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Journal

Biological Research For Nursing, 16(2)

ISSN

1099-8004

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Publication Date

2014-04-01

DOI

10.1177/1099800413478827

Peer reviewed

HHS Public Access

Author manuscript

Biol Res Nurs. Author manuscript; available in PMC 2017 November 21.

Published in final edited form as:

Biol Res Nurs. 2014 April ; 16(2): 143–151. doi:10.1177/1099800413478827.

Polymorphisms of Interleukin-1 Beta and Interleukin-17Alpha Genes Are Associated With Restless Legs Syndrome

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Abstract

Objective—Dopamine, iron, and inflammatory pathways are considered important to the development of restless legs syndrome (RLS). Recent genetic studies support involvement of dopamine and iron; however, cytokine gene variation in the inflammatory component remains unexplored. A recent study reported a high prevalence of RLS among HIV-infected adults. We estimate occurrence of RLS in an ethnically diverse sample of HIV-infected adults and examine differences in demographic factors, clinical characteristics, and biomarkers relating to dopamine, iron, and inflammation between adults with and without RLS symptoms.

Design—A prospective longitudinal study aimed at identifying biomarkers of RLS symptom experience among HIV-infected adults.

Method—316 HIV-positive adults were evaluated using International RLS Study Group criteria. Genes were chosen for hypothesized relationships to dopamine (*NOS1, NOS2*), iron (*HFE*) or inflammation-mediated by cytokine genes (interferon [*IFN*], interleukin [*IL*], nuclear factor kappa-B [*NFKB*], and tumor necrosis factor alpha [*TNFA*]).

Results—Similar to general population estimates, 11% of the sample met all four RLS diagnostic criteria. Controlling for race, gender, and hemoglobin, carrying two copies of the minor allele for *IL1B* rs1143643, rs1143634, or rs1143633 or carrying the minor allele for *IL17A* rs8193036 was associated with increased likelihood of meeting RLS diagnostic criteria.

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Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Conclusion—This study provides preliminary evidence of a genetic association between IL1B and IL17A genes and RLS.

Keywords

restless legs syndrome; inflammation; HIV/AIDS; genes

Restless legs syndrome (RLS) is a neurological movement disorder characterized by a strong urge to move the legs that is worse in the evening and at rest and is relieved with movement. The disorder was highly prevalent (33%) in a German cohort of adults with HIV (Happe, Kundmuller, Reichelt, Husstedt, & Evers, 2007) compared to the general population (5–11%; Allen et al., 2005; Bjorvatn et al., 2005). Investigators have speculated that RLS symptoms may be associated with basal ganglia involvement of the virus (Cardoso, 2002). Genetic studies of the disorder have focused on European cohorts with little racial heterogeneity (Winkelmann et al., 2008). Examining RLS in a more racially and ethnically diverse population of individuals infected with HIV may reveal important factors for RLS etiology.

The pathophysiology of RLS is poorly understood. Prior research has demonstrated that its pathophysiology involves dopamine and iron regulation, with patients responding positively to dopaminergic medications (Hening, Allen, Earley, Picchietti, & Silber, 2004). However, objective measures of dopamine function are not clinically available, and results of radionuclide imaging of dopamine receptors are conflicting (Allen, 2007; Earley et al., 2011). Iron is a cofactor in dopamine metabolism (Patrick, 2007). There is support for central nervous system (CNS) iron deficiency in RLS pathophysiology (Earley et al., 2011), and among individuals with the disorder, periodic CNS iron deficiency can occur when peripheral serum iron levels are low (e.g., the evening nadir of iron's circadian rhythm; Connor et al., 2011). Human hemochromatosis protein (*HFE*) regulates iron absorption (Ajioka, Levy, Andrews, & Kushner, 2002).

High rates (60%) for family history of RLS (Winkelmann et al., 2002) among patients with the disorder suggest a genetic link. To date researchers have identified five loci associated with RLS at regions on chromosomes 12q, 14q, 9p, 2q, and 20p (RLS1-5; Bonati et al., 2003; Desautels et al., 2001; Levchenko et al., 2006). There is also evidence for involvement of the neuronal nitric oxide synthase (*NOS1*) pathway (12q; Winkelmann et al., 2008) as well as other molecules (Schormair et al., 2011). Nitric oxide (NO) is an important cellular signaling molecule that controls CNS development and is associated with pain perception as well as sleep–wake regulation (Gautier-Sauvigne et al., 2005). The NO–arginine pathway modulates dopaminergic transmission (Kiss, Zsilla, & Vizi, 2004) and opioid regulation (Hervera, Negrete, Leanez, Martin-Campos, & Pol, 2011).

