were tested, and the model that best fit the data by maximizing the significance of the *p*-value (barring trivial improvements of delta < 10%) was reported for each SNP. Genetic markers were further evaluated in adjusted logistic regression models, controlling for relevant covariates. Given evidence that sleep phenotypes differ by ancestry [42] all regression models controlled for genomic estimates of ancestry (GEA, described below) as well as self-reported race/ethnicity (i.e., White/Caucasian, Black/African American, other). In addition, all demographic and clinical variables associated ($p < 0.10$) with long daytime napping were evaluated as potential covariates. Covariates were retained if their significance was $p < 0.05$ prior to including genotype in the model. A model was fit for each genetic marker to estimate its unique contribution to long napping when controlling for relevant demographic and clinical covariates. To evaluate the potentially different genetic associations with long napping (≥60 min) and short napping (15–59 min), sensitivity analyses were conducted to compare each napping group to the non-napping group (<15 min of daytime sleep).

Each polymorphism associated with long daytime napping 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) were used to minimize bias due to population substructure [43–45]. Homogeneity in ancestry among participants was estimated by principal component (PC) analysis with orthogonal rotation [46] using HelixTree software (GoldenHelix, Bozeman, MT). With 106 AIMs included in this analysis, PCs were sought that distinguished the major racial/ethnic groups in the sample (i.e., White/Caucasian, Black/African American, other) by visual inspection of scatterplots of orthogonal PCs (e.g., 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 PC coefficients for the AIMs were included as genomic estimates of ancestry (GEA) in all adjusted regression models to allow 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). An additional 28 participants were excluded due to incomplete or invalid actigraphy, and 32 were excluded due to missing data for daytime napping, cognitive function, CD4+ T-cell count, or viral load. Valid actigraphy data for the initial visit were missing for 11 participants, and data from a subsequent visit were used. The remaining 257 adults were included in the final sample, although sample

sizes for each analysis varied (n=253 – 257) due to random missing data for individual SNPs and plasma cytokines. Demographic and clinical characteristics are presented in Table 1. The mean age was 44.8 ± 8.6 (SD) years. Most (67%) were men and the sample was ethnically diverse.

3.2 Daytime Napping

Of the 257 participants in the final sample, 83 (32%) napped an average of 60 minutes or longer per day over the three days of actigraphy monitoring. Mean nap time for the entire sample was 53.2 min ± 57.7 (SD). As shown in Table 1 bivariate analyses, the group that napped 60 minutes or longer was more recently diagnosed as HIV-positive than the group that napped <60 minutes. In addition, sleep medication use, antidepressant use, opiate medication use, cognitive problems, depression, and poor nocturnal sleep quality were associated with long daytime napping. Other clinical characteristics (including CD4+ T-cell count, viral load and antiretroviral therapy) and sleep latency on the PSQI were not associated with long daytime napping. Rather than poor nocturnal sleep (ie, short sleep duration or more sleep disruption), long daytime nappers had more total sleep time (TST) and less sleep disruption (WASO) at night than the short nap group with <60 minutes of daytime sleep. As shown in Table 2, compared to the group who slept <60 minutes during the day, the long napping group had significantly higher plasma levels for two (IL-1β and IL-2) of the six plasma measures (IL-1β, IL-2, IL-6, IL-10, IL-13, TNFα): IL-1β and IL-2.

3.3 Genetic Associations with Long Daytime Napping

In the unadjusted analyses, eight SNPs (*IFNG* rs2069728; *IL2* rs2069763; *IL4* rs2243263; *IL6* rs4719714 and rs2069845; *IL13* rs1800925; *NFKB1* rs4648068; *NFKB2* rs1056890) mapping seven genes (*IFNG, IL2, IL4, IL6, IL13, NFKB1,* and *NFKB2*) were significantly associated with long daytime napping (Supplementary Table). To better estimate the magnitude of genetic associations when adjusting for relevant covariates, multivariate logistic regression models were fit to predict long daytime napping. GEA and self-reported race/ethnicity were forced into all models. In addition, years since HIV diagnosis, sleep medication use, antidepressant use, opiate medication use, cognitive problems, depression, perceived sleep quality (PSQI), objective sleep quality (WASO), and objective sleep duration (TST) were evaluated as potential covariates, but only years since HIV diagnosis, antidepressant and opiate medication use, and WASO met the criterion for retention ($p < 0.05$) in the final models.

