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Cytokine Polymorphisms are Associated with Poor Sleep Maintenance in Adults Living with Human Immunodeficiency Virus/Acquired Immunodeficiency Syndrome

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Study Objectives: Cytokine activity and polymorphisms have been associated with sleep outcomes in prior animal and human research. The purpose of this study was to determine whether circulating plasma cytokines and cytokine polymorphisms are associated with the poor sleep maintenance commonly experienced by adults living with human immunodeficiency virus/acquired immunodeficiency syndrome (HIV/AIDS).

Design: Cross-sectional descriptive study.

Setting: HIV clinics and community sites in the San Francisco Bay area.

Participants: A convenience sample of 289 adults (193 men, 73 women, and 23 transgender) living with HIV/AIDS.

Interventions: None.

Measurements and Results: A wrist actigraph was worn for 72 h to estimate the percentage of wake after sleep onset (WASO%) and total sleep time (TST), plasma cytokines were analyzed, and genotyping was conducted for 15 candidate genes involved in cytokine signaling: interferon-gamma (*IFNG*), IFNG receptor 1 (*IFNGR1*), interleukins (*IL1B, IL1R2, 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*). Controlling for demographic variables such as race and sex, and clinical variables such as CD4+ count and medications, higher WASO% was associated with single nucleotide polymorphisms (SNPs) of *IL1R2* rs11674595 and *TNFA* rs1041981 and less WASO% was associated with *IL2* rs2069776. *IL1R2* rs11674595 and *TNFA* rs1041981 were also associated with short sleep duration.

Conclusions: This study strengthens the evidence for an association between inflammation and sleep maintenance problems. In this chronic illness population, cytokine polymorphisms associated with wake after sleep onset provide direction for intervention research aimed at comparing anti-inflammatory mechanisms with hypnotic agents for improving sleep maintenance and total sleep time.

Keywords: Actigraphy, cytokine, genetic, HIV, inflammation, sleep maintenance

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INTRODUCTION

Sleep disturbance is a common symptom in chronic illness populations, and it is estimated that up to 75% of adults living with human immunodeficiency virus/acquired immunodeficiency syndrome (HIV/AIDS) experience sleep problems.[1] The most common type of sleep problem in HIV disease is difficulty with sleep maintenance.[2-4] Inflammatory processes and cytokine activity have been implicated in sleep disturbance in animal models,[5-8] healthy populations,[9-12] and adults with chronic illness.[13-16] Chronic illness often involves inflammatory processes and cytokine function, particularly tumor necrosis factor alpha (*TNFA*)[17,18] and interleukin-6 (*IL6*),[15,16] yet prior studies have not directly compared the contribution of both plasma levels of various inflammatory markers, such as cytokines and cytokine gene polymorphisms, to sleep disturbance in samples of adults with chronic illness. Therefore, the purpose of this study was to confirm prior associations between cytokines and sleep maintenance, and to describe the relative contributions of cytokines in accounting for the variance in sleep maintenance in a sample of adults living with HIV infection.

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METHODS

Participants and Setting

The Symptom and Genetic Study was a longitudinal study aimed at identifying biomarkers of symptom experience among HIV-infected adults.[19] This analysis focuses on cytokine-related biomarkers of insomnia related to poor sleep maintenance. The Committee on Human Research at the University of California at 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 y of age in whom HIV had been diagnosed 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 4 h between 12:00 and 06:00); reported having bipolar disorder, schizophrenia, or dementia; or were pregnant within the prior 3 mo. Participants were not excluded for insomnia, but were excluded for other diagnosed sleep disorders, such as apnea or narcolepsy.

Measures

Demographic, Clinical, and Laboratory Characteristics

A demographic questionnaire was used to collect information about the participant's age, sex, race/ethnicity, and employment status. Health history (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, anxiolytic, antidepressant, neuroleptic, opiate, antiemetic, or anti-histamine based on the potential for such medications to affect sleep. Lifestyle factors likely to exacerbate sleep disturbance (smoking and daily consumption of caffeine and alcohol) were assessed using a 3-day diary. Trained research staff obtained measures of body mass index (BMI, weight in kilograms divided by squared height in meters) and waist circumference 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.

Biomarkers

Fasting blood samples were obtained from each participant during the CRC visit. Plasma levels of six cytokine analytes (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).

Gene Selection and Genotyping: 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 and maintained by the UCSF Genomic Markers of Symptoms Tissue Bank[16,18] 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; Thermo Fisher Scientific, Waltham, MA) and normalized to a concentration of 50 ng/μL (diluted in 10 mM Tris/1 mM ethylenediaminetetraacetic acid [EDTA]). Samples were genotyped using the GoldenGate genotyping platform (Illumina, San Diego, CA) and processed according to the standard protocol using GenomeStudio (Illumina). 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.

Selection of SNPs: A combination of tagging SNPs and literature driven SNPs (i.e., SNPs reported as being associated with altered function) were selected for analysis. Tagging SNPs were required to be common (defined as having a minor allele frequency ≥ 0.05) in public databases (e.g., HapMap [http://www.hapmap.org]). 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 < 5% (n = 10) or with fewer than three individuals homozygous for the rare allele (n = 19) were also excluded from analysis. In order to control for potential confounding due to population substructure (e.g., race, ethnicity), 106 ancestry informative marker (AIM) SNPs were genotyped. Eighty SNPs among the 15 candidate cytokine genes passed all quality-control filters and were included in the genetic association analyses.

Actigraphy and Sleep Diary

Sleep and activity were estimated with a noninvasive battery-operated wrist actigraph microprocessor with a piezoelectric beam that detects movement and acceleration (Mini Motionlogger Actigraph model AAM-32, Ambulatory Monitoring, Inc. Ardsley, NY). Actigraphy provides continuous movement counts and data were sampled in 30-sec epochs using zero-crossing mode. The actigraphy monitor was worn continuously on the nondominant wrist for 72 h 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. Sleep diaries were also completed each morning and evening of the actigraphy monitoring period for the purpose of cross-validating bedtimes and wake times. Wrist actigraphy has been validated with polysomnography measures of sleep and wake time for healthy and disturbed sleepers.[20-22] Bedtime and final wake times were determined by one of two approaches: (1) participant pressing the event marker on the actigraph to indicate "lights out" and "lights on" or (2) if no reliable event marker indication, the diary entry of clock time was used if it matched with a 50% change in movement during the same 10-min block of time on actigraphy.

The primary sleep outcomes were wake after sleep onset (WASO) and total sleep time (TST) in min. The Cole-Kripke algorithm was used to calculate WASO and TST using an automatic sleep scoring program (Action4® Software Program, Ambulatory Monitoring Inc.) to reduce researcher scoring bias. WASO was standardized as a percentage of the person's TST to control for varying sleep durations. The intraclass correlation coefficient across the 3 nights was 0.83 for WASO and 0.76 for TST. The 3-night means for WASO and TST were used for all analyses. Because WASO% was highly correlated with TST (rho = -0.711, P < 0.0001), we focused our analyses on WASO% as the dependent variable of interest for relationships with plasma cytokines and cytokine candidate genes.