Cytokines, which have a major influence on inflammatory processes, may induce symptoms associated with RLS (Weinstock, Walters, Mullin, & Duntley, 2010) by dysregulating dopamine receptors (Aguilar-Valles, Flores, & Luheshi, 2010; Machado et al., 2011). HIV infection, itself, with immunological involvement of the CNS, may increase risk of RLS (Happe et al., 2007; Weinstock, Bosworth et al., 2010; Weinstock, Walters, et al., 2010). To date, no RLS and cytokine genetic association studies have been published. The purpose of

the present study was to estimate the prevalence of RLS in a diverse sample of adults living with HIV in the United States and determine whether participants with RLS differed from those without RLS on selected demographic factors, clinical characteristics, and biomarkers relating to iron, dopamine, and cytokine inflammation.

Method

Participants and Setting

The Symptom and Genetic Study was a prospective longitudinal study aimed at identifying biomarkers of symptom experience among HIV-infected adults (Lee et al., 2009). The present analysis of data from that study reports on the association of RLS symptoms with demographic and clinical characteristics and biomarkers in a sample of adults living with HIV in the San Francisco area. The Committee on Human Research at the University of California, San Francisco (UCSF), approved the study protocol. Participants were recruited using flyers posted at local HIV clinics and community sites, provided written informed consent, and signed a Health Insurance Portability and Accountability Act release for use of their protected medical information in research. Study visits were conducted at the UCSF General Clinical Research Center.

Eligible participants were English-speaking adults who were at least 18 years old and had been diagnosed with HIV at least 30 days before enrollment. To specifically address HIV-related symptoms, 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 hr between 12 a.m. and 6a.m.); reported a diagnosis of bipolar disorder, schizophrenia or dementia; or were pregnant. Participants were not excluded for RLS or insomnia but were excluded for diagnosed sleep disorders (apnea or narcolepsy). All participants completed a baseline evaluation, and those who did not report sleep or fatigue problems at baseline (approximately 50%) completed up to four follow-up visits at 6-month intervals to describe the trajectory of symptoms over time.

Measures

RLS Status—At each assessment, participants completed a brief questionnaire assessing their experience of the four RLS symptom criteria during the previous week. Participants were first asked whether they experienced an abnormal urge to move their legs in the past week. If participants responded “yes” to this first symptom, they were asked about the three other symptom criteria: (2) Was it worse at night? (3) did the symptom come on at rest? and (4) was the symptom relieved with movement? Based on responses to these diagnostic criteria, participants were classified into one of three groups: (1) RLS negative—met none or only the first diagnostic criterion at each study visit, (2) RLS indeterminate— two or three of the four diagnostic criteria at any one of the study visits, or (3) RLS positive—met all four diagnostic criteria during at least one study visit.

Demographic, Clinical, and Laboratory Characteristics—At the baseline assessment, a demographic questionnaire was used to collect information about age, gender, race/ethnicity, employment status, and monthly income. Time since HIV diagnosis and prior

AIDS diagnosis were obtained by self-report. Current medication regimen was assessed by self-report at each assessment, and medications were categorized as anti-retroviral therapy (ART), RLS ameliorating (e.g., dopaminergic agents, anticonvulsants, opiates), or RLS exacerbating (e.g., antihistamines, antidepressants, neuroleptics). Lifestyle factors likely to exacerbate RLS were assessed at each assessment using a 3-day diary and included smoking and daily consumption of caffeine and alcohol. CD4+ T-cell count, HIV viral load, and hemoglobin were obtained at each assessment from the most recent laboratory report in participants' medical records.

Blood Collection—At the baseline assessment, a fasting blood sample was obtained for analysis of plasma levels of interleukin (IL) cytokines (IL1-B, IL-2, IL-6, IL-10), tumor necrosis factor (TNF)–alpha and C-reactive protein (CRP). Assays were done using the Luminex xMAP multiplex platform by Millipore, Inc. (BioMarker Services, EMD Millipore, St. Charles, MO).

Genomic Data—Genomic DNA was extracted from peripheral blood mononuclear cells and maintained by the UCSF Genomic Markers of Symptoms Tissue Bank (Aouizerat et al., 2009; Miaskowski et al., 2010) using the PUREGene DNA Isolation System (Invitrogen, Carlsbad, CA). Of the samples drawn from the 350 participants recruited, DNA was isolated from 348. We selected 18 candidate genes for analysis. We included *NOS1* due to recent evidence of a genetic association with RLS (Winkelmann et al., 2008), and *NOS2* catalyzes the production of NO and is cytokine inducible (Knowles & Moncada, 1994). *HFE* is a protein that functions to regulate iron absorption. Cytokines and their receptors are polypeptides that exercise a major influence on inflammatory processes.