Of the eight SNPs associated with long daytime napping in the unadjusted analyses, five SNPs among four genes (*IFNG* rs2069728; *IL2* rs2069763; *IL6* rs4719714 and rs2069845; *NFKB2* rs1056890) remained significant after adjusting for GEA, self-reported race/ethnicity, years since HIV diagnosis, antidepressant and opiate medication, and WASO (Table 3). In addition, seven SNPs among three genes (ie, *IL1B* rs1143642, rs1143627, and rs16944; *IL6* rs1554606; *IL17A* rs3819024 and rs8193036; *NFKB1* rs4648110) that were not associated with long daytime napping in the bivariate analysis were significantly associated with long napping after adjusting for relevant covariates. Of 12 cytokine polymorphisms associated with long daytime napping, ten polymorphisms were associated

with greater odds of long napping and two polymorphisms were associated with lower odds of long napping.

Sensitivity analyses were conducted to evaluate whether the 12 genetic associations evident in the main analyses persisted when short daytime napping (15–59 minutes by actigraphy) was distinguished from non-napping (<15 minutes by actigraphy). In adjusted models predicting long daytime napping (n=83) relative to non-napping (n=78), eight of the original 12 genetic associations remained significant, one (*IFNG* rs2069728) had a similar odds ratio (9.73) but did not reach significance (p =0.053) in this reduced sample (n=159–161), and three (*IL2* rs2069763, *IL17A* rs8193036, and *NFKB1* rs4648110) had odds ratios that were attenuated and no longer significant. In adjusted models predicting short daytime napping (n=96) relative to non-napping (n=78), only one of the original 12 associations was significant (*IL6* rs4719714, OR=0.41, p =0.044); odds ratios for the other 11 SNPs were substantially reduced and not significantly associated with short daytime napping.

Cytokine plasma levels were available for 3 of the genes associated with long daytime napping (i.e., IL-1β, IL-2, IL-6). IL-Iβ plasma levels differed significantly by genotype *IL1B* rs1143642 (n=253, β: −0.94, Z= −2.77, p=0.006) after adjusting for GEA, self-reported race/ethnicity, years since HIV diagnosis, and WASO.

4. DISCUSSION

About one third of the participants in this sample of HIV-infected adults napped for 60 minutes or longer during the day. In our sample, 12 polymorphisms from seven cytokine-related genes (*IFNG* rs2069728; *IL1B* rs1143642, rs1143627, and rs16944; *IL2* rs2069763; *IL6* rs4719714, rs1554606, and rs2069845; *IL17A* rs3819024 and rs8193036; *NFKB1* rs4648110; and *NFKB2* rs1056890) were associated with long daytime napping, after adjusting for GEA, self-reported race/ethnicity, and relevant demographic and clinical variables. These results are especially noteworthy considering that we controlled for nocturnal sleep parameters, which have also been associated with cytokine polymorphisms. Sensitivity analyses indicated that the associations observed in this study are specific to long daytime napping, as these polymorphisms were generally not associated with short daytime napping (15–59 minutes). Cytokine plasma levels of IL-1β and IL-2 were elevated in participants who napped 60 minutes or longer compared to those who napped < 60 minutes. IL-1β plasma levels differed significantly by *IL1B* rs1143642 after adjusting for GEA, self-reported race/ethnicity, years since HIV diagnosis, and nocturnal sleep disruption (WASO).

Up to 75% of adults with HIV experience sleep disturbance [47]. We previously reported an association in this sample between nocturnal sleep disturbance and cytokine polymorphisms such as *IL1R2, IL2* [17], *TNFA* [17, 24], *IL1B, IL6, IL13,* and *NFKB1* [24]. Others have reported similar findings for *IL6, TNFA,* and *NFKB2* in adults with cancer [26, 27, 29]. Among 12 SNPs associated with long napping in our study, *IL1B* rs1143642, *IL6* rs4719714, and *NFKB1* rs4648110 have been previously associated with sleep onset insomnia in this sample [24]. Miaskowski et al. reported an association between *IL6* rs4719714 and fatigue and sleep disturbance in oncology patients and their family caregivers [27]. We also previously reported an association between *IL1B* rs1143627 and fatigue in

HIV/AIDS [28]. Given these results, and despite different SNPs for *IL1B, IL2, IL6, NFKB1,* and *NFKB2*, it is plausible that these polymorphisms also play a role in regulating daytime sleep in our sample of adults with HIV infection. The associations with different SNPs in the same genes may relate to differences in linkage disequilibrium between causal SNPs and the associated SNPs examined in other studies.

