Periodic Leg Movements during Sleep Are Associated with Polymorphisms in BTBD9, TOX3/BC034767, MEIS1, MAP2K5/SKOR1, and PTPRD

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Study Objectives: To examine association between periodic leg movements (PLM) and 13 single nucleotide polymorphisms (SNPs) in 6 loci known to increase risk of restless legs syndrome (RLS).

Setting: Stanford Center for Sleep Sciences and Medicine and Clinical Research Unit of University of Wisconsin Institute for Clinical and Translational Research.

Patients: Adult participants (n = 1,090, mean age = 59.7 years) from the Wisconsin Sleep Cohort (2,394 observations, 2000-2012).

Design and Interventions: A previously validated automatic detector was used to measure PLMI. Thirteen SNPs within BTBD9, TOX3/BC034767, MEIS1 (2 unlinked loci), MAP2K5/SKOR1, and PTPRD were tested. Analyses were performed using a linear model and by PLM category using a 15 PLM/h cutoff. Statistical significance for loci was Bonferroni corrected for 6 loci (P < 8.3 × 10^{-3}). RLS symptoms were categorized into four groups: likely, possible, no symptoms, and unknown based on a mailed survey response.

Measurements and Results: Prevalence of PLMI ≥ 15 was 33%. Subjects with PLMs were older, more likely to be male, and had more frequent RLS symptoms, a shorter total sleep time, and higher wake after sleep onset. Strong associations were found at all loci except one. Highest associations for PLMI > 15/h were obtained using a multivariate model including age, sex, sleep disturbances, and the best SNPs for each loci, yielding the following odds ratios (OR) and P values: BTBD9 rs3923809(A) OR = 1.65, P = 1.5×10^{-8}; TOX3/BC034767 rs3104788(T) OR = 1.35, P = 9.0 × 10^{-5}; MEIS1 rs12469063(G) OR = 1.38, P = 2.0 × 10^{-4}; MAP2K5/SKOR1 rs6494696(G) OR = 1.24, P = 1.3×10^{-2}; and PTPRD(A) rs1975197 OR = 1.31, P = 6.3×10^{-3}. Linear regression models also revealed significant PLM effects for BTBD9, TOX3/BC034767, and MEIS1. Co-varying for RLS symptoms only modestly reduced the genetic associations.

Conclusions: Single nucleotide polymorphisms demonstrated to increase risk of RLS are strongly linked to increased PLM as well, although some loci may have more effects on one versus the other phenotype.

Keywords: periodic leg movements, restless legs syndrome, polysomnography, Wisconsin Sleep Cohort, genetics

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INTRODUCTION

Periodic limb movements (PLMs) are episodic, involuntary muscle contractions that occur during sleep, most notably NREM sleep. Restless legs syndrome (RLS) is often associated with PLMs, with four out of five patients diagnosed with RLS exhibiting PLMs in one study (≥ 5/h)[1] and 85% to 95% in others.[2,3] PLM can occur during sleep (PLMS) or wake (PLMW), and much research has been conducted to examine and understand the physiology of PLM in RLS (e.g., circadian effect, significance of PLMW, higher periodicity index).[1,4-7] However, PLMs can also occur without RLS symptoms, though little is known about the cause of PLMs or their impact on daytime sleepiness or insomnia symptoms in the absence of RLS. A study review of cardiac risk for RLS and PLMS found associations between PLMS and congestive heart failure.[8] Additionally, patients with RLS were at higher risk for heart disease and hypertension.[8] While this association is controversial, another recent prospective study found increased mortality in men

with RLS.[9] Further, PLMs have been associated with increased risk of atrial fibrillation[10] and coronary heart disease.[11] PLM are known to be associated with several other disorders and pathologies such as depression, cardiovascular disease, REM behavior disorder, narcolepsy, Parkinson disease, and multiple system atrophy.[12-15] Patients with REM behavior disorder and narcolepsy have more PLMs occurring in REM.[16,17]

Recent investigations have revealed strong genetic components for RLS and PLMI. Most notably, single nucleotide polymorphisms (SNPs) were identified as RLS susceptibility markers in a genome-wide association study (GWAS) described in 2011 (with related work shown earlier[19-22]), identifying nine genome wide significant loci with best effects at rs2300478 in MEIS1; rs3923809 in BTBD9; rs1975197 in PTPRD; rs12593813 in MAP2K5/SKOR1; rs6747972 in intergenic region on chromosome 2 (presumably another distant regulator of MEIS1); and rs3104767 in TOX3/BC034767.[18] Association of the BTBD9 and MEIS1 genes with RLS and end-stage renal disease was reported in a German study (200 RLS cases, 443 controls) with replication in a Greek sample (141 RLS cases, 393 controls).[23] Another team, using an Icelandic discovery sample with replications in Icelandic and U.S. samples found association of BTBD9 in PLM in sleep (PLMS)[22] apart from RLS diagnosis as adopted from a 1995 criteria.[24] PLMS were measured using a small tri-axial accelerometer worn on the ankle that could not distinguish sleep from wake. PLMS were classified whenever the ambulatory device detected five or more PLM during an

hour while participants were recumbent during their major rest period.[22] These studies did not use PSG measures of PLM or account for additional effects on PLMS, such as use of medication that can aggravate or inhibit factors contributing to RLS.