Genotyping was performed blinded to clinical status and included positive and negative controls. DNA samples were quantitated with 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 Golden-Gate 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 adjudicated by a third reviewer.

SNP Selection—A combination of tagging SNPs and SNPs associated with altered function were selected for analysis. Tagging SNPs were required to be common (having a minor allele frequency ≥ .05) in public databases (e.g., HapMap). In order to ensure robust genetic association analyses, quality control filtering of SNPs was performed. SNPs with call rates of <95% ($n = 0$) or Hardy-Weinberg p values of < .001 ($n = 4$) were excluded. In order to maximize the power to detect genetic associations due to common genetic risk factors, SNPs with allele frequencies <5% ($n = 10$) or with <3 individuals homozygous for the rare allele ($n = 17$) were excluded. As shown in the Supplementary Table, >123 SNPs among the 18 candidate genes passed all quality-control filters and were included in the analyses; excluded SNPs are listed in the supplementary table footnote. Potential functional roles for SNPs associated with RLS were examined using PUPASuite 2.0 (Conde et al., 2006), a comprehensive search engine that screens for a series of functional effects.

Statistical Analyses

Except where indicated below, all analyses were conducted using STATA (version 11.2, College Station, TX). Descriptive statistics were used to summarize demographic, clinical, and biomarker characteristics. Differences between RLS-positive and RLS-negative groups were evaluated using χ^2 and analysis of variance. CD4+ T-cell count, viral load, and hemoglobin were analyzed as continuous variables and in clinically meaningful categories. Because reference ranges for hemoglobin values differ for men and women, values are also reported by sex.

Allele and genotype frequencies were determined by gene counting. Hardy-Weinberg equilibrium was assessed by the chi-square exact test. Measures of linkage disequilibrium (D' and r^2) were computed from participants' genotypes with Haploview 4.1. Unadjusted genetic associations with RLS group were determined using logistic regression models predicting positive RLS status. The RLS-negative group served as the reference group. Given uncertainty around the RLS-indeterminate group, this group was excluded from regression analyses. 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. Adjustments were not made for missing data or multiple testing (Rothman, 1990).

Each genetic marker was further evaluated in a multivariate logistic regression model controlling for relevant covariates. As in the unadjusted regression analyses, the adjusted models predicted RLS-positive status, the RLS-negative group served as the reference group, and the RLS-indeterminate group was excluded. All adjusted regression models controlled for population substructure (i.e., ancestry informative markers [AIMs] for race/ethnicity, as described below), and self-reported race (i.e., Black/African American, White/Caucasian, Other). All demographic, clinical, and laboratory variables associated with RLS status ($p < .10$) were evaluated as potential covariates. Variables were retained as covariates in all adjusted models if significance was $p < .05$ prior to including genotype in the model. A model was fit for each genetic marker to estimate its unique contribution when controlling for relevant covariates.

AIMs can be used to minimize bias due to population stratification in case–control association studies (Halder, Shriver, Thomas, Fernandez, & Frudakis, 2008; Hoggart et al., 2003; Tian, Gregersen, & Seldin, 2008). Homogeneity in AIMs was verified by cluster and principal component (PC) analysis (Price et al., 2006) using HelixTree software (GoldenHelix, Bozeman, MT). PCs were sought that distinguished major racial groups (i.e., Black/African American, White/Caucasian, Other) by visual inspection of scatterplots of orthogonal PCs. This procedure was repeated until no discernible clustering of participants by self-reported race/ethnicity was possible. The first three PCs were selected to adjust for potential confounding due to population substructure (i.e., race/ethnicity) and included in all multiple regression models. We included 106 AIMs in the PC analysis.

Results

Sample Characteristics

A convenience sample of 350 adults with HIV was enrolled (April 2005 to December 2007). Of these, 34 participants were excluded for positive drug screening ($n = 31$), missing urine sample ($n = 1$), or missing genetic or RLS-symptom data ($n = 3$). Of the 316 participants remaining for analysis, 177 completed one study visit, 54 completed two to three visits, and 85 completed four to five visits. Demographic and clinical characteristics are presented in Table 1. The sample was ethnically diverse and predominantly male, reflecting the local population of adults with HIV. Of the 77 women in the sample, more than half (62%) were African American. A total of 23 participants identified themselves as male-to-female transgender, and we also categorized a genetic male who identified as a woman as transgender. Most participants had been living with HIV for many years, 52% had been diagnosed with AIDS, and 28% of those with an AIDS diagnosis had a current CD4 count of less than 200 cells/mm^3.