IL-1β is an important modulator of inflammatory pathways [48]. In our study, *IL1B* rs1143642, rs1143627, and rs16944 were associated with increased risk of long daytime napping. Cytokine plasma levels of IL-1β were also elevated in the long napping group in the bivariate analysis and differed significantly by *IL1B* rs1143642 genotype after adjusting for GEA, self-reported race/ethnicity, and clinical variables. *IL1B* rs1143642, which is located in an intronic region, is also associated with increased C-reactive protein levels over time in obese populations [49]. Our findings suggest IL-1β plasma levels and the *IL1B* gene may be involved in regulating daytime napping in adults living with HIV/AIDS.

Although there is relatively little evidence regarding the role of IL-2 in physiological regulation of sleep compared to other cytokines such as IL-1β or TNFα, it has been previously suggested that IL-2 may be involved in sleep regulation [21]. In rabbits, IL-2 enhances non-REM sleep [50]. In our previous study, *IL2* rs2069776 was associated with nocturnal sleep maintenance [17]. In this study, *IL2* rs2069763, a synonymous SNP in exon 1, was associated with long daytime napping and IL-2 cytokine plasma levels were elevated in participants who napped 60 minutes or longer compared to those who napped < 60 minutes. However, the genetic association was considerably attenuated when short daytime nappers were excluded in a sensitivity analysis, suggesting that the *IL2* association with long daytime napping may be less robust than other genetic associations reported in this study.

IFNγ may influence circadian activity of the suprachiasmatic nucleus, the circadian pacemaker [51]. IFNγ is mainly released by T-lymphocytes and natural killer cells. However, IFNγ is also released by the central nervous system, and is involved in acute or chronic or latent infection in brain tissue [51, 52]. Long-term IFNγ treatment can alter and decrease circadian clock gene expression in the suprachiasmatic nucleus in rats [51]. In our study, *IFNG* rs2069728, located 3' of the gene, was associated with long daytime napping. It is unclear if this SNP influences circadian activity or sleep-wake patterns, but our findings suggest that this SNP may influence daytime napping in adults with HIV.

IL17A rs3819024 and rs8193036 may increase the likelihood of long daytime napping among adults with HIV. IL-17 is an important proinflammatory cytokine involved in the expression of many other proinflammatory cytokines, including IL-1β, IL-6, and TNFα, chemokines and mediators of tissue destruction in different cell types [53, 54]. IL-17 also has a synergistic effect with other cytokines, particularly with TNFα, and upregulates many target genes such as IL-6 [53, 55]. In human macrophages, IL-17A activates the expression of IL-1β and TNFα [55, 56]. In our study, cytokine polymorphisms of *IL-1β* and *IL-6* were also associated with long daytime napping. Because IL-17 plasma cytokine was not measured in our study, the effects of IL17A on IL-1β and IL-6 on daytime napping could not be evaluated and remain unclear. Rapid-eye-movement (REM) sleep deprivation was shown to induce elevations in IL-17 in rats [57]. In human studies, messenger RNA and IL-17 were

elevated after sleep restriction in an experimental setting, and after a recovery period, IL-17 levels remained elevated [58]. These findings would suggest that long-term sleep restriction may lead to increased production of IL-17 [58]. The function of *IL17A* rs3819024 and rs8193036 on sleep in humans remains unknown, and our use of actigraphy did not allow for assessment of sleep stages during naps.

Nuclear factor-kappa B (NF-κB) regulates many proinflammatory genes, inflammatory cytokines, adhesion molecules, and activation of adaptive immunity [59]. In our study, *NFKB1* rs4648110 and *NFKB2* rs1056890 were associated with longer nap time. *NFKB1* rs4648110 has been associated with decreased risk of colon cancer [60]. Although the function of *NFKB2* (rs1056890) on sleep is not known, it has been associated with bortezomib (a proteasome inhibitor) treatment outcome for multiple myeloma in a Chinese population [61] and inflammatory responses to the development of lymphedema following breast cancer surgery [62]. Based on the results of the current study, these *NFKB1* and *NFKB2* SNPs may also influence daytime napping in adults with HIV.