In this study, we report on the genetic association of PLM with and without the presence of RLS symptoms from DNA samples and automatically obtained PLM metrics from the Wisconsin Sleep Cohort, an epidemiological sample totaling 1,086 subjects and 2,394 nocturnal polysomnography (PSG) based sleep studies conducted between 2000 and 2012.

METHODS

Cohort Used in the Analysis

A total of 2,394 nocturnal PSG (NPSG) studies from 1,086 Wisconsin Sleep Cohort (WSC) participants were used in this study. Electroencephalography (EEG), electrooculography (EOG), and chin electromyography (EMG) were used to score sleep stages for each 30-s epoch using standard Rechtschaffen and Kales criteria.[25]

The WSC is a longitudinal study of sleep habits and disorders in the general population that was established in 1988 from a sample of employees of 4 state agencies in south central Wisconsin, USA, aged 30-60 years.[26] In 2000, WSC began digitally storing PSGs, thus the study dates for this paper are from 2000 and 2012. Participants in this time line without ≥ 2 h scored sleep were excluded. NPSGs were exported as EDF files and paired with scoring files of stages and events (e.g., apneas and hypopneas). PLM and SDB were characterized using a 16-channel PSG recording system (16-channel Grass-Telefactor Heritage digital sleep system Model 15). Arterial oxyhemoglobin saturation was measured by pulse oximetry using a 3-s averaging rate. Oral and nasal airflow were measured using thermocouples (ProTech). Nasal air pressure was measured with a pressure transducer (Validyne, Northridge). Thoracic cage and abdominal respiratory motion were measured with inductance plethysmography (Respitrace, Ambulatory Monitoring). These signals were used to identify SDB events. Apnea was defined as a cessation of airflow lasting ≥ 10 sec. Hypopnea was defined as a decrease in airflow accompanied by ≥ 4% reduction in oxyhemoglobin saturation and is close to the 2007 AASM recommended (Medicare) criteria for scoring hypopneas.[27]

A single-leg EMG channel, combined from the left and right anterior tibialis (LAT/RAT) EMGs, was used to determine significant leg movements according to the 2007 AASM Manual for Scoring Sleep (AASM Manual) with slight modifications. The AASM Manual defines a significant leg movement (LM) as a period of 0.5-10 s where LAT/RAT EMG activity exceeds 8 μV above baseline and then falls below 2 μV from baseline for 0.5 sec or longer.[28] PLM are further defined as the consecutive sequence of ≥ 4 LMs whose inter-movement intervals are between 5 and 90 sec. This definition of PLM is consistent with the clinical guidelines set for by the World Association of Sleep Medicine in 2006 and the American Sleep Disorders Association in 1993.[29,30]

The AASM Manual does not allow LMs occurring 0.5 s before, during, or 0.5 s after a respiratory event to be included in PLM.[28] Our PLM classifier modifies these rules to only exclude LM detected at the edges of respiratory events. Any

LM occurring 5 s prior to the onset of a respiratory event until 0.5 sec after its onset are excluded from PLM, as are any LM occurring 0.5 s prior to offset of a respiratory event through 5.0 s after offset. This adjustment is based on empirical analysis of time-locked respiratory events and leg EMG activity in patients stratified by an apnea-hypopnea index (AHI) of 15. The analysis defined AHI as the average number of apneas and hypopneas per hour of objectively measured sleep.[31]

Data pertaining to the intake of drugs known to modulate RLS symptoms were also collected, and variable created if the subject took any of these drugs. RLS symptoms aggravators included antidepressants (e.g., selective serotonin reuptake inhibitors, tricyclics), antipsychotics, and antihistamines. RLS inhibitors include benzodiazepines, opiates, antiseizure medication, and medication for Parkinson disease.

RLS Symptoms

WSC participants were stratified according to RLS symptoms based on questionnaire responses from a previous study of the cohort in 2003.[32] Patients were asked to provide the frequency with which they felt (a) repeated urge to move legs, (b) strange and uncomfortable feelings in the legs, (c) periods of several leg jumps or jerks. The choice of frequency included never, less than once a month, monthly, weekly, and nightly. Two further questions were asked to describe responses other than "never": (d) Do these feelings just mentioned get better when you get up and start walking? (e) Do these feelings just mentioned disrupt your sleep? The questionnaire did not completely address all RLS diagnostic criteria put forth by the National Institutes of Health (NIH)[31]; notably it did not ask for the symptoms to be worse at night. As the circadian component of RLS is essential to the diagnosis, the term "RLS symptoms" is used rather than RLS.