The RLS-positive group comprised the 11% ($n = 36$) of participants who met all four RLS diagnostic criteria (26 at baseline and an additional 10 at a follow-up visit). The RLS-indeterminate group comprised the 16% ($n = 50$) of participants who met two or three of the four RLS diagnostic criteria (40 at baseline and 10 at a follow-up visit). The remaining 73% ($n = 230$) of participants met none or only one of the RLS diagnostic criteria at all study visits and were classified as RLS negative.

The only clinical characteristics that differed between the RLS-positive and RLS-negative groups were estrogen therapy and hemoglobin. Genetic sex was unrelated to RLS status. However, the 24 transgender adults in this sample had an unusually high rate of RLS (29%). Transgender adults also had lower hemoglobin levels than males ($F = 12.7$, $p = .0005$) and were less likely than males to be Caucasian (12% vs. 43%; $\chi^2 = 12.4$, $p < .001$). Transgender status was associated with RLS even after controlling for race and hemoglobin level. There were no associations between cytokine genes and cytokine serum levels.

Genetic Associations

The RLS-positive and RLS-negative groups differed in their distributions of nine SNPs in bivariate analyses: one SNP mapping to *NOS1*, one to *NOS2*, five to *IL1B*, one to *IL1R2*, and one to *IL17A* (Table 2). In order to estimate the magnitude of the association of genotype with RLS group when adjusting for relevant covariates, we used logistic regression models to compare the RLS-negative ($n = 230$) and RLS-positive ($n = 36$) groups. In addition to gene SNPs, the phenotypic variables we evaluated in all adjusted models included race/ethnicity, gender, and hemoglobin. The SNP associations in *NOS1* ($p = .215$), *NOS2* ($p = .230$), and *IL1R2* ($p = .077$) were attenuated and no longer significant after adjusting for relevant covariates (AIMs, self-reported race/ethnicity, gender, and hemoglobin).

The models' fit for four *IL1B* SNPs (rs1071676, rs1143634, rs1143643, and rs1143633) as genotype predictors of RLS group remained significant after adjusting for race/ethnicity, gender, and hemoglobin. The overall models explained 15–17% of the variance in RLS

diagnostic group membership. Controlling for AIMs, self-reported race/ethnicity, gender, and hemoglobin, there was an increased likelihood of belonging to the RLS-positive group if a participant carried two copies of the minor allele for any of these four *IL1B* SNPs. Due to the near complete pairwise linkage disequilibrium among *IL1B* rs1071676, rs1143634, rs1143643, and rs1143633 (all pairwise D' = 1.0), we show only the model for rs1143643 in Table 3.

The model fit for *IL17A* rs8193036 also remained significant after adjusting for race/ethnicity, gender, and hemoglobin level. The overall model explained 16% of the variance in RLS diagnostic group membership, and carriers of the minor allele for this *IL17A* SNP were more likely to be in the RLS-positive group (Table 3).

Discussion

The prevalence estimate of RLS in our sample was 11%, which is similar to general population estimates (Allen et al., 2005; Bjorvatn et al., 2005) but lower than the 33% reported in an HIV clinic in Germany (Happe et al., 2007). The German cohort was similar to our cohort for age and proportion of participants on antiretroviral therapy. In contrast, the German cohort was 100% Caucasian and had a lower average CD4 cell count. Their higher estimated prevalence may be due to the difference in methods for determining the presence of RLS: In Happe et al.'s study, participants were screened by an experienced neurologist, while we relied on a self-report questionnaire. Alternatively, our racially diverse sample may have had a decreased prevalence compared to their 100% Caucasian sample. In the present study, RLS-ameliorating or RLS-exacerbating medications were not associated with RLS status. However, medication dose and duration were not available, and future studies should consider dose and duration of ameliorating and exacerbating medications.

Iron levels in the brain can influence dopamine metabolism, and measures of iron status (lower hemoglobin) have been associated with RLS (Lee, Zaffke, & Baratte-Beebe, 2001). In our sample, it was only the men in the RLS-positive group that had lower hemoglobin levels compared to the men in the RLS-negative group. We cannot ascertain whether this hemoglobin difference was associated with iron and ferritin levels low enough to affect CNS iron function, and it remains unclear why similar differences were not evident for the women in our sample.