Given the documented associations between *TNFA* and other sleep outcomes, it was somewhat surprising that *TNFA* was not associated with long daytime napping in this study. This lack of association between *TNFA* polymorphisms and long daytime napping may be due to the small number of participants who napped for 60 minutes or longer. It is also possible that the tagSNPs selected for analysis in our cohort failed to capture a subset of the risk alleles that are associated with sleep. Although we adjusted for relevant demographic and clinical characteristics, it is possible that other factors in this sample, such as chronic illness, comorbidities or lifestyle, may have greater impact on long daytime napping than *TNFA*.

Contrary to expectations, the group that napped 60 minutes or longer during the day had less WASO and longer TST during the night. In older adults, more nighttime movement and fragmentation have been associated with increased risk for napping; however, movement and fragmentation of sleep were not associated with daytime nap duration [63]. Cohen-Mansfield and Perach reported that nighttime sleep duration of more than nine hours was associated with increased mortality risk among older adults who napped during the day [64]. The underlying mechanism is unclear, but some adults may need more sleep at night as well as naps during the day, underscoring the importance of assessing 24-hour sleep duration and timing when examining health status in chronic illness.

The major limitations of this study included the modest sample size for a genetic-association study. Some SNPs were excluded due to low minor allele frequencies, and it is possible these SNPs may also be associated with long daytime napping. The number of participants who napped 60 minutes or longer was also relatively small for a genetic association study even though it was a substantial percentage of the sample. Although the distribution of age and gender in our sample reflects the HIV population in the San Francisco Bay Area, the numbers of female participants and both younger and older adults were relatively small. It is also worth noting that the genetic associations identified in this study of long daytime napping differ somewhat from those associated with nocturnal sleep parameters in prior studies [17, 24]. Given the multiple SNPs and outcomes analyzed across the studies, the risk

of type I error needs to be considered. We compared long nappers to short nappers and non-nappers in our sample of adults with HIV infection, but did not include a healthy control group. Persons with HIV may have an increased risk for long daytime napping compared to the general population, but this was not evaluated in the current study. Thus, replication studies with larger sample sizes and with other patient populations as well as healthy control groups would be warranted.

Cytokine plasma levels were elevated in participants who napped ≥60 minutes, but only IL-1β and IL-2 levels were significantly higher compared to those who napped <60 minutes. Despite the associations between polymorphisms in *IL6* and daytime napping, plasma cytokine levels of IL-6 were not associated with daytime napping. The weak relationship between cytokine plasma levels and daytime napping may have been influenced by the small number of participants who napped for 60 minutes or longer. Furthermore, plasma cytokines levels have circadian rhythm variations, whereas cytokine polymorphisms do not present the same challenge [17]. Variations in the timing for collecting plasma samples and the single time-point measure rather than a 24-hour sample may alter results. Furthermore, because chronic HIV infection perturbs many of these cytokines, detecting variations in plasma cytokines may be challenging in this population, while genetic associations may be more easily detected. The multiplex assay we used, rather than more sensitive assays, may have also attenuated the associations of cytokine plasmas and daytime napping. In addition, because the timing of the CD4, viral load, and hemoglobin values were determined by the participant's clinical care, the lag between these values and the data collection visit may have limited the associations observed these values and other variables, particularly among participants with highly fluctuating lab values.

Although the inclusion of an objective measure of daytime sleep was a strength of our study, daytime napping was assessed using wrist actigraphy rather than the gold-standard polysomnography assessment. We used a self-reported sleep log to validate bed times and final wake times for wrist actigraphy assessments, but actigraphy can overestimate sleep in sedentary adults. Other chronic illnesses and conditions such as cardiovascular disease, obstructive sleep apnea or insomnia can influence daytime napping. We excluded patients with a history of sleep apnea or narcolepsy. However, individuals with undiagnosed obstructive sleep apnea or chronic insomnia could have been included in this sample, and these comorbid sleep disorders may have confounded the study findings. We considered other factors that can affect daytime napping, such as cognitive function, depression and pain, but daytime napping research with more rigorous assessment of comorbidities is warranted. Sleep quality and daytime dysfunction were evaluated with actigraphy and self-report (PSQI), but daytime sleepiness was not assessed with objective measures such as a multiple sleep onset latency test (MSLT) in a controlled laboratory setting. Future research using polysomnography for daytime napping or assessment of excessive daytime sleepiness with an MSLT may be warranted.