Pittsburgh Sleep Quality Index

Subjective experience of recent sleep quality was assessed with the Pittsburgh Sleep Quality Index (PSQI). The PSQI's 19 self-rated items are rated on a four-point scale (not at all, less than once a week, once or twice a week, and three or more time a week) according to their frequency of occurrence during the previous month. The PSQI yields global scores that range from 0 to 21. A global score > 5 has been found to distinguish good and poor sleepers,[23] although higher scores have been suggested for specific populations.[24-26] In addition to the global

score, the PSQI yields seven component scores addressing sleep quality (one item), sleep latency (two items), sleep duration (one item), habitual sleep efficiency (calculation based on three items), sleep disturbance (nine items), use of sleeping medication (one item), and daytime dysfunction (two items). Component scores range from 0 to 3, with higher scores indicating greater sleep disturbance. Internal consistency reliability (Cronbach alpha coefficient) was 0.67 in this sample. Both the global score and habitual sleep efficiency were used to describe the sample.

Fatigue

The Fatigue Severity Scale (FSS) was used to assess the perceived effect of fatigue over the past week.[27] Findings from recent studies in patients with stroke[28] and patients with HIV[29] indicate that a seven-item version of the FSS has better psychometric properties; thus, the seven-item version (FSS-7) was used. Each item is rated from 1 (strongly disagree) to 7 (strongly agree), and the seven items are averaged to yield a score from 1 (no functional problems due to fatigue or lack of energy) to 7 (unable to function due to fatigue or lack of energy). A mean score of 5 or higher was considered indicative of severe fatigue.[30-32] The FSS has well-established test-retest reliability and clearly differentiates between patients with chronic disease and healthy adults.[27] In this study, the Cronbach alpha for the FSS-7 was 0.93.

Depressive Symptoms

The Center for Epidemiologic Studies – Depression Scale (CES-D) was used to assess frequency of depressive symptoms in the past week.[33] 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 construct validity.[33] In this study, the Cronbach alpha coefficient for the CES-D was 0.88.

Anxiety

The Profile of Moods State (POMS) Tension-Anxiety subscale[34] was used to assess anxiety in the past week. The subscale consists of nine items representing the major symptoms of anxiety. Scores can range from 0 to 36, and a mean of 8.6 has been documented in HIV-infected outpatients.[35] The POMS has well-established concurrent and construct validity.[34] In this study, the Cronbach alpha coefficient for the POMS subscale was 0.86.

Pain and Nausea Symptoms

The Memorial Symptom Assessment Scale (MSAS) was used to assess pain, nausea, and other symptoms.[36] It is a reliable and valid self-report measure of symptoms that has been used in a variety of clinical populations,[37,38] including patients with HIV.[39] The MSAS evaluates symptom occurrence in the past week using an endorsement of yes/no, and includes additional follow-up questions about the symptom's frequency, severity, and distress. For this analysis, the "pain" and "nausea" occurrence items were used to determine whether the participant had experienced these symptoms in the previous week (yes/no).

Statistical Analysis

All analyses were conducted using Stata (version 11.2, College Station, TX) except where indicated in the next paragraphs. Descriptive statistics were used to summarize demographic, clinical, and biomarker characteristics. Square root transformation was sufficient to normalize skewed distributions for WASO and CD4+ T-cell count, and log transformation was sufficient to normalize HIV viral load values. CD4+ T-cell count and HIV viral load were analyzed both as continuous variables and in clinically meaningful categories. Demographic and clinical associations with WASO were evaluated using Spearman correlations, independent sample *t*-tests, or analysis of variance with Scheffé *post hoc* tests. Mann-Whitney U tests were used for group comparisons of cytokine plasma levels. 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.[40]

Unadjusted genetic associations with WASO were determined using linear regression models predicting WASO. 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. Each genetic marker with a significant bivariate association with WASO was further evaluated in a multiple-variable linear regression model controlling for relevant covariates. As in the unadjusted regression analyses, the adjusted models predicted WASO. All regression models controlled for race and ethnicity as a form of population substructure with the potential to confound genetic associations (described in the next sentences) as well as self-reported race/ethnicity (i.e., White/Caucasian, Black/African American, other). In addition, all demographic, clinical, and laboratory variables associated with WASO ($P < 0.10$) were evaluated as potential covariates. Variables were retained as covariates in all adjusted models if their significance was $P < 0.05$ prior to including genotype in the model. A model was fit for each genetic marker to estimate its unique contribution to sleep disruption (WASO) when controlling for relevant demographic and clinical covariates.

AIMs can be used as a tool to minimize bias due to population stratification.[41-43] Homogeneity in ancestry among participants was verified by cluster and principal component analysis,[44] using HelixTree software (GoldenHelix, Bozeman, MT). One hundred six AIMs were included in this analysis, and principal components (PC) 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 (PC1 versus PC2, PC2 versus PC3). This procedure was repeated until no discernible clustering of participants by self-report race/ethnicity was possible. The first three PCs were included in all adjusted regression models to allow for potential confounding due to population substructure (i.e., race/ethnicity).

RESULTS

Sample Characteristics

A convenience sample of 350 adults with HIV was enrolled in the study, and 61 participants were excluded prior to analysis for either screening positive for illicit drugs (n = 31),

Table 1—Wake after sleep onset by demographic and clinical characteristics (n = 289)

	N	n (%) or mean (SD)	WASO% mean (SD)	Statistics
Demographic characteristics				
Age, y (range 22-77)	289	44.9 (8.4)		rho = 0.028, P = 0.634
Sex	289			F(2,286) = 1.11, P = 0.332
Male		192 (66%)	21.1 (15.5)	
Female		74 (26%)	19.1 (12.5)	
Transgender		23 (8%)	24.3 (15.0)	
Race	**289**			**F(2,286) = 11.4, P < 0.001**
Caucasian		118 (41%)	16.1 (12.0)	
African American		110 (38%)	24.6 (14.4)	
Other		61 (21%)	23.1 (17.8)	
Employment	**289**			**t(287) = 3.15, P = 0.002**
Employed or in school		46 (16%)	15.3 (12.9)	
Unemployed or on disability		243 (84%)	21.9 (14.9)	
Clinical characteristics				
CD4+ T-cell count (cells/mm^3)	276			
Mean (SD)		**453 (267)**		**rho = -0.166, P = 0.006**
< 200		47 (17%)	23.5 (15.2)	t(274) = 1.49, P = 0.139
≥ 200		229 (83%)	20.2 (14.6)	
Viral load (log_{10} copies/mL)	270			
Mean (SD)		2.64 (1.20)		rho = 0.102, P = 0.096
Undetectable		137 (51%)	18.7 (13.0)	t(268) = 1.87, P = 0.062
Detectable		133 (49%)	22.5 (16.0)	
Antiretroviral therapy (ART)	289			t(287) = 1.35, P = 0.177
Not on treatment		85 (29%)	21.9 (13.3)	
On treatment		204 (71%)	20.4 (15.3)	
Sleep medication use	289			t(287) = 1.52, P = 0.129
No		240 (83%)	20.2 (14.5)	
Yes		49 (17%)	23.9 (15.9)	
Neuroleptic medication use	288			t(286) = 1.74, P = 0.083
No		261 (91%)	21.3 (15.1)	
Yes		27 (9%)	15.5 (9.5)	
Opiate medication use	**288**			**t(286) = 3.41, P < 0.001**
No		210 (73%)	19.1 (14.3)	
Yes		78 (27%)	25.2 (15.2)	
Antiemetic medication use	**288**			**t(286) = 2.67, P = 0.008**
No		277 (96%)	20.3 (14.5)	
Yes		11 (4%)	32.6 (15.9)	
Plasma cytokine levels	286			
IL-1β (pg/mL)		4.20 (3.64)		rho = -0.020, P = 0.735
IL-2 (pg/mL)		8.91 (14.2)		rho = -0.065, P = 0.271
IL-6 (pg/mL)		21.0 (35.4)		rho = 0.003, P = 0.959
IL-10 (pg/mL)		24.1 (49.4)		rho = -0.037, P = 0.537
IL-6 / IL-10 ratio		1.96 (3.78)		rho = 0.044, P = 0.462
IL-13 (pg/mL)		5.92 (9.85)		rho = -0.111, P = 0.061
TNFα (pg/mL)		**12.4 (11.7)**		**rho = 0.121, P = 0.042**
C-reactive protein (ng/mL)		**12.6 (19.0)**		**rho = 0.135, P = 0.023**
Anthropometrics	289			
Body mass index		27.0 (5.5)		rho = 0.108, P = 0.066
Waist circumference (cm)		**93.7 (12.9)**		**rho = 0.146, P = 0.013**
Other symptoms				
Depression in past week	287			F(1,285) = 1.16, P = 0.282
CES-D < 16		147 (51%)	21.6 (14.8)	
CES-D ≥ 16		140 (49%)	19.9 (14.7)	
Anxiety in past week	281			F(1,279) = 0.04, P = 0.848
POMS < 8.6		161 (57%)	21.0 (15.0)	
POMS ≥ 8.6		120 (43%)	20.6 (14.8)	
Fatigue in past week	289			F(1,287) = 0.02, P = 0.885
FSS7 < 5		215 (74%)	20.8 (14.8)	
FSS7 ≥ 5		74 (26%)	21.0 (14.6)	
Pain in past week	288			F(1,286) = 0.72, P = 0.397
No		131 (45%)	20.1 (14.6)	
Yes		157 (55%)	21.4 (14.9)	
Nausea in past week	286			F(1,284) = 0.28, P = 0.594
No		224 (78%)	20.5 (14.2)	
Yes		62 (22%)	21.2 (16.7)	