Transgender participants were more likely to report RLS symptoms and to have lower hemoglobin levels than the other participants. Although this was an unexpected finding, it has been reported that 20% of estrogen-treated transsexuals experience RLS (Fulda, Stalla, & Wetter, 2007). In our sample, 58% ($n = 14$) of transgender participants were taking estrogen compared to only 6% ($n = 3$) of the women and 0% of the men. Of the 19 participants taking estrogen, 5 (26%) met criteria for RLS compared to only 31 of the 297 (10%) not taking estrogen. Although the prevalence of RLS is higher in the general population among women than among men (Allen et al., 2005), this was not evident in our sample. With the probable confounding of transgender status and estrogen use in our sample, additional research to evaluate estrogen's role in RLS is warranted.

Our study may not have had sufficient statistical power for us to find significant relationships between RLS and *NOS1, NOS2, HFE*, or most of the cytokine genes. We were unable to replicate a previous finding of a genetic association between *NOS1* and RLS (Winkelmann et al., 2008) after controlling for race, gender, and hemoglobin level, due, perhaps, to smaller sample sizes or population differences by race. Winkelmann and colleagues examined *NOS1* in a large European cohort in which controlling for race was not necessary. However, population substructure AIMs can still occur in seemingly homogeneous samples (Salmela et al., 2008). It is possible that *NOS1* could be a European-cohort population-specific risk factor. The unique contribution of the present study is that our sample consisted of varied racial backgrounds, which authors of a prior study had suggested (Winkelmann et al., 2008).

The present study is the first to identify the importance of *IL1B* and *IL17A* cytokine genes associated with RLS symptoms in a diverse sample of adults with HIV. After controlling for race, gender, and hemoglobin, an increased likelihood of meeting diagnostic criteria for RLS was associated with carrying two copies of the minor allele for *IL1B* (rs1143643, rs1143634, or rs1143633) or carrying at least one copy of the minor allele for *IL17A* (rs8193036). These data are particularly intriguing, given the recent discoveries of links between RLS and inflammatory and immunological conditions, such as inflammatory bowel disease (Weinstock, Bosworth, et al., 2010), multiple sclerosis (Manconi et al., 2008), rheumatoid arthritis (Salih, Gray, Mills, & Webley, 1994), and narcolepsy (Plazzi et al., 2010). Inflammation increases levels of hepcidin and CNS iron deficiency, which in turn could initiate RLS, with auto-antibodies directly attacking the CNS (Weinstock & Walters, 2011; Weinstock, Walters, & Paueksakon, 2012).

The differences we observed between the RLS groups in *IL1B* and *IL17A* genes may provide insight into mechanisms that lead to development of cytokine-mediated treatments. In a small study describing cytokines in 22 patients with and without RLS, investigators found no significant difference in serum levels of six cytokines (Siddiqui, Strus, Sun, & Walters, 2007). That finding is in accord with our finding in the present study of no difference in serum cytokine levels between the RLS-positive and RLS-negative groups.

IL1B gene encodes for the *IL1B* cytokine protein, an important mediator of both pro- and anti-inflammatory responses. IL-1β increases iron efflux from glial cells by inducing ferroportin-1 (Fpn) synthesis (di Patti et al., 2004). Fpn transports iron from the cell and is inhibited by hepcidin (Nemeth & Ganz, 2006). The expression of Fpn in response to IL-1β requires activation of mitogen-activated protein (MAP) kinase pathways, and IL-1β regulates expression of *Fpn* genes (Persichini et al., 2010). Of note, investigators recently linked a related gene, *MAP2K5*, to RLS in a genome-wide association study (Winkelmann et al., 2007).

IL17A gene encodes for the pro-inflammatory cytokine IL-17. It is produced by activated T cells and upregulated during inflammation. IL-17 is important in pro-inflammatory responses and enhances NO production (Krstic et al., 2010). High levels of IL-17 are associated with several chronic inflammatory diseases, including rheumatoid arthritis and inflammatory bowel disease (Kim et al., 2011; Nakano et al., 2011). IL-17 is also involved

in the regulation and production of blood cells (Krstic et al., 2010). Therefore, *IL17A* may be an important genetic component of the development of RLS through regulation of iron-containing red blood cells. *IL17A* may also play a role in RLS as part of the dopamine pathway. Dopamine increases IL-17 production and increases inflammation in patients with rheumatoid arthritis by inducing IL-17 release from immune system cells (Nakano et al., 2011). Likewise, treatment with a D1-like receptor antagonist inhibits IL-17 expression (Okada, Inoue, Hashimoto, Suzuki, & Matsushita, 2009) and dopamine-antagonist medications can worsen RLS symptoms (Chokroverty, 2009).