5. Conclusions

Cytokine polymorphisms were associated with long daytime napping, after adjusting for demographic and clinical characteristics in adults with HIV. Our findings suggest that

cytokine polymorphisms may be involved in physiological regulation related to a propensity for daytime sleep regardless of nocturnal sleep duration. However, given the weak associations between plasma cytokine levels and long daytime napping, the inflammatory mechanisms underlying the associations between cytokine polymorphisms and daytime sleep remain unclear. Further research, including studies in other populations, is needed to elucidate potential mechanisms for these associations. Nonetheless, the cytokine polymorphisms related to long daytime napping in our sample may help identify adults with HIV who may benefit from targeted therapeutic interventions. Our findings also suggest possible areas for interventions for adults with HIV to reduce the duration of their daytime napping and improve quality of life and social interactions as well as adherence to medical treatment. Anti-inflammatory medication has been shown to reduce sleepiness and fatigue in patients with sleep apnea [65], and blocking inflammatory cytokines may be helpful for reducing the duration of daytime napping. Such interventions may be particularly warranted given that long napping was associated with more cognitive problems in this sample and that a prior study found that daytime sleepiness is associated with poorer medication adherence [14]. Further research is needed to determine whether assessing and treating the inflammatory response using a more targeted approach helps to reduce daytime nap duration and improve health outcomes and quality of life in adults with HIV.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Highlights

- Daytime napping is associated with *IFNG, IL1B, IL2, IL6, IL17A, NFKB1*, and *NFKB2* polymorphisms in adults with HIV.
- Cytokine genetic variations may have a role in physiological regulation of daytime sleep as well as nocturnal sleep.
- Napping-related polymorphisms may help identify adults with HIV who may benefit from targeted therapeutic interventions.

Table 1

Demographic and clinical characteristics by daytime napping group (n=257)

	Total Sample (n=257)	Napping		Statistics
		< 60 min (n=174)	≥ 60 min (n=83)	
Demographics				
Age (years), mean (SD) (range 22 – 77)	44.8 (8.6)	45.0 (8.6)	44.2 (8.6)	$t_{(255)} = .66$, $p = .509$
Gender, n (%)				$\chi^2_{(2,257)} = 0.30$, $p = .859$
Male	172 (67%)	116 (67%)	56 (67%)	
Female	63 (25%)	44 (25%)	19 (23%)	
Transgender	22 (9%)	14 (8%)	8 (10%)	
Race/ethnicity, n (%)				$\chi^2_{(2,257)} = 4.89$, $\mathbf{p = .087}$
Caucasian	111 (43%)	77 (44%)	34 (41%)	
African-American	92 (36%)	67 (39%)	25 (30%)	
Other	54 (21%)	30 (17%)	24 (29%)	
Education, n (%)				$\chi^2_{(1,257)} = 0.02$, $p = .892$
High school or less	113 (44%)	76 (44%)	37 (45%)	
More than high school	144 (56%)	98 (56%)	46 (55%)	
Employment, n (%)				$\chi^2_{(2,257)} = 3.83$, $p = .148$
Employed/student	43 (17%)	31(18%)	12 (14%)	
Unemployed	24 (9%)	20 (11%)	4 (5%)	
Disability	190 (74%)	123 (71%)	67 (81%)	
Income, n (%)				$\chi^2_{(1,257)} = 1.03$, $p = .310$
< $1,000/month	178 (69%)	117 (67%)	61 (74%)	
≥ $1,000/month	79 (31%)	57 (33%)	22 (27%)	
Clinical Characteristics				
CD4+ T-cell count, n (%)				$\chi^2_{(1,257)} = 0.04$, $p = .852$
< 200 cells/mm^3	45 (18%)	31 (18%)	14 (17%)	
≥ 200 cells/mm^3	212 (82%)	143(82%)	69 (83%)	
Viral load, n (%)				$\chi^2_{(1,257)} = 0.07$, $p = .786$
Undetectable	127 (49%)	87 (50%)	40 (48%)	
Detectable	130 (51%)	87 (50%)	43 (52%)	
Years since HIV diagnosis				$t_{(255)} = 2.52$, $\mathbf{p = .012}$
Mean (SD)	12.2 (6.8)	13.0(6.5)	10.7(7.2)	
Anti-retroviral therapy, n (%)				$\chi^2_{(1,257)} = 0.52$, $p = .473$
Not on treatment	76 (30%)	49 (28%)	27 (33%)	
On treatment	181 (70%)	125 (72%)	56 (67%)	
Sleep medication use, n (%)				$\chi^2_{(1,257)} = 6.10$, $\mathbf{p = .014}$
No	155 (60%)	114 (66%)	41 (49%)	
Yes	102 (40%)	60 (34%)	42 (51%)	