WASO and CD4+ T-cell count analyses were conducted with square root-transformed values. WASO was unrelated to use of antidepressant and anxiolytic medications (data not shown). CES-D, Center for Epidemiologic Studies – Depression Scale; FSS7, 7-item Fatigue Severity Scale; IL, interleukin; pg/mL, picograms per milliliter; POMS, Profile of Mood States, Tension-Anxiety subscale; SD, standard deviation; TNFα, tumor necrosis factor alpha; WASO, wake after sleep onset. Bolded variables are associated with WASO (P < 0.05).

being unable to submit a urine or blood sample (n = 2), or having incomplete or invalid actigraphy data (n = 28). Twelve participants were missing valid actigraphy data for the initial visit, and actigraphy data were used from a subsequent visit. Demographic and clinical characteristics for the 289 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 12.1 ± 6.9 y; AIDS had been diagnosed in 51%, and 29% with an AIDS diagnosis had current CD4+ T-cell counts below 200 cells/mm^3. Most (75%) were receiving medical disability assistance, 71% were currently receiving ART, and they were taking 5.9 ± 4.0 medications (median 6, range 0-20).

Almost half (45%) of the sample averaged less than 6 h of sleep at night (Table 2). As previously reported,[2] the majority of the sample experienced significant WASO, with 56% (n = 162) having mean WASO values of more than 15% of their TST, and 35% (n = 101) having more than 25% WASO. As shown in Table 1, race and employment status were associated with WASO. Of the clinical variables, CD4+ T-cell count was correlated with WASO, and participants with detectable HIV viral load levels had slightly more WASO than participants with undetectable viral loads. Waist circumference was also associated with WASO. Of the medication classes examined, only opiates and antiemetic medications were significantly associated with higher WASO. WASO was not associated with symptoms of fatigue, depression, anxiety, pain, or nausea and was unrelated to use of medications for sleep, depression, or anxiety. Lifestyle factors likely to exacerbate sleep disturbance (smoking and daily consumption of caffeine and alcohol) were unrelated to WASO in this sample.

Biomarkers: Plasma Cytokines

None of the Spearman correlations (rho) between cytokine plasma levels (IL-1β, IL-2, IL-6, IL-10, IL-13, or TNFα) and WASO (see Table 1) or TST (data not shown) exceeded 0.20. The strongest associations between inflammatory markers and WASO were with C-reactive protein (rho = 0.135) and plasma TNFα (rho = 0.125). Although few of the correlations were significant, there was a general pattern of positive correlations for proinflammatory cytokines and negative correlations for anti-inflammatory cytokines. In addition, TNFα plasma levels were lower among participants who were taking ART (10.6 ± 9.42 versus 16.9 ± 15.2, P = 0.001), had CD4+ T-cell counts of at least 200 (11.7 ± 11.5 versus 17.4 ± 12.7, P = 0.0003), or had undetectable viral load values (9.24 ± 8.22 versus 16.2 ± 14.0, P < 0.0001). Participants with an undetectable viral load also had lower IL-1β (P = 0.046) and IL-6 to

IL-10 ratio (P = 0.007) levels compared to those with detectable viral load values.

Biomarkers: Genetic Cytokines

Of the 80 SNPs examined for the cytokine candidate genes, 19 SNPs among seven genes (i.e., *IL1B, IL1R2, IL2, IL8, IL13, IL17A, NFKB1, NFKB2, TNFA*) were significantly associated with WASO in unadjusted analyses (see Table 3). To better estimate the magnitude of the association between genotype and WASO when adjusting for relevant covariates, multiple linear regression models were fit. AIMs, self-reported race, and sex were forced into all models. Significant covariates from Table 1 included CD4+ T-cell count, waist circumference, opiate use, and antiemetic use. Employment status, HIV viral load, BMI, and neuroleptic use, symptoms of depression and anxiety, and plasma TNFA levels were evaluated as potential covariates but did not meet the criterion for retention (P < 0.05) in the model prior to inclusion of genotype.

Of the 19 SNPs associated with WASO in unadjusted analyses (Table 3), four SNPs (*IL1R2* rs11674595, *IL2* rs2069776, and *TNFA* rs1800683 and rs1041981) remained significant after adjusting for race, sec, CD4+ T-cell count, waist circumference, and medication use (Table 4). Due to the near complete collinearity, or shared variance, between

Table 2—Sleep characteristics (n = 289)

Sleep characteristics	n (%) or mean (SD)	Association with WASO%
Wake after sleep onset (%WASO)		
Mean (SD)	20.8 (14.8)	
Range	0.6-78.9	
< 15%	127 (44%)	
15-24.9%	61 (21%)	
≥ 25%	101 (35%)	
TST (h)		
Mean (SD)	6.18 (1.64)	rho = -0.711, P < 0.0001
Range	1.30-11.4	
< 5	70 (24%)	
5-5.9	60 (21%)	
6-6.9	69 (24%)	
7-7.9	52 (18%)	
≥ 8	38 (13%)	
PSQI global score	n = 288	
Mean (SD)	7.44 (3.70)	rho = 0.109, P = 0.064
Range (0-21)	1-18	
≤ 5 (below clinical threshold)	100 (35%)	
> 5 (at or above threshold)	188 (65%)	t(286) = 1.54, P = 0.126
PSQI habitual sleep efficiency	n = 284	
Mean (SD)	84.1 (15.1)	rho = 0.118, P = 0.052
Range (0-100%)	40-100	

PSQI *t*-test was conducted with square root-transformed values. PSQI, Pittsburgh Sleep Quality Index; SD, standard deviation; TST, total sleep time; WASO, wake after sleep onset.