There are limitations to this study that warrant discussion. The RLS-positive group status was determined by the International Restless Legs Syndrome Study Group (IRLSSG) self-report screening measure, the gold standard at the time of the study, but the diagnosis was not confirmed by a neurologist. In addition, a family history of RLS was not obtained from participants. The modest sample size may have limited statistical power to detect more subtle associations as well as our ability to control for additional covariates. Only one baseline measure for plasma biomarkers was available, and longitudinal measures were not possible. Hemoglobin levels were not available for all 316 participants, further decreasing the sample size for adjusted analyses. Although we used hemoglobin as a proxy for iron status, ferritin and serum transferrin receptor are more accurate measures of iron, especially within the inflammatory environment characteristic of the HIV population (Baynes, 1996; Ferguson, Skikne, Simpson, Baynes, & Cook, 1992). Evaluation of hepcidin and gonadal hormones (estrogen and testosterone) in future studies could shed light on iron status in relation to RLS. Finally, in our study, we assessed RLS symptoms across 1 week. Symptoms of RLS vary in frequency and severity (Milligan & Chesson, 2002); therefore, participants may not have reported symptoms if their symptoms were relatively mild or infrequent at the time of assessment.

The present study is the first to identify associations between *IL1B* and *IL17A* genes and RLS. The sample included ethnically diverse adults with HIV infection from one area of the United States. The null findings for *NOS1* are equivocal and warrant further study. The overactive inflammatory reaction in an HIV-positive sample may cause a lower threshold for developing RLS and alter the relative importance of *IL1B* and *IL17A* genes in developing the disorder. Clinicians should consider the medical history of all patients presenting with symptoms of RLS, particularly patients with chronic inflammatory conditions, as these other conditions may include neuropathies that are difficult to distinguish from RLS. Future studies should examine differences between patients with neuropathy and RLS and consider other medical conditions that may mimic RLS. Future studies should also include larger diverse samples and more specific measures of gonadal hormones as well as more complete measures of iron status and hepcidin levels.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

Funding

The author(s) disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: This research was supported by a grant from the National Institute of Mental Health (NIMH, 5 R01 MH074358). Data collection was supported by the General Clinical Research Center in the UCSF CTSA (1 UL RR024131). Dr. Aouizerat is supported by an NIH Roadmap K12 (KL2 RR024130). Dr. Hennessy was supported by T32 Nurse Research Training in Symptom Management (5 T32 NR07088).

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Table 1

Sample Demographic and Clinical Characteristics by Restless Legs Syndrome (RLS) Group, Mean (*SD*) or *n* (%).

		RLS status			
Characteristic	**Total sample *N* = 316**	**Negative *n* = 230**	**Indeterminate *n* = 50**	**Positive *n* = 36**	**Comparison of RLS-positive and RLS-negative groups[a]**
Age (years)	45.2 (8.4)	45.2 (8.1)	44.6 (8.0)	45.6 (10.5)	$F(1, 264) = 0.06$; $p = .805$
Gender					$\chi^2(2) = 4.41$; $p = .110$
Male	215 (68%)	157 (68%)	36 (72%)	22 (61%)	
Female	77 (24%)	58 (25%)	11 (22%)	8 (22%)	
Transgender	24 (8%)	15 (7%)	3 (6%)	6 (17%)	
Race/ethnicity					$\chi^2(2) = 0.57$; $p = .752$
Black/African American	122 (39%)	90 (39%)	19 (38%)	13 (36%)	
White/Caucasian	129 (41%)	95 (41%)	20 (40%)	14 (39%)	
Other	65 (21%)	45 (20%)	11 (22%)	9 (25%)	
Monthly income					$\chi^2(1) = 0.23$; $p = .633$
<$1,000	219 (69%)	157 (68%)	36 (72%)	26 (72%)	
≥$1,000	97 (31%)	73 (32%)	14 (28%)	10 (28%)	
Years since HIV diagnosis	12.1 (7.0)	11.7 (6.8)	12.9 (7.7)	13.3 (6.9)	$z = -1.22$; $p = .224$
AIDS diagnosis	164 (52%)	109 (47%)	32 (64%)	23 (64%)	$\chi^2(1) = 3.39$; $p = .066$
On antiretroviral therapy	223 (71%)	158 (69%)	38 (76%)	27 (75%)	$\chi^2(1) = 0.53$; $p = .466$
Estrogen therapy	19 (6%)	11 (5%)	3 (6%)	5 (14%)	$\chi^2(1) = 4.57$; $p = .033$
RLS-ameliorating medications[b]	0.81 (1.03)	0.74 (1.01)	1.12 (1.12)	0.81 (0.98)	$z = -0.47$; $p = .642$
RLS-exacerbating medications[c]	0.68 (0.90)	0.70 (0.92)	0.62 (0.75)	0.64 (0.96)	$z = 0.64$; $p = .522$
Exacerbating lifestyle factors					
Caffeine servings/day	1.49 (1.37)	1.49 (1.36)	1.64 (1.61)	1.22 (1.01)	$z = 1.22$; $p = .221$
Alcohol servings/day	0.22 (0.67)	0.21 (0.65)	0.25 (0.73)	0.18 (0.72)	$z = 0.78$; $p = .437$
Cigarettes/day	5.86 (7.13)	5.65 (6.82)	6.15 (8.65)	6.80 (6.83)	$z = -0.98$; $p = .328$
CD4+ T-cells (cells/mm^3; $n = 301$)	454 (270)	460 (266)	439 (305)	436 (254)	$z = 0.38$; $p = .708$
Viral load ($\log_{10}$ copies/ml; $n = 294$)	2.61 (1.19)	2.58 (1.18)	2.68 (1.22)	2.70 (1.28)	$z = -0.53$; $p = .599$
Detectable (≥1.70)	142 (48%)	101 (47%)	24 (51%)	17 (52%)	$\chi^2(1) = 0.21$; $p = .644$