	Total Sample (n=257)	Napping		Statistics
		< 60 min (n=174)	≥ 60 min (n=83)	
Anti-depressant Use, n (%)				$\chi^2_{(1,257)} = 7.02$, ***p* = .008**
No	154 (60%)	114 (66%)	40 (48%)	
Yes	103 (40%)	60 (34%)	43 (52%)	
Opiate medication use, n (%)				$\chi^2_{(1,257)} = 7.03$, ***p* = .008**
No	191 (74%)	138 (79%)	53 (64%)	
Yes	66 (26%)	36 (21%)	30 (36%)	
Anemia, n (%)	n = 158[a]	n=113[a]	n = 45[a]	$\chi^2_{(1,158)} = 1.35$, $p = .246$
No	109 (69%)	81 (72%)	28 (62%)	
Yes	49 (12%)	32 (28%)	17 (38%)	
Body mass index,				$t_{(255)} = 0.68$, $p = .498$
mean (SD)	59.7 (27.0)	27.2 (5.4)	26.7 (6.0)	
Blood pressure (mmHg)				
Systolic	125.5 (16.0)	125.9 (15.8)	124.7 (16.2)	$t_{(255)} = 0.57$, $p = .573$
Diastolic	74.6 (9.9)	74.5 (9.5)	75.0 (10.7)	$t_{(255)} = 0.40$, $p = .687$
Other Symptoms				
Cognitive problems				$t_{(255)} = 2.27$, ***p* = .014**
Mean score (SD)	70.9 (24.1)	73.5 (22.9)	65.6 (25.8)	
Depression, n (%)				$\chi^2_{(1,257)} = 4.48$, ***p* = .034**
CES-D score < 16	136 (53%)	100 (57%)	36 (43%)	
CES-D score ≥ 16	121 (47%)	74 (43%)	47 (57%)	
Pain, n (%)	n=247[b]	n=167[b]	n=80[b]	$\chi^2_{(1,247)} = 0.03$, $p = .864$
No	110 (45%)	75 (45%)	35 (44%)	
Yes	137 (55%)	92 (55%)	45 (56%)	
Perceived Sleep Quality				$\chi^2_{(1,257)} = 5.0$, ***p* = .026**
PSQI ≤ 5, n (%)	93 (36%)	71 (41%)	22 (27%)	
PSQI > 5, n (%)	164 (64%)	103 (59%)	61 (74%)	
PSQI total score, mean (SD)	7.27 (3.6)	6.9 (3.5)	8.0 (4.0)	$t_{(255)} = 2.11$, ***p* = .036**
PSQI	n = 250[c]	n = 167[c]	n = 83[c]	$\chi^2_{(1,250)} = 0.12$, $p = .726$
SOL ≤ 30 minutes, n (%)	196 (78%)	132 (79%)	64 (77%)	
SOL > 30 minutes, n (%)	54 (22%)	35 (21%)	19 (23%)	
SOL, mean (SD)	25.7 (23.1)	24.9 (23.6)	27.3 (22.3)	$t_{(248)} = 0.78$, $p = .436$
Nocturnal Sleep				
WASO (%), mean (SD)	20.6 (14.8)	22.4 (15.5)	17.0 (12.5)	$t_{(255)} = 2.77$, ***p* = .006**
TST (hours), mean (SD)	6.2 (1.7)	6.0 (1.6)	6.6 (1.7)	$t_{(255)} = 2.54$, ***p* = .012**
Daytime Sleep (minutes)				$t_{(91)} = 15.8$[d], $p < .001$
Mean (SD)	53.2 (57.7)	21.4 (18.3)	119.8 (55.2)	

Note: SD = standard deviation; CES-D = Center for Epidemiologic Studies – Depression Scale; PSQI = Pittsburgh Sleep Quality Index; WASO = wake after sleep onset (sleep disruption); TST = total sleep time (sleep duration); SOL = sleep onset latency. **Bolded** *p*-values indicate variables that were associated ($p < 0.10$) with long napping and were evaluated as potential covariates in adjusted regression models.