Table 3—Unadjusted associations between wake after sleep onset and cytokine polymorphisms (n = 289)

Gene	SNP	HGVS Description	HuRef Position	Chr	MAF	b	95% CI	P	Model
IFNG	rs2069728	(3' of gene, G > A)	65598422	12	0.151	0.398	-0.027, 0.824	0.066	D
	rs2069727	(3' of gene, A > G)	65598861	12	0.322	-0.232	-0.519, 0.056	0.114	A
	rs2069718	c.367-895T > C	65600800	12	0.486	-0.227	-0.687, 0.233	0.331	R
	rs1861493	c.366+497C > T	65601835	12	0.222	-0.238	-0.624, 0.147	0.225	D
	rs1861494	c.366+284G > A	65602048	12	0.233	-0.191	-0.573, 0.191	0.325	D
	rs2069709[a,b]	(5' of gene, G > T)	65604339	12	0.014				
IFNGR1	rs9376268	c.86-4537C > T	135094927	6	0.185	0.194	-0.515, 0.127	0.235	A
IL1B	rs1071676	c.*505G > C	106042060	2	0.160	0.303	-0.077, 0.683	0.118	A
	rs1143643	c.598-152G > A	106042929	2	0.260	-0.577	-1.234, 0.080	0.085	R
	rs1143642	**c.597+316T > C**	**106043180**	**2**	**0.141**	**0.454**	**0.056, 0.851**	**0.025**	**A**
	rs1143634	c.315C > T	106045017	2	0.157	0.212	-0.171, 0.596	0.277	A
	rs1143633	**c.302-64G > A**	**106045094**	**2**	**0.274**	**-0.680**	**-1.323, -0.037**	**0.038**	**R**
	rs1143630	c.100-503A > C	106046282	2	0.150	0.299	-0.072, 0.670	0.114	A
	rs3917356	c.99+780G > A	106046990	2	0.373	-0.256	-0.637, 0.126	0.188	D
	rs1143629	c.47+242C > T	106048145	2	0.420	-0.123	-0.388, 0.141	0.359	A
	rs1143627	(5' of gene, T > C)	106049014	2	0.495	0.065	-0.196, 0.326	0.624	A
	rs16944	(5' of gene, G > A)	106049494	2	0.463	0.002	-0.265, 0.270	0.986	A
	rs1143623	**(5' of gene, G > C)**	**106050452**	**2**	**0.232**	**-0.489**	**-0.791, -0.188**	**0.002**	**A**
	rs13032029	(5' of gene, C > T)	106055022	2	0.354	-0.213	-0.475, 0.048	0.109	A
IL1R1	rs949963	(5' of gene, G > A)	96533648	2	0.228	0.651	-0.226, 1.528	0.145	R
	rs2228139[a,b]	c.371C > G	96545511	2	0.038				
	rs3917320[b]	c.1366A > C	96556738	2	0.073				
	rs2110726	c.*1063G > A	96558145	2	0.234	-0.279	-0.580, 0.021	0.069	A
	rs3917332	(3' of gene, T > A)	96560387	2	0.155	-1.772	-3.625, 0.080	0.061	R

Table 3 continues on the following page.

Table 3 (*continued*)—Unadjusted associations between wake after sleep onset and cytokine polymorphisms (n = 289)

Gene	SNP	HGVS Description	HuRef Position	Chr	MAF	b	95% CI	P	Model
IL1R2	rs4141134	(5' of gene, T > C)	96370336	2	0.300	-0.202	-0.582, 0.177	0.295	D
	rs11674595	**c.-62+2519T > C**	**96374804**	**2**	**0.196**	**1.465**	**0.449, 2.480**	**0.005**	**R**
	rs7570441	**c.-62+1417G > A**	**96380807**	**2**	**0.456**	**0.307**	**0.034, 0.579**	**0.027**	**A**
IL2	**rs1479923**	**(3' of gene, C > T)**	**119096993**	**4**	**0.211**	**-0.574**	**-0.960, -0.189**	**0.004**	**D**
	rs2069776	**(3' of gene, T > C)**	**119098582**	**4**	**0.186**	**-1.588**	**-2.723, -0.454**	**0.006**	**R**
	rs2069772	c.352-116A > G	119099739	4	0.188	-0.196	-0.596, 0.204	0.336	D
	rs2069777[a,b]	c.207+862C > T	119103043	4	0.049				
	rs2069763[c]	c.114G > T	119104088	4	0.302				
IL4	rs2243248	(5' of gene, T > G)	127200946	5	0.111	0.156	-0.309, 0.621	0.510	D
	rs2243250[c]	(5' of gene, C > T)	127201455	5	0.380				
	rs2070874	c.-33C > T	127202011	5	0.280	-0.147	-0.526, 0.232	0.445	D
	rs2227284[c]	c.183+2527T > G	127205027	5	0.460				
	rs2227282[c]	c.184-2227C > G	127205481	5	0.451				
	rs2243263	c.184-2107C > G	127205601	5	0.146	0.128	-0.262, 0.518	0.518	A
	rs2243266	c.184-1617G > A	127206091	5	0.264	-0.210	-0.590, 0.170	0.277	D
	rs2243267	c.184-1520G > C	127206188	5	0.263	-0.208	-0.586, 0.171	0.281	D
	rs2243274	c.184-574G > A	127207134	5	0.377	0.150	-0.104, 0.403	0.246	A
IL6	rs4719714	(5' of gene, A > T)	22643793	7	0.210	-0.110	-0.455, 0.236	0.532	A
	rs2069827[b]	(5' of gene, G > T)	22648536	7	0.052				
	rs1800796	(5' of gene, G > C)	22649326	7	0.097	-0.106	-0.523, 0.311	0.618	A
	rs1800795	(5' of gene, C > G)	22649725	7	0.240	-0.163	-0.546, 0.219	0.401	D
	rs2069835[b]	c.211-441T > C	22650951	7	0.076				
	rs2066992	c.211-63G > T	22651329	7	0.098	-0.174	-0.598, 0.250	0.420	A
	rs2069840	c.324+147C > G	22651652	7	0.262	-0.126	-0.428, 0.177	0.415	A
	rs1554606	c.324+282T > G	22651787	7	0.379	-0.410	-0.940, 0.120	0.129	R
	rs2069845	c.471+870G > A	22653229	7	0.375	-0.454	-0.998, 0.091	0.102	R
	rs2069849[b]	c.603C > T	22654236	7	0.092				
	rs2069861[a,b]	(3' of gene, C > T)	22654734	7	0.044				
	rs35610689	(3' of gene, A > G)	22656903	7	0.231	-0.562	-1.505, 0.381	0.242	R
IL8	rs4073	(5' of gene, T > A)	70417508	4	0.443	-0.219	-0.475, 0.038	0.095	A
	rs2227306	**c.65-204C > T**	**70418539**	**4**	**0.276**	**-0.515**	**-0.889, -0.140**	**0.007**	**D**
	rs2227543	**c.284+161C > T**	**70419394**	**4**	**0.275**	**-0.511**	**-0.885, -0.138**	**0.007**	**D**
IL10	rs3024505	(3' of gene, C > T)	177638230	1	0.122	0.853	-0.761, 2.467	0.299	R
	rs3024498	c.*452A > G	177639855	1	0.189	-0.293	-1.324, 0.738	0.576	R
	rs3024496	c.*117T > C	177640190	1	0.457	0.168	-0.096, 0.432	0.211	A
	rs1878672	c.379-474C > G	177642039	1	0.395	0.070	-0.321, 0.462	0.724	D
	rs3024492	c.378+140A > T	177642438	1	0.157	-0.672	-1.757, 0.413	0.224	R
	rs1518111	c.225+56A > G	177642971	1	0.292	-0.202	-0.579, 0.175	0.293	D
	rs1518110	c.166-101T > G	177643187	1	0.289	-0.198	-0.576, 0.181	0.304	D
	rs3024491	c.166-286G > T	177643372	1	0.392	0.026	-0.240, 0.293	0.847	A
IL13	rs1881457	(5' of gene, A > C)	127184713	5	0.210	-0.088	-0.478, 0.301	0.656	D
	rs1800925	**(5' of gene, C > T)**	**127185113**	**5**	**0.291**	**0.413**	**0.038, 0.787**	**0.031**	**D**
	rs2069743[b]	(5' of gene, A > G)	127185579	5	0.082				
	rs1295686	c.334-24T > C	127188147	5	0.432	0.212	-0.041, 0.464	0.100	A
	rs20541	c.431A > G	127188268	5	0.226	-0.598	-1.445, 0.249	0.166	R
IL17A	**rs4711998**	**(5' of gene, G > A)**	**51881422**	**6**	**0.375**	**0.481**	**0.103, 0.860**	**0.013**	**D**
	rs8193036	(5' of gene, T > C)	51881562	6	0.245	0.227	-0.083, 0.538	0.150	A
	rs3819024	(5' of gene, A > G)	51881855	6	0.303	-0.180	-0.469, 0.110	0.222	A
	rs2275913	**(5' of gene, G > A)**	**51882102**	**6**	**0.217**	**-0.488**	**-0.790, -0.186**	**0.002**	**A**
	rs3804513[a,b]	c.230+594A > T	51884266	6	0.024				
	rs7747909	**c.*159G > A**	**51885318**	**6**	**0.181**	**-0.564**	**-0.949, 0.144**	**0.008**	**D**