Characteristic	Total sample *N* = 316	RLS status			Comparison of RLS-positive and RLS-negative groups[a]
		Negative *n* = 230	Indeterminate *n* = 50	Positive *n* = 36	
Hemoglobin (g/dl)					
Total sample (*n* = 186)	13.9 (1.8)	13.9 (1.7)	14.3 (1.9)	13.1 (1.6)	$F(1, 159) = 4.48$; $p = .036$
Male (*n* = 129)	14.5 (1.6)	14.5 (1.6)	15.0 (1.6)	13.6 (1.6)	$F(1, 108) = 3.18$; $p = .077$
Female (*n* = 42)	12.3 (1.3)	12.4 (1.3)	11.7 (0.4)	12.0 (1.9)	$F(1, 37) = 0.33$; $p = .571$
Transgender (*n* = 15)	13.0 (1.0)	13.3 (1.0)	12.6 (1.3)	12.8 (1.1)	$F(1, 19) = 0.77$; $p = .401$
C-reactive protein (ng/ml; *n* = 313)	12,824 (19,458)	12,234 (18,790)	15,140 (21,467)	13,327 (20,973)	$z = -0.61$; $p = .543$
IL-1β (IU/ml; *n* = 314)	4.10 (3.49)	4.01 (3.33)	4.16 (3.61)	4.57 (4.28)	$z = -0.72$; $p = .471$
IL-2 (IU/ml; *n* = 314)	8.55 (13.7)	8.17 (13.1)	10.34 (15.6)	8.46 (14.6)	$z = 0.60$; $p = .549$
IL-6 (IU/ml; *n* = 314)	21.2 (35.1)	22.7 (36.3)	14.4 (25.4)	20.6 (38.8)	$z = 1.51$; $p = .132$
IL-10 (IU/ml; *n* = 314)	24.0 (48.7)	27.6 (55.9)	16.5 (19.1)	11.4 (10.8)	$z = 1.50$; $p = .133$
TNF-α (IU/ml; *n* = 314)	12.4 (11.5)	12.2 (11.6)	11.9 (8.69)	14.1 (14.4)	$z = -0.38$; $p = .701$

Note. Sample size = 316, unless otherwise indicated. Participants in the RLS-negative group met none or only the first RLS diagnostic criterion at each study visit; participants in the RLS-indeterminate group met two to three RLS diagnostic criteria at any one of the study visits; and participants in the RLS-positive group met all four diagnostic criteria at any one of the study visits. *z* scores are reported for the Mann–Whitney *U* test in the event of nonnormality

[a] No differences between RLS-negative, RLS-indeterminate, and RLS-positive groups except for AIDS diagnosis [$\chi^2(2) = 6.88$; $p = .032$] and RLS-ameliorating medications ($z = 6.08$; $p = .048$). The last column comparing the positive and negative RLS groups determined which variables were included in the regression models.

[b] RLS-ameliorating medications include anti-convulsants, dopaminergic agents, benzodiazepines, opiates, bupropion, zolpidem, zaleplon, baclofen, clonidine, folic acid, and iron.