RLS symptoms were split into 4 categories (Table S1, supplemental material). Category A (n = 186, observations = 360), likely RLS symptoms, was defined as response to (a) as weekly or more often, (d) yes, and (e) yes. The term RLS(A) is used to refer to this category. Category B (n = 190, observations = 384), possible RLS symptoms, was defined as response (a) monthly or more frequent, and (d) yes. Category B did not include members already in Category A. The term RLS(B) is used to refer to this category. Category C (n = 523, observations = 997), no RLS symptoms, was defined by response (a) as less than monthly and response (b) as either missing or less than monthly. The term RLS(C) is used to refer to this category. Remaining subjects (n = 171, observations = 321) were excluded from RLS symptom stratification (i.e., missing responses or responses that did not fit into categories A, B, or C).

RLS symptom categories were used as a single ordinal variable. As few differences were found between categories A and B, notably in terms of PLMI variable and association (Table S1), RLS A and B categories were most often combined in all analysis, although the effect of category A alone was also systematically studied. The term RLS(AB) is used to refer to the combination of these two categories.

PLM Phenotype

PSGs were processed for PLM using our described Stanford PLM automatic detector (S-PLMAD)[31] that uses manually

scored staging and respiratory event files in determining PLMI. The PLM detector was implemented in the SEV, a MATLAB toolbox for automating pattern recognition and biomarker classification algorithms in NPSG sleep cohorts.[31]

We define PLMI as the average number of PLMs occurring per hour of sleep and intermittent wake (i.e. wake after sleep onset [WASO]). PLMI is calculated automatically using a previously designed detection algorithm that was validated against manually scored data from 2 cohorts,[31] and which is briefly described here. First, the algorithm removes cardiac interference from the EMG channel using an adaptive filter that reduces false positive detections. Next, a two-pass noise floor calculation is performed and variable amplitude thresholds generated to account for changes in the baseline noise. Candidate LMs are identified where the filtered signal (i.e., adaptively cleaned and root-mean-square filtered) meets the amplitude and duration which fall in line with AASM Manual criteria for significant LM as described in the original manuscript.[31] LMs whose area under the curve is too small or that fall within the respiratory exclusion window of respiratory events described above are removed from candidacy. Remaining LMs are deemed significant and scored for PLM using AASM Manual criteria and inclusion of WASO.

A PLMI cutoff of 15 was used to distinguish between presence (PLMI ≥ 15) and absence (PLMI < 15) of the PLM phenotype (i.e., PLM+ or PLM−). Tables S1 and S2 (supplemental material) show the high association of PLMI with the presence of RLS symptoms, confirming the validity of our detector and the association of these phenotypes. The cutoff of 15 was used because OR were similar (~2.0) across PLMI indices cutoffs above 10, 15, and 30, and because population frequency was approximately 33%, a reasonable portion of the population. Further, a PLMI cutoff of 15 is frequently used clinically in older individuals. A cutoff of ≥ 5 would have meant including more than 50% of the population as affected. Results with this cutoff did not change substantially the results, but led to lower ORs.

Table 1 describes the WSC stratified by PLMI ≥ 15 and shows several additional PLM metrics that were automatically obtained using this algorithm in order to determine a suitable PLM phenotype. The periodicity index is the number of inter-PLM-movement intervals > 10 s divided by the total number of inter-movement intervals (i.e. the total LM count less one). It is based on the observation that RLS patients exhibit longer inter-movement intervals than non-RLS patients.[1] The PLM night ratio quantifies the circadian or diurnal effect observed in PLM[26,27] and is presented here as the PLM count from the first half of the PSG sleep study divided by the PLM count measured in the second half. The PLM ratio uses sleep onset to define a study's start, and total sleep time plus WASO to determine the study midpoint. PLM attrition is the per hour change in PLM as determined by linear regression.

Genotypes

Thirteen SNPs from 6 genes are examined. SNPs from BTBD9 on chromosome 6 include rs935271, rs9296249, and rs392809. TOX3/BC034767 (chromosome 16) SNPs include rs3104767, rs3104774, and rs3104788. Two genes from chromosome 2 are examined: MEIS1 (rs6710341, rs12469063, rs2300478), and "no gene" (rs6747972, a SNP located a

megabase away from MEIS1 but involved in its regulation). Also examined are chromosome 15 gene MAP2K5/SKOR1 (rs649469) and chromosome 9 gene PTPRD (rs4626664 and rs1975197). All 13 SNPs have previously been shown to increase susceptibility for RLS,[18] while BTBD9 SNP rs392809 has been shown to increase susceptibility for PLM apart from RLS.[22] Genotyping was performed on the MassARRAY system using MALDI-TOF mass spectrometry with the iPLEX Gold chemistry (Sequenom Inc, San Diego, CA, USA).