Table 3 continues on the following page.

Table 3 (*continued*)—Unadjusted associations between wake after sleep onset and cytokine polymorphisms (n = 289)

Gene	SNP	HGVS Description	HuRef Position	Chr	MAF	b	95% CI	P	Model
NFKB1	rs3774933	c.-8+3394T > C	99162722	4	0.363	0.303	-0.297, 0.903	0.321	R
	rs170731	c.40-2090T > A	99185284	4	0.266	-0.377	-0.753, 0.000	0.050	D
	rs17032779	c.255+7137T > C	99202630	4	0.066	0.497	-0.014, 1.007	0.056	A
	rs230510	**c.256-11978T > A**	**99212552**	**4**	**0.343**	**-0.586**	**-0.960, -0.212**	**0.002**	**D**
	rs230494	c.256-1175G > A	99223356	4	0.375	0.221	-0.325, 0.767	0.426	R
	rs4648016[a,b]	c.404+1378C > T	99226057	4	0.036				
	rs4648018[a,b]	c.404+1908G > C	99226587	4	0.049				
	rs3774956	c.1063+2549C > T	99244914	4	0.391	0.093	-0.437, 0.623	0.730	R
	rs10489114[a,b]	c.1064-3194T > C	99247604	4	0.049				
	rs4648068	c.1493-372A > G	99254521	4	0.304	-0.189	-0.566, 0.188	0.325	D
	rs4648095[a,b]	c.1951+22T > C	99264093	4	0.040				
	rs4648110	c.2589+58T > A	99270046	4	0.258	0.142	-0.240, 0.524	0.465	D
	rs4648135[b]	c.2747-921A > G	99272895	4	0.069				
	rs4648141[c]	c.2747-690G > A	99273126	4	0.328				
	rs1609798	c.2747-149C > T	99273667	4	0.253	-0.184	-0.568, 0.200	0.347	D
NFKB2	**rs12772374**	**c.395+99A > G**	**97790120**	**10**	**0.132**	**-1.630**	**-3.066, -0.193**	**0.026**	**R**
	rs7897947	c.662-27T > G	97790920	10	0.286	0.344	-0.033, 0.721	0.073	D
	rs11574849[b]	c.1470-141G > A	97792905	10	0.071				
	rs1056890	c.*187C > T	97795944	10	0.259	-0.318	-1.000, 0.365	0.360	R
TNFA	**rs2857602**	**g.31473378G > A**	**31533378**	**6**	**0.316**	**-0.643**	**-1.231, -0.054**	**0.032**	**R**
	rs1800683	**c.-18G > A**	**31540071**	**6**	**0.440**	**0.659**	**0.145, 0.664**	**0.005**	**A**
	rs2239704[d]	**c.-92A > C**	**31540141**	**6**	**0.310**	**-0.702**	**-1.308, -0.097**	**0.023**	**R**
	rs2229094[d]	c.37T > C	31540556	6	0.257	-0.265	-0.647, 0.117	0.173	D
	rs1041981[d]	**c.179C > A**	**31540784**	**6**	**0.442**	**0.406**	**0.149, 0.663**	**0.002**	**A**
	rs1799964	g.31482308T > C	31542308	6	0.182	-0.091	-0.423, 0.241	0.591	A
	rs1800750[a,b]	g.31482963G > A	31542963	6	0.017				
	rs1800629	c.-308G > A	31543031	6	0.137	0.304	-1.556, 2.164	0.748	R
	rs1800610[b]	c.186+123G > A	31543827	6	0.077				
	rs3093662[b]	c.187-122A > G	31544189	6	0.082				

All analyses were conducted with square root-transformed WASO values. Bold SNPs have P < 0.05. [a]SNP excluded from analysis because MAF < 0.05 (n = 10). [b]SNP excluded from analysis because one of the genotypes had a frequency < 3 (n = 19). [c]SNP excluded from analysis because distribution violated Hardy-Weinberg equilibrium (n = 5). [d]This TNFA SNP uses NM_000595.2. b, regression coefficient; Chr, chromosome; CI, confidence interval; 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; SNP, single nucleotide polymorphism; TNFA, tumor necrosis factor alpha. Models: A, additive model; D, dominant model; R, recessive model.

TNFA SNPs rs1800683 and rs1041981 (i.e., linkage disequilibrium $r^2 = 0.99$), we selected rs1041981 as a surrogate for rs1800683, and the model for rs1800683 is not shown. For the SNPs in Table 4, the overall models explained 24-28% of the variance in WASO, with genotype accounting for 1.4-5.4% of the variance. Unadjusted differences in WASO by genotype are illustrated in Figure 1.

Cytokine plasma levels were available for two of the genes associated with WASO in Table 4 (i.e., *IL2*, *TNFA*). However, neither of the plasma levels differed significantly by genotype (data not shown).

Because sleep disturbance often results in shorter sleep duration, the three SNPs uniquely associated with WASO were also evaluated for associations with TST. Of the three, *IL1R2* rs11674595 and *TNFA* rs1800683 were found to have significant associations with TST in addition to their association with WASO (Figure 2).