[c] RLS-exacerbating medications include antihistamines, anti-depressants (except bupropion and phenylpiperazine), neuroleptics (except aripiprazole), anti-emetics, and thyroxine.

Table 2

Significant Unadjusted Associations Between Restless Legs Syndrome (RLS) Status and Genotypes (n = 266).

Gene	SNP	HGVS description	Position	Chr	MAF	OR	*SE*	95% CI	p	Model
NOS1	rs2293054	c.2202T>C	117701714	12	.230	2.56	0.94	[1.25, 5.28]	.010	D
NOS2	rs10459953	c.−227G>C	26127518	17	.306	1.74	0.49	[1.00, 3.00]	.048	A
IL1B	rs1071676	c.*505G>C	106042060	2	.160	10.3	9.61	[1.66, 64.1]	.012	R
IL1B	rs1143643	c.598-152G>A	106042929	2	.265	3.00	1.47	[1.14, 7.84]	.025	R
IL1B	rs1143634	c.315C>T	106045017	2	.157	10.3	9.61	[1.66, 64.1]	.012	R
IL1B	rs1143633	c.302-64G>A	106045094	2	.280	2.68	1.30	[1.04, 6.93]	.042	R
IL1B	rs1143630	c.100-503A>C	106046282	2	.138	2.03	0.65	[1.08, 3.82]	.029	A
IL1R2	rs4141134	(5' of gene, T>C)	96370336	2	.297	2.53	1.16	[1.03, 6.21]	.042	R
IL17A	rs8193036	(5' of gene, T>C)	51881562	6	.241	2.20	0.80	[1.08, 4.50]	.030	D

Note. A = Additive; Chr = chromosome; CI = confidence interval; D = Dominant; HGVS = Human Genome Variation Society; MAF = minor allele frequency; OR = odds ratio; R = Recessive; *SE* = standard error; SNP = single nucleotide polymorphism. Genome Build 37.1 (www.ncbi.nlm.nih.gov). Odds ratios reflect risk of RLS positive relative to doses of the minor allele; negative RLS group served as the reference and RLS-indeterminate group was excluded. All genotypes evaluated are reported in the supplemental table available online.

Table 3

Significant Adjusted Logistic Regression Analyses of Restless Legs Syndrome (RLS) Status on Genotype (n = 160).

Predictor	OR	*SE*	95% CI	*Z*	*p*	ΔR^2	Full model
Race						.019	χ^2 = 21.4, p = .011
African American	1.25	1.53	[0.11, 13.8]	0.18	.856		Total R^2 = .167
Other	1.87	1.52	[0.38, 9.21]	0.77	.440		
Gender						.056	
Female	0.88	0.62	[0.22, 3.51]	−0.18	.859		
Transgender	7.52	6.16	[1.51, 37.5]	2.46	.017		
Hemoglobin	0.67	0.14	[0.44, 1.00]	−1.97	.049	.049	
IL1B rs1143643 (REC)	**6.08**	**4.50**	**[1.42, 26.0]**	**2.43**	**.015**	**.043**	
Race						.019	χ^2 = 20.1, p = .017
African American	0.99	1.13	[0.11,9.28]	−0.01	0.995		Total R^2 = .157
Other	1.74	1.34	[0.38, 7.91]	0.71	.476		
Gender						.056	
Female	1.08	0.74	[0.28. 4.17]	0.11	.910		
Transgender	7.32	6.14	[1.41, 37.9]	2.37	.018		
Hemoglobin	0.67	0.13	[0.47,0.98]	−2.09	.037	.044	
IL17A rs8193036 (DOM)	**3.12**	**1.64**	**[1.11, 8.75]**	**2.16**	**.031**	**.038**	

Note. Figures in boldface imply significant gene findings. CI = confidence interval; DOM = dominant genetic model; OR = odds ratio; REC = recessive genetic model; *SE* = standard error.

SNPs IL1B rs1143634 (REC) and IL1B rs1143633 (REC) also yielded significant results similar to IL1B rs1143643 (REC). Only SNPs with significant adjusted associations with RLS status are included in this table (IL1B rs1071676 is not reported given its near complete linkage disequilibrium with IL1B rs1143634). The RLS-negative group was used as the reference, and the RLS-indeterminate group was excluded. Odds ratios for the relationship between SNPs and RLS status are based on doses of the minor allele. Ancestry informative markers (AIMS) and race were included in all models, and gender and hemoglobin met the significance threshold for covariate retention. AIDS diagnosis was not a significant predictor and was not retained in the final models.