[a]The sample size for anemia was reduced due to missing hemoglobin values for 99 participants.

[b]The sample size for pain was reduced due to missing values for ten participants.

[c]The sample size for pain was reduced due to missing values for seven participants.

[d]Separate variance t-test with adjusted degrees of freedom due to unequal variances.

Table 2

Plasma cytokine values by daytime napping group

	Total Sample (n=255)	Napping < 60 min (n = 173)	Napping ≥ 60 min (n = 82)	Statistics
IL-1β (pg/mL)	4.11 (3.42)	3.70 (2.48)	4.97 (4.74)	MWU ***p* = .015**
IL-2 (pg/mL)	8.77(13.97)	7.32 (11.31)	11.84 (18.05)	MWU ***p* = .045**
IL-6 (pg/mL)	20.44 (34.12)	19.72 (33.56)	21.96 (35.43)	MWU *p* = .241
IL-10 (pg/mL)	22.63 (45.04)	16.20 (22.64)	36.18 (70.71)	MWU *p* = .378
IL-13 (pg/mL)	5.91 (10.25)	4.85 (6.06)	8.15 (15.62)	MWU *p* = .358
TNFα (pg/mL)	12.79 (12.16)	12.93 (11.78)	12.50 (13.00)	MWU *p* = .389

Note: Sample size is 255 due to missing plasma cytokine values for two participants. IL = interleukin; MWU = Mann Whitney *U* Test; pg/mL = picograms per milliliter; TNFα = tumor necrosis factor alpha. Bolded variables differed by daytime napping group using MWU.

Table 3

Significant adjusted associations between cytokine-related genotypes and long daytime napping

Gene	SNP	Model	OR	95% CI	*p*	Full models
IFNG	rs2069728	R	8.31	1.42, 48.7	.019	$\chi^2 = 44.2$, $p < .001$
IL1B	rs1143642	D	2.34	1.15, 4.74	.019	$\chi^2 = 44.1$, $p < .001$
	rs1143627	A	1.65	1.07, 2.53	.023	$\chi^2 = 43.8$, $p < .001$
	rs16944	A	1.54	1.01, 2.35	.045	$\chi^2 = 41.9$, $p < .001$
IL2	rs2069763	R	3.50	1.03, 11.9	.045	$\chi^2 = 42.6$, $p < .001$
IL6	rs4719714	A	1.77	1.06, 2.96	.028	$\chi^2 = 43.3$, $p < .001$
	rs1554606	R	0.38	0.15, 0.99	.047	$\chi^2 = 42.6$, $p < .001$
	rs2069845	R	0.31	0.11, 0.87	.025	$\chi^2 = 44.3$, $p < .001$
IL17A	rs3819024	A	1.72	1.09, 2.69	.019	$\chi^2 = 44.1$, $p < .001$
	rs8193036	D	1.98	1.09, 3.60	.025	$\chi^2 = 43.6$, $p < .001$
NFKB1	rs4648110	A	1.61	1.02, 2.55	.043	$\chi^2 = 42.6$, $p < .001$
NFKB2	rs1056890	A	1.77	1.11, 2.82	.016	$\chi^2 = 44.4$, $p < .001$

Note: Sample size for each model varies (n=253–255) due to missing data. Models predict membership in the group with napping ≥ 60 min, with the napping < 60 min group serving as the reference. All models adjusted for genomic estimates of ancestry, self-reported race/ethnicity, years since HIV diagnosis, use of antidepressant or opiate medication, and nocturnal wake after sleep onset. A = additive model; CI = confidence interval; D = dominant model; IL = interleukin; *INFG* = interferon-gamma; *NFKB* = nuclear factor of kappa light polypeptide gene enhancer in B cells; OR = odds ratio based on dose of minor allele; R = recessive model.