DISCUSSION

Poor sleep maintenance, as indicated by high WASO values, was a common problem in this sample of adults living with HIV/AIDS, and 56% experienced WASO of 15% or more of their total sleep period. This finding is consistent with other studies.[3,4] Poor sleep maintenance was also significantly associated with a number of demographic and clinical factors, including race, unemployment, low CD4+ T-cell count, use of opiates or antiemetic medication, and larger waist circumference. To our knowledge, this is the first study to compare objective sleep measures with both cytokine plasma levels and polymorphisms in cytokine genes in a population of adults with a chronic illness. WASO was associated with plasma levels of C-reactive protein and TNFα, but was unrelated to levels of other plasma inflammatory markers. These findings are not inconsistent with other reports,[45] including studies that have examined sleep using other parameters, such as perceived sleep

Table 4—Significant adjusted associations between wake after sleep onset and cytokine genotype

Gene	SNP	Model	b	95% CI	t	P	ΔR^2	R^2	Full models
IL1R2	rs11674595	R	2.138	1.174, 3.102	4.37	< 0.001	0.054	0.277	F(16,256) = 6.14
IL2	rs2069776	R	-1.359	-2.596, -0.122	2.16	0.031	0.014	0.237	F(16,256) = 4.98
TNFA	rs1041981	A	0.303	0.050, 0.556	2.36	0.019	0.017	0.238	F(16,254) = 4.97

All models adjusted for population substructure, self-reported race, sex, interaction of race and sex, CD4+ T-cell count, waist circumference, use of opiate medication, and use of antiemetic medication. All full models, P < 0.001. Sample sizes for each analysis: n = 273 for *IL1R2* and *IL2* models, and n = 271 for *TNFA* model. b, regression coefficient; CI, confidence interval; IL, interleukin; IL1R2, interleukin 1 receptor 2; R^2, proportion of variance in wake after sleep onset explained by the full model; ΔR^2, proportion of variance in wake after sleep onset accounted for by genotype when adjusting for covariates; SNP, single nucleotide polymorphism; TNFA, tumor necrosis factor alpha. Models: A, additive model; R, recessive model.

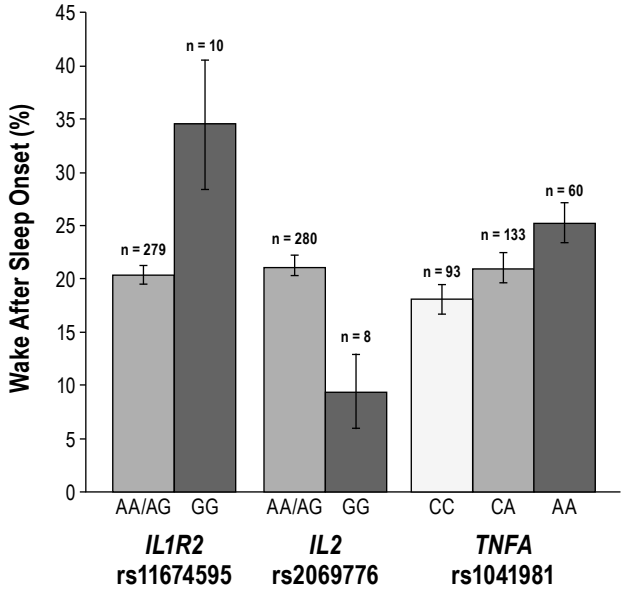

Figure 1—Wake after sleep onset by genotype. Carriers of two doses of the minor allele for *IL1R2* rs11674595 had significantly more wake after sleep onset than carriers of the major allele. In contrast, carriers of two doses of the minor allele for *IL2* rs2069776 had less wake after sleep onset than carriers of the major allele. For *TNFA* rs1041981, each dose of the minor allele was associated with an increase in wake after sleep onset. All P values < 0.01. IL, interleukin; IL1R2, interleukin 1 receptor 2; TNFA, tumor necrosis factor alpha.

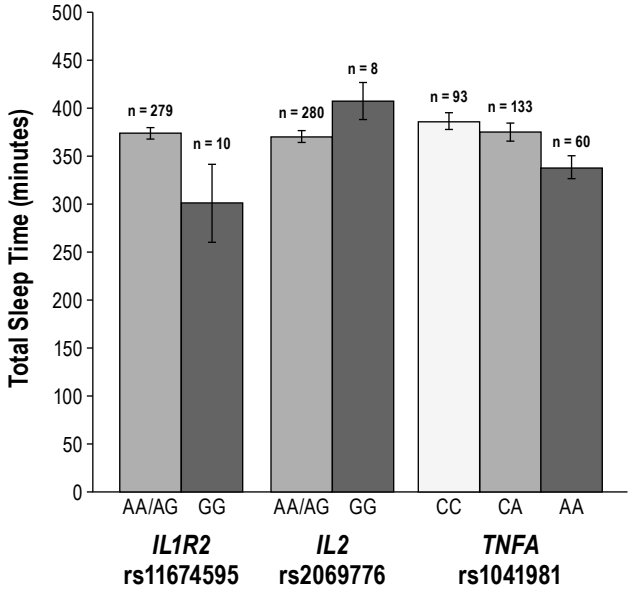

Figure 2—Total sleep time by genotype. Carriers of two doses of the minor allele for *IL1R2* rs11674595 had significantly less total sleep night per night than carriers of the major allele (P = 0.022). There was no significant difference in total sleep time by *IL2* rs2069776 genotype. For *TNFA* rs1041981, each dose of the minor allele was associated with a decrease in total sleep time (P = 0.009). IL, interleukin; IL1R2, interleukin 1 receptor 2; TNFA, tumor necrosis factor alpha.

quality[46,47] or sleep duration.[48] Findings may vary by researchers examining acute laboratory-induced sleep disturbance[49,50] or chronic sleep disturbance related to illness.[51]

Results from this study would suggest that cytokine gene variations (particularly *IL1R2*, *IL2*, and *TNFA*) influence sleep maintenance. The *IL2* polymorphism was associated with less WASO but was not associated with TST. The *IL1R2* and *TNFA* polymorphisms were significantly associated with more WASO (and hence less TST) and the association remained after adjusting for known demographic and clinical factors associated with poor sleep (race, sex, CD4+ T-cell, BMI, waist circumference, and medication use). Although the actual SNPs for these genes may differ from SNPs reported in other studies, the genes involved remain the same. Associations observed among different SNPs within the same genes may be due to differences in linkage disequilibrium between the causal SNPs and those measured in each study or may reflect different susceptibility alleles among populations studied.

The genetic associations observed in this study suggest that the inflammatory pathway is involved in regulating sleep-wake cycles. This finding supports prior research with animal models, as well as laboratory-induced sleep deprivation studies in healthy adults.[6,11,52] An advantage in characterizing cytokine susceptibility genes rather than circulating plasma levels is that genetic polymorphisms do not suffer from the measurement challenges that exist for cytokines (i.e., differences in central versus peripheral levels, fasting requirement, circadian fluctuation, pleiotropic function of cytokines). In addition to plasma levels of certain cytokines, a knowledge of cytokine genotype may be important for understanding poor sleep maintenance in chronic illness populations as demographic and clinical correlates, and may help to explain why hypnotic agents or medications for anxiety and depression fail to improve sleep in chronic

illness populations. Additional research is needed to determine whether anti-inflammatory medication is effective for treating sleep disturbance among adults with HIV/AIDS, and if so, whether the effectiveness varies by cytokine genotype.

In this sample of adults living with HIV disease, markers in the following nine cytokine genes were associated with WASO in unadjusted analyses: *IL1B, IL1R2, IL2, IL8, IL13, IL17A, NFKB1, NFKB2,* and *TNFA*. However, after controlling for race/ethnicity and other significant covariates, only three SNPs explained a unique and significant proportion of the variance in WASO. For the *IL1R2* SNP (i.e., rs11674595), carrying two doses of the minor "G" allele was associated with more WASO. Although rs1295686 is located in intron 1 of the *IL1R2* gene and has no reported function, it occurs within a region of *IL1R2* that undergoes proven deoxyribonucleic acid (DNA) methylation (i.e., an epigenetic modulator of gene expression). For the *IL2* SNP (i.e., rs2069776), carrying two doses of the minor "G" allele was associated with less WASO. *IL2* rs1518111 is located in the promoter region of the gene; although no functional effect of this SNP on the expression of the gene has been reported, it is predicted to occur in an active CCCTC-binding factor (CTCF) transcription factor binding site. Although the function of the *IL1R2* and *IL2* polymorphisms is unknown, a possibility remains that they may be in linkage disequilibrium with one or more functional SNPs that were not examined in this study.