Analysis

PLM observations (more versus less than 15 PLM/h [PLM+ versus PLM−]) were first compared using t-test and χ^2 for basic demographic variables and other variables that could influence the phenotype (Table 1). These basic comparisons were used for descriptive purposes and to identify variables to potentially control for in subsequent models. Table 1 also compares allele frequencies by group and allelic OR derived for each SNP.

Following these basic comparisons, allelic ORs of each SNP were calculated using logistic regressions on repeated measure observations with allele dose of the RLS risk allele as a linear variable, and controlling for covariates identified in Table 1. This was done in 2 adjusted models predicting PLM, with and without additional adjustment of RLS symptoms (Table 2). The models are solved with generalized estimating equations (GEEs) using a Markov correlation assumption for repeated measures as implemented in GEEQBOX, a MATLAB toolbox.[33] To account for multiple observations, we used the Markov structure, a generalization of the first-order autoregressive that assumes the correlation between repeated measures is based on the timing between those measures (which can be unequal in the case of the WSC). Confidence intervals and P-values are determined using a robust estimate of the covariance matrix (i.e., *sandwich* method). Of notes, these results were similar using a *model-based* estimate, further confirming our choice of this correlation structure.

Finally, a linear trend test of each SNP on PLMI in repeated observations was done by linear regression and selected covariates, including RLS symptoms (ordinal categories, or considering likely RLS or likely and possible RLS as positive for RLS symptoms).

RESULTS

Prevalence and Associations of PLM in the Wisconsin Sleep Cohort

Prevalence of PLMI ≥ 15/h was 33% (Table 1). As expected, subjects with PLM were significantly older (about 4 years as a mean). They were also more frequently male (OR = 1.5) and significantly reported RLS symptoms—OR = 1.46 to 1.71, $P < 10^{-8}$ for RLS(AB) versus RLS(C)—more frequently. Finally, we found that these subjects had a shorter total sleep time (TST) and higher wake after sleep onset (WASO) ($P < 10^{-13}$ and 10^{-18}, respectively), possibly reflecting disturbed sleep.

Unadjusted SNP Associations with PLM

PLM+ versus PLM− revealed association for almost all SNPs (Table 1): rs9357271(T), rs9296249(T), rs3923809(A) for BTBD9 (OR = 1.42-1.46, strongest for rs3923809); rs3104767(G),

Table 1—Characteristics of the Wisconsin Sleep Cohort observations stratified by the presence of PLM.