For *TNFA* rs1800683, each dose of the minor "A" allele was associated with an increase in wake after sleep onset. *TNFA* rs1800683 is located in the promoter region of the gene and has been associated with differences in inflammatory response in different phenotypes.[53] Although the exact function of this SNP has not been reported, it is predicted to occur in a proven site of DNA methylation as well as in a region of the promoter that is known to be bound by several transcription factors (i.e., NF*K*β, polymerase 2 [Pol2], octamer-binding transcription factor 2 [Oct-2], POU class 2 homeobox 2 [POU2F2], TATA box binding protein [TBP]). Although no studies were found that described an association between sleep disturbance and *IL1R2* or *IL2*, *TNFA* has been associated with poor self-reported sleep quality in a prior study of cancer patients and their family caregivers.[18] In addition, *IL6* and *NFKB2* have recently been associated with self-reported sleep disturbance in the same sample of oncology patients and caregivers.[15]

Although the function of these cytokine polymorphisms remains unknown, there is considerable evidence that cytokine plasma levels are associated with sleepiness, both in healthy adults after sleep deprivation[11] and in adults with sleep apnea.[54] Furthermore, reduction of cytokine plasma levels through the administration of anti-inflammatory medication has also been found to reduce sleepiness and fatigue.[55] In our sample of adults living with HIV/AIDS, cytokine plasma levels were weakly associated with sleep maintenance, although several clinical factors, including ART, CD4+ T-cell count, and viral load, were associated with circulating cytokine levels. It is possible that these clinical characteristics may have attenuated the relationship between cytokine plasma levels and sleep maintenance in this study. Future research should explore perception of sleepiness and fatigue symptom experience in addition to objective sleep disturbance.

There are limitations to this study that need to be acknowledged. Participants were screened for sleep disorders by self-report, and actigraphy estimates of sleep related parameters do not substitute for the gold standard polysomnography assessment used in prior studies[11,55] describing associations between plasma cytokines and sleep disturbance. Moreover, cytokine plasma levels were based on a single time-point rather than 24-h plasma sampling. A multiplex assay was used rather than more sensitive assays, which may have attenuated associations between cytokine plasma levels and sleep maintenance in this study. Despite the association between the inflammatory system and stress hormones, this study did not include an assessment of cortisol secretion, which is known to be disturbed in individuals with sleep disturbance and individuals with a chronic illness. Although the representative sample of adults living with HIV infection in the United States was adequate, the sample size was modest for a genetic association study, and larger samples are needed to extend these findings and validate associations found in our sample. Furthermore, this study did not include a control group of HIV seronegative adults, and thus, it remains unclear whether the findings are specific to adults with HIV/AIDS or can be generalized to other populations. Finally, the tagSNP approach used may have resulted in failure to identify additional associations poorly captured by the tagSNP set selected for analysis and some associations may have been missed by limiting adjusted analyses to SNPs with significant unadjusted associations with WASO. Findings from this study do, however, contribute to the growing evidence for an association between both proinflammatory and anti-inflammatory pathways and poor sleep maintenance, regardless of clinical characteristics in a chronic illness population. Further research is warranted to compare the therapeutic effects of anti-inflammatory medications to therapeutic effects of hypnotics to reduce WASO and increase TST in this patient population.

ABBREVIATIONS

AIM, ancestry informative marker
ART, antiretroviral therapy
BMI, body mass index
CES-D, Center for Epidemiologic Studies – Depression Scale
CRC, Clinical Research Center
IFNG, interferon-gamma
IFNGR1, interferon-gamma receptor 1
IL, interleukin
IL1R2, interleukin 1 receptor 2
MSAS, Memorial Symptom Assessment Scale
NFKB, nuclear factor of kappa light polypeptide gene enhancer in B cells
PC, principal components
POMS, Profile of Mood State, Tension-Anxiety subscale
PSQI, Pittsburgh Sleep Quality Index
SNP, single nucleotide polymorphism
TNFA, tumor necrosis factor alpha (gene symbol in italics, no greek letters)
TNFα, tumor necrosis factor alpha (protein not italicized, with greek letters)
TST, total sleep time
UCSF, University of California, San Francisco
WASO, wake after sleep onset