	PLM+ (n = 797)	PLM− (n = 1,597)	All (n = 2,394)	PLM+ vs. PLM−
Demographics/Clinical				
Age	62.2 ± 0.30	58.4 ± 0.20	59.7 ± 0.17	**P < 10⁻¹⁶**
Sex, Male (%)	60.70%	50.70%	54.00%	**OR = 1.51, P < 10⁻⁵**
BMI (kg/m²)	31.5 ± 0.24 (762)	31.7 ± 0.19 (1570)	31.6 ± 0.15 (2332)	P = 0.538
AHI (events/h)	12.8 ± 0.56 (728)	12.5 ± 0.41 (1506)	12.6 ± 0.33 (2234)	P = 0.625
AHI > 15 (%)	29.0% (728)	27.5% (1506)	28.0% (2234)	OR = 1.08, P = 0.461
RLS(A) (%)	21.20%	15.50%	17.40%	**OR = 1.46, P < 10⁻³**
RLS(B) (%)	23.10%	16.20%	18.50%	**OR = 1.55, P < 10⁻⁴**
RLS(AB) (%)	44.30%	31.70%	35.90%	**OR = 1.71, P < 10⁻⁸**
RLS− (%)	40.30%	53.10%	48.80%	**OR = 0.60, P < 10⁻⁸**
Polysomnogram				
Hours evaluated	7.54 ± 0.03	7.54 ± 0.02	7.54 ± 0.02	P = 0.895
TST (h)	5.85 ± 0.04	6.20 ± 0.02	6.08 ± 0.02	**P < 10⁻¹³**
Stage 1 (%)	11.5 ± 0.24	10.1 ± 0.17	10.6 ± 0.14	**P < 10⁻⁵**
Stage 2 (%)	66.9 ± 0.33	65.6 ± 0.23	66.0 ± 0.19	**P = 0.002**
SWS (%)	6.33 ± 0.25	7.54 ± 0.20	7.13 ± 0.16	**P < 10⁻³**
REM (%)	15.1 ± 0.22	16.5 ± 0.16	16.0 ± 0.13	**P < 10⁻⁶**
WASO (h)	1.45 ± 0.03	1.13 ± 0.02	1.24 ± 0.02	**P < 10⁻¹⁸**
Medication				
RLS symptom aggravators	38.50%	34.60%	35.90%	OR = 1.19, P = 0.057
RLS symptom inhibitors	12.20%	10.60%	11.20%	OR = 1.16, P = 0.264
Periodic Leg Movement				
PLMI	38.93 ± 0.83	5.15 ± 0.13	16.40 ± 0.44	n.a.
Periodicity Index	0.66 ± 0.00	0.25 ± 0.00 (1447)	0.39 ± 0.01 (2244)	n.a.
PLM stage 1	20.84 ± 0.92	2.56 ± 0.13 (1447)	9.05 ± 0.38 (2244)	n.a.
PLM stage 2	163.92 ± 4.77	13.49 ± 0.61 (1447)	66.92 ± 2.31 (2244)	n.a.
PLM SWS	16.22 ± 1.04	1.36 ± 0.14 (1447)	6.64 ± 0.41 (2244)	n.a.
PLM REM	9.32 ± 0.65	2.55 ± 0.17 (1447)	4.95 ± 0.27 (2244)	n.a.
PLM WASO	83.02 ± 2.57	23.00 ± 0.53 (1447)	44.32 ± 1.15 (2244)	n.a.
PLM night ratio (first half/second half)	3.22 ± 0.23 (777)	1.80 ± 0.09 (1013)	2.41 ± 0.11 (1790)	n.a.
Attrition (PLM/hour)	-5.00 ± 0.21	-0.37 ± 0.03 (1595)	-1.91 ± 0.08 (2392)	n.a.
Heart rate (delta)	22.61 ± 0.60	34.81 ± 0.63 (1447)	30.48 ± 0.48 (2244)	n.a.
Heart rate (slope)	2.31 ± 0.07	3.52 ± 0.07 (1447)	3.09 ± 0.05 (2244)	n.a.
SNP				
BTBD9(6)				
rs9357271(T)	0.811 (734)	0.752 (1533)	0.771 (2267)	**OR = 1.42, P < 10⁻⁵**
rs9296249(T)	0.811 (695)	0.747 (1434)	0.768 (2129)	**OR = 1.45, P < 10⁻⁵**
rs3923809(A)	0.744 (728)	0.666 (1525)	0.691 (2253)	**OR = 1.46, P < 10⁻⁶**
TOX3/BC034767(16)				
rs3104767(G)	0.610 (738)	0.551 (1535)	0.571 (2273)	**OR = 1.27, P < 10⁻³**
rs3104774(G)	0.618 (731)	0.554 (1513)	0.575 (2244)	**OR = 1.31, P < 10⁻⁴**
rs3104788(T)	0.621 (743)	0.554 (1529)	0.576 (2272)	**OR = 1.32, P < 10⁻⁴**
MEIS1(2)				
rs6710341(G)	0.143 (735)	0.140 (1532)	0.141 (2267)	OR = 1.02, P = 0.820
rs12469063(G)	0.271 (737)	0.223 (1529)	0.239 (2266)	**OR = 1.30, P < 10⁻³**
rs2300478(G)	0.266 (713)	0.224 (1464)	0.238 (2177)	**OR = 1.26, P = 0.002**
Intergenic region(2)				
rs6747972(A)	0.441 (740)	0.441 (1533)	0.441 (2273)	OR = 1.00, P = 0.971
MAP2K5/SKOR1(15)				
rs6494696(G)	0.719 (735)	0.669 (1523)	0.685 (2258)	**OR = 1.27, P < 10⁻³**
PTPRD(9)				
rs4626664(A)	0.150 (735)	0.155 (1514)	0.153 (2249)	OR = 0.96, P = 0.628
rs1975197(A)	0.176 (732)	0.145 (1527)	0.155 (2259)	**OR = 1.26, P = 0.006**

PLM+ is defined as a PLMI ≥ 15, PLM− as PLMI < 15. Data are mean ± standard error of mean or percent with observation count (n) shown in parenthesis. Single nucleotide polymorphisms (SNP) are grouped according to gene (chromosome in parenthesis) and with the risk allele in parenthesis odd ratios are calculated using the risk allele for PLM phenotype. TST, total sleep time; BMI, body mass index; AHI, apnea hypopnea index; SWS, slow wave sleep; REM, rapid eye movement sleep; WASO, wake after sleep onset; RLS, restless legs syndrome; PLMI, periodic leg movement index; RLS(A), RLS symptoms likely; RLS(B), RLS symptoms possible; RLS(AB), RLS(A) and RLS(B) combined; RLS(C), no RLS symptoms. Statistical significance (bold) was set to P < 0.0083 after Bonferroni correction for multiple testing.

Table 2—Associations of various SNPs with PLMs (PLMI ≥ 15 versus PLMI < 15)

	PLM+ (n = 797) vs Controls (PLM−, n = 1,597) (Adjust for gender, age, TST, and WASO)		PLM+ (n = 674) vs Controls (PLM−, n = 1,355) (Adjust for gender, age, TST, WASO, and RLS symptoms)	
	OR	P	OR	P
BTBD9(6)				
rs9357271(T)	1.45 (1.27, 1.63)	**3.63 × 10⁻⁵**	1.38 (1.18, 1.57)	**0.00114**
rs9296249(T)	1.49 (1.31, 1.67)	**1.36 × 10⁻⁵**	1.43 (1.23, 1.62)	**3.32 × 10⁻⁴**
rs3923809(A)	1.57 (1.40, 1.74)	**1.76 × 10⁻⁷**	1.46 (1.28, 1.64)	**4.70 × 10⁻⁵**
TOX3/BC034767(16)				
rs3104767(G)	1.29 (1.14, 1.44)	**6.24 × 10⁻⁴**	1.30 (1.14, 1.46)	**0.00114**
rs3104774(G)	1.32 (1.17, 1.46)	**1.98 × 10⁻⁴**	1.34 (1.18, 1.50)	**2.98 × 10⁻⁴**
rs3104788(T)	1.33 (1.19, 1.48)	**1.08 × 10⁻⁴**	1.34 (1.18, 1.50)	**2.98 × 10⁻⁴**
MEIS1(2)				
rs6710341(G)	1.03 (0.82, 1.24)	0.785	1.05 (0.83, 1.28)	0.645
rs12469063(G)	1.33 (1.16, 1.50)	**0.00118**	1.29 (1.10, 1.48)	**0.00738**
rs2300478(G)	1.28 (1.10, 1.45)	**0.0056**	1.24 (1.05, 1.43)	0.0287
Intergenic region(2)				
rs6747972(A)	1.04 (0.89, 1.20)	0.575	1.01 (0.85, 1.18)	0.863
MAP2K5/SKOR1(15)				
rs6494696(G)	1.24 (1.07, 1.41)	0.0129	1.13 (0.94, 1.31)	0.201
PTPRD(9)				
rs4626664(A)	0.95 (0.75, 1.15)	0.63	0.97 (0.75, 1.18)	0.746
rs1975197(A)	1.30 (1.11, 1.49)	**0.00605**	1.35 (1.15, 1.55)	**0.00388**
Multivariate Model				
BTBD9 rs3923809(A)	1.65 (1.47, 1.82)	**1.52 × 10⁻⁸**	1.51 (1.32, 1.69)	**1.21 × 10⁻⁵**
TOX3 rs3104788(T)	1.35 (1.20, 1.50)	**8.95 × 10⁻⁵**	1.34 (1.17, 1.50)	**5.10 × 10⁻⁴**
MEIS1 rs12469063(G)	1.39 (1.21, 1.57)	**2.70 × 10⁻⁴**	1.34 (1.15, 1.53)	**0.00299**
MAP2K5/SKOR1 rs6494696(G)	1.24 (1.07, 1.41)	0.0133	1.13 (0.94, 1.32)	0.197
PTPRD rs1975197(A)	1.31 (1.12, 1.50)	**0.00626**	1.34 (1.14, 1.55)	**0.00498**

SNP odds ratios (OR) are shown for PLM+ versus PLM− using two adjusted logistic regression models. PLM+ is defined as a PLMI ≥ 15; PLM− as PLMI < 15. Data shown after adjustment for gender, age, total sleep time (TST), and duration of wake after sleep onset (WASO) (left), and then with additional adjusting for RLS symptoms (right, which reduces the sample size). Statistical significance (bold) was set to $P < 0.0083$ after Bonferroni correction for multiple testing.

rs3104774(G), rs3104788(T) for TOX3/BC034767 (OR = 1.27-1.32, strongest for rs3104788); rs12469063(G), and rs2300478(G) for MEIS1 (OR = 1.25-1.30, strongest for rs12469063 but more significant for rs2300478); rs6494696(G) for MAP2K5/SKOR1 (OR = 1.27) and rs1975197(A) for PTPRD (OR = 1.26). The SNP in the intergenic region of Chromosome 2 known to regulate MEIS1 was not significantly associated. The top association and allelic directions revealed here with rs3923809(A) in BTBD9; rs3104788(T) in TOX3/BC034767; rs2300478(G) in MEIS1; and rs1975197(A) in PTPRD are all in the same direction as those associated with these loci in RLS.[18] Regarding MAP2K5/SKOR1, the highest reported SNP in the Winkelmann study,[18] rs12593813(G) was not tested, but we found a similarly high association with rs6494696(G) a SNP with almost complete linkage disequilibrium (LD) with it across ethnic groups ($r^2 = 0.91$).

SNP Associations with PLM Adjusted for Age, Sex, and Sleep Disturbances

Categorical PLM associations with the various SNPs had similar effect sizes and P values to unadjusted models (Table 2). Association was most remarkable at rs39238809(A) when adjusted for age, sex, TST, and WASO (Table 2). In multivariate analysis where all significant SNPs (one per locus) except rs6747972(A) (no gene, a region presumably regulating MEIS1 but never significant in any of our models) were added in addition to age, sex, TST, and WASO, significance was improved in most cases, although rs6747972(A) remained nonsignificant (Table 2). Finally, the effects of each SNP (as a linear dose variable) on PLM index were tested using a linear regression models with adjustment of covariates with very similar effects (Table 3).

SNP Associations with RLS Symptoms Adjusted for Covariates

SNP associations with RLS were next tested after adjustment of covariates detected in Table S1, with and without further adjustment with PLM (Table S2). The presence of RLS symptoms in these models was tested using multiple definitions for RLS, including RLS(A) and RLS(AB) versus RLS(C). Surprisingly, none of the SNPs were found to be associated with RLS symptoms in our cohort (Table S2A and S2B).

Table 3—Associations of various SNPs with PLMs using PLMI as a linear outcome

	PLMI (n = 2,394) Adjustment without RLS symptoms		PLMI (n = 2,029) Adjustment includes RLS symptoms	
	$\hat{\beta}$	P	$\hat{\beta}$	P
BTBD9(6)				
rs9357271(T)	3.01	**7.02×10^{-5}**	2.49	**0.00123**
rs9296249(T)	3.15	**2.98×10^{-5}**	2.8	**2.48×10^{-4}**
rs3923809(A)	3.21	**3.52×10^{-6}**	2.51	**2.43×10^{-4}**
TOX3/BC034767(16)				
rs3104767(G)	1.66	**0.00783**	1.47	0.0245
rs3104774(G)	1.63	**0.00823**	1.51	0.0207
rs3104788(T)	1.73	**0.00554**	1.59	0.0152
MEIS1(2)				
rs6710341(G)	-0.55	0.562	0.16	0.875
rs12469063(G)	3.04	**6.79×10^{-4}**	2.72	**0.00165**
rs2300478(G)	2.77	**0.0025**	2.36	**0.00751**
Intergenic region(2)				
rs6747972(A)	1.02	0.156	1.02	0.171
MAP2K5/SKOR1(15)				
rs6494696(G)	1.59	0.0354	1.15	0.125
PTPRD(9)				
rs4626664(A)	-0.85	0.365	-0.46	0.647
rs1975197(A)	1.44	0.0967	2.02	0.0257
Multivariate Model				
BTBD9 rs3923809(A)	3.51	**3.58×10^{-7}**	2.79	**3.89×10^{-5}**
TOX3 rs3104788(T)	1.97	**0.00177**	1.69	0.0105
MEIS1 rs12469063(G)	3.16	**4.09×10^{-4}**	2.73	**0.00146**
MAP2K5/SKOR1 rs6494696(G)	1.66	0.0264	1.21	0.101
PRPRD rs1975197(A)	1.29	0.126	1.8	0.0405

Coefficient estimates ($\hat{\beta}$, in movements per hour) and associated P values for all SNPs are provided after adjusting for age, gender, AHI, BMI, TST, and WASO duration (left), and then after additional adjustment for RLS effecting medications and RLS symptoms (i.e., RLS+ or RLS−, right). Statistical significance (bold) was set to P < 0.0083 after Bonferroni correction for multiple testing of six genes.

SNP Associations with PLM after Adjustment for the Presence of RLS Symptoms

Significant SNP associations with PLM were next tested after adjustment for RLS symptoms (Tables 2 and 3). The presence of RLS in these models was tested using multiple definitions for RLS, including RLS(A) and RLS(AB), and using RLS symptom categories as an ordinal variable, as presented in Tables 2 and 3. In all cases, the presence of RLS in the model only modestly reduced strong SNP associations with PLMs.

The sample was next stratified by PLM and RLS status (Table S3, supplemental material). Subjects reporting no RLS symptoms and with a low PLM index ≤ 15/h (PLM−) constituted the reference group. Subjects with possible RLS symptoms and PLM index > 15/h (PLM+), subjects with possible RLS symptoms and PLM−, and subjects with PLM+ and possible RLS were compared to this group, with adjustment of covariates. The same risk alleles previously reported for RLS and found to be associated with PLM in Tables 1-3 were used here. Similar models were also run with RLS(A) versus RLS(C), with similar results, although power was decreased due to smaller sample size.

As expected, subjects with both RLS and PLM showed stronger association with the BTBD9 locus, and similar associations with TOX3/BC034767 (Table S3). As these two loci together with MEIS1 are the most strongly associated in this study (see Tables 2-4), the result was expected. In subjects with PLM but no RLS symptoms, associations with BTBD9 and TOX3/BC034767 were present but with a reduced effect. Surprisingly however, MEIS1 SNPs were more associated in subjects with PLM without RLS symptoms, suggesting this locus may regulate PLM more strongly than RLS symptoms. Another unusual observation concerned the TOX3/BCO34767 locus, where PLM-associated SNP alleles were weakly protective in subjects with RLS and no PLM, an unusual combination that many researchers may consider a misdiagnosis.

Comparison of the Effects of These Loci on PLMs (This Study) versus RLS (Prior Studies)

Table 4 contrasts the effects of the SNPs most associated with RLS in recent studies[18,21] with corresponding data obtained for PLM as typed in this study. For better comparison, models obtained in prior tables for PLM most associated SNPs were recalculated using the previously reported top RLS markers rather than our top PLM markers, if applicable. As these SNPs

Table 4—Comparative effects of loci on PLM (this study) and RLS (from Winkelmann et al., 2011)

Gene	Comparison marker	Allele	OR SNP alone*	OR multi loci*	OR SNP alone RLS†
BTBD9	rs3923809	T	1.45 (1.27, 1.63)	1.70 (1.52, 1.87)	1.47 (1.35, 1.47)
TOX3/BC034767	rs3104767	G	1.29 (1.14, 1.44)	1.33 (1.18, 1.49)	1.33 (1.25, 1.43)
MEIS1	rs2300478	G	1.28 (1.10, 1.45)	1.34 (1.16, 1.52)	1.68 (1.57, 1.81)
Intergenic region	rs6747972	A	1.04 (0.89, 1.20)	1.05 (0.89, 1.20)	1.23 (1.16, 1.31)
MAP2K1/SKOR1	rs6494696	G	1.24 (1.07, 1.41)	1.23 (1.06, 1.40)	1.41 (1.32, 1.52)
PTPRD	rs1975197	A	1.30 (1.11, 1.49)	1.29 (1.10, 1.49)	1.29 (1.19, 1.40)

Effects for each locus on periodic leg movements (this study) for SNPs that are most closely comparable/linked to top RLS markers reported by Winkelmann et al., 2011. *Models rerun with top marker for RLS Winkleman et al., 2011 study, when possible. †From Winkelmann et al., 2011.

are identical or in high LD ($r^2 > 0.9$) with each other however, OR reported for each locus are essentially identical to those reported in prior tables and all these can be considered as proxies of each other.

OR reported for RLS are derived from Winkelmann et al. (2007)[21] for rs3923809 in BTBD9 and from Winkelmann et al. (2011)[18] for other loci, namely rs3104767 for TOX3/BC034767, rs2300478 for MEIS1, rs6747972 for the intergenic region suggested to regulate MEIS1, rs6494696 for MAP2K1/SKOR1, and rs1975197 for PTPRD. The rationale for using rs3923809 data from the 2007 study[21] rather than the 2011 bigger sample study[18] is that it is known rs3923809 is the most associated BTBD9 marker,[21] and only rs9357271, a marker partially associated with rs3923809 ($r^2 = 0.429$) was typed in the larger 2011 sample.[18]

As can be seen effects of rs9357271 are similar for PLM and RLS, but the effect of our top SNP (rs3923809) is stronger for PLM. Ranking the effects of the same SNPs on RLS and PLM, the effects of the various loci on RLS were: MEIS1 > BTBD9 ≥ MAP2K1/SCOR1 > TOX3/BC034767 ≥ PTPRD and intergenic region, while ranking for PLM was BTBD9 ≥ TOX3/BC034767 and MEIS1 > PTPRD and MAP2K1/SCOR1.

DISCUSSION

In this study, we found that all loci known to be associated with RLS in prior studies were also associated with PLM except for rs6747972, an SNP located in an intergenic region distal of MEIS1 that has been suggested to modulate this gene. The stronger association with PLM was found with BTBD9 [rs3923809(A) OR = 1.57 in the single SNP model and 1.65 in the multi SNP model]. A similar association with PLM (PLMS > 5/h) was reported in Stefansson et al.[22] for rs3923809(A), a marker also associated with RLS. In addition to this observation, we also found that MEIS1 [rs12469063(G) OR = 1.33 and 1.39], TOX3/BC034767 [rs3104788(T) OR = 1.33 and 1.35], PTPRD [rs1975197(A) OR = 1.30 and 1.31] and MAP2K1/SKOR1 [rs6494