DISCLOSURE STATEMENT

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REFERENCES

1. Rubinstein ML, Selwyn PA. High prevalence of insomnia in an outpatient population with HIV infection. J Acquir Immune Defic Syndr Hum Retrovirol 1998;19:260-5.
2. Lee KA, Gay C, Portillo CJ, et al. Types of sleep problems in adults living with HIV/AIDS. J Clin Sleep Med 2012;8:67-75.
3. Phillips KD, Sowell RL, Boyd M, Dudgeon WD, Hand GA. Sleep quality and health-related quality of life in HIV-infected African-American women of childbearing age. Qual Life Res 2005;14:959-70.
4. Gamaldo CE, Spira AP, Hock RS, et al. Sleep, Function and HIV: A Multi-Method Assessment. AIDS Behav 2013;17:2808-15.
5. Kapas L, Bohnet SG, Traynor TR, et al. Spontaneous and influenza virus-induced sleep are altered in TNF-alpha double-receptor deficient mice. J Appl Physiol 2008;105:1187-98.
6. Taishi P, Davis CJ, Bayomy O, et al. Brain-specific interleukin-1 receptor accessory protein in sleep regulation. J Appl Physiol 2012;112:1015-22.
7. Zhu B, Dong Y, Xu Z, et al. Sleep disturbance induces neuroinflammation and impairment of learning and memory. Neurobiol Dis 2012;48:348-55.
8. Krueger JM, Taishi P, De A, et al. ATP and the purine type 2 X7 receptor affect sleep. J Appl Physiol 2010;109:1318-27.
9. Krueger JM, Clinton JM, Winters BD, et al. Involvement of cytokines in slow wave sleep. Prog Brain Res 2011;193:39-47.
10. Clinton JM, Davis CJ, Zielinski MR, Jewett KA, Krueger JM. Biochemical regulation of sleep and sleep biomarkers. J Clin Sleep Med 2011;7(5 Suppl):S38-42.
11. Vgontzas AN, Zoumakis E, Bixler EO, et al. Adverse effects of modest sleep restriction on sleepiness, performance, and inflammatory cytokines. J Clin Endocrinol Metab 2004;89:2119-26.
12. Vgontzas AN, Zoumakis M, Bixler EO, et al. Impaired nighttime sleep in healthy old versus young adults is associated with elevated plasma interleukin-6 and cortisol levels: physiologic and therapeutic implications. J Clin Endocrinol Metab 2003;88:2087-95.
13. McNicholas WT. Chronic obstructive pulmonary disease and obstructive sleep apnea: overlaps in pathophysiology, systemic inflammation, and cardiovascular disease. Am J Respir Crit Care Med 2009;180:692-700.
14. Greenberg DB, Gray JL, Mannix CM, Eisenthal S, Carey M. Treatment-related fatigue and serum interleukin-1 levels in patients during external beam irradiation for prostate cancer. J Pain Symptom Manage 1993;8:196-200.
15. Miaskowski C, Cooper BA, Dhruva A, et al. Evidence of associations between cytokine genes and subjective reports of sleep disturbance in oncology patients and their family caregivers. PLoS One 2012;7:e40560.
16. Miaskowski C, Dodd M, Lee K, et al. 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;40:531-44.
17. Lee YH, Ji JD, Song GG. Tumor necrosis factor-alpha promoter -308 A/G polymorphism and rheumatoid arthritis susceptibility: a metaanalysis. J Rheumatol 2007;34:43-9.
18. Aouizerat BE, Dodd M, Lee K, et al. 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.
19. Lee KA, Gay C, Portillo CJ, et al. Symptom experience in HIV-infected adults: a function of demographic and clinical characteristics. J Pain Symptom Manage 2009;38:882-93.
20. Ancoli-Israel S, Clopton P, Klauber MR, Fell R, Mason W. Use of wrist activity for monitoring sleep/wake in demented nursing-home patients. Sleep 1997;20:24-7.
21. Cole RJ, Kripke DF, Gruen W, Mullaney DJ, Gillin JC. Automatic sleep/wake identification from wrist activity. Sleep 1992;15:461-9.
22. Lichstein KL, Stone KC, Donaldson J, et al. Actigraphy validation with insomnia. Sleep 2006;29:232-9.
23. Buysse DJ, Reynolds CF, 3rd, Monk TH, Berman SR, Kupfer DJ. The Pittsburgh Sleep Quality Index: a new instrument for psychiatric practice and research. Psychiatry Res 1989;28:193-213.
24. Backhaus J, Junghanns K, Broocks A, Riemann D, Hohagen F. Test-retest reliability and validity of the Pittsburgh Sleep Quality Index in primary insomnia. J Psychosom Res 2002;53:737-40.
25. Carpenter JS, Andrykowski MA. Psychometric evaluation of the Pittsburgh Sleep Quality Index. J Psychosom Res 1998;45:5-13.
26. Berger AM, Parker KP, Young-McCaughan S, et al. Sleep wake disturbances in people with cancer and their caregivers: state of the science. Oncol Nurs Forum 2005;32:E98-126.
27. Krupp LB, LaRocca NG, Muir-Nash J, Steinberg AD. The fatigue severity scale. Application to patients with multiple sclerosis and systemic lupus erythematosus. Arch Neurol 1989;46:1121-3.
28. Lerdal A, Kottorp A. Psychometric properties of the Fatigue Severity Scale-Rasch analyses of individual responses in a Norwegian stroke cohort. Int J Nurs Stud 2011;48:1258-65.
29. Lerdal A, Kottorp A, Gay C, Aouizerat BE, Portillo CJ, Lee KA. A 7-item version of the fatigue severity scale has better psychometric properties among HIV-infected adults: an application of a Rasch model. Qual Life Res 2011;20:1447-56.
30. Lerdal A, Wahl A, Rustoen T, Hanestad BR, Moum T. Fatigue in the general population: a translation and test of the psychometric properties of the Norwegian version of the fatigue severity scale. Scand J Public Health 2005;33:123-30.
31. Bakshi R, Miletich RS, Henschel K, et al. Fatigue in multiple sclerosis: cross-sectional correlation with brain MRI findings in 71 patients. Neurology 1999;53:1151-3.
32. Roelcke U, Kappos L, Lechner-Scott J, et al. Reduced glucose metabolism in the frontal cortex and basal ganglia of multiple sclerosis patients with fatigue: a 18F-fluorodeoxyglucose positron emission tomography study. Neurology 1997;48:1566-71.
33. Radloff LS. The CES-D scale: A self-report depression scale for research in the general population. Appl Psychol Meas 1977;1:385-401.
34. McNair DM, Lorr, M., Droppleman, L. F. Manual for the Profile of Mood States. San Diego, CA: Educational and Industrial Testing Services, 1971.
35. Illa L, Brickman A, Saint-Jean G, et al. Sexual risk behaviors in late middle age and older HIV seropositive adults. AIDS Behav 2008;12:935-42.
36. Portenoy RK, Thaler HT, Kornblith AB, et al. The Memorial Symptom Assessment Scale: an instrument for the evaluation of symptom prevalence, characteristics and distress. Eur J Cancer 1994;30A:1326-36.
37. Blinderman CD, Homel P, Billings JA, Portenoy RK, Tennstedt SL. Symptom distress and quality of life in patients with advanced congestive heart failure. J Pain Symptom Manage 2008;35:594-603.
38. Sawicki GS, Sellers DE, Robinson WM. Self-reported physical and psychological symptom burden in adults with cystic fibrosis. J Pain Symptom Manage 2008;35:372-80.
39. Harding R, Molloy T, Easterbrook P, Frame K, Higginson IJ. Is antiretroviral therapy associated with symptom prevalence and burden? Int J STD AIDS 2006;17:400-5.
40. Barrett JC, Fry B, Maller J, Daly MJ. Haploview: analysis and visualization of LD and haplotype maps. Bioinformatics 2005;21:263-5.
41. 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;29:648-58.
42. Hoggart CJ, Parra EJ, Shriver MD, et al. Control of confounding of genetic associations in stratified populations. Am J Hum Genet 2003;72:1492-504.
43. Tian C, Gregersen PK, Seldin MF. Accounting for ancestry: population substructure and genome-wide association studies. Hum Mol Genet 2008;17:R143-50.
44. 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;38:904-9.
45. Liu L, Mills PJ, Rissling M, et al. Fatigue and sleep quality are associated with changes in inflammatory markers in breast cancer patients undergoing chemotherapy. Brain Behav Immun 2012;26:706-13.
46. Taraz M, Khatami MR, Hajiseyedjavadi M, et al. Association between antiinflammatory cytokine, IL-10, and sleep quality in patients on maintenance hemodialysis. Hemodial Int 2013;17:382-90.

47. Chiu YL, Chuang YF, Fang KC, et al. Higher systemic inflammation is associated with poorer sleep quality in stable haemodialysis patients. Nephrol Dial Transplant 2009;24:247-51.
48. Miller MA, Kandala NB, Kivimaki M, et al. Gender differences in the cross-sectional relationships between sleep duration and markers of inflammation: Whitehall II study. Sleep 2009;32:857-64.
49. Irwin MR, Wang M, Ribeiro D, et al. Sleep loss activates cellular inflammatory signaling. Biol Psychiatry 2008;64:538-40.
50. Mullington JM, Haack M, Toth M, Serrador JM, Meier-Ewert HK. Cardiovascular, inflammatory, and metabolic consequences of sleep deprivation. Prog Cardiovasc Dis 2009;51:294-302.
51. Lange T, Dimitrov S, Born J. Effects of sleep and circadian rhythm on the human immune system. Ann N Y Acad Sci 2010;1193:48-59.
52. Krueger JM, Majde JA, Rector DM. Cytokines in immune function and sleep regulation. Handb Clin Neurol 2011;98:229-40.
53. Velez DR, Fortunato S, Thorsen P, Lombardi SJ, Williams SM, Menon R. Spontaneous preterm birth in African Americans is associated with infection and inflammatory response gene variants. Am J Obstet Gynecol 2009;200:209.e1-27.
54. Vgontzas AN, Papanicolaou DA, Bixler EO, et al. Sleep apnea and daytime sleepiness and fatigue: relation to visceral obesity, insulin resistance, and hypercytokinemia. J Clin Endocrinol Metab 2000;85:1151-8.
55. Vgontzas AN, Zoumakis E, Lin HM, Bixler EO, Trakada G, Chrousos GP. Marked decrease in sleepiness in patients with sleep apnea by etanercept, a tumor necrosis factor-alpha antagonist. J Clin Endocrinol Metab 2004;89:4409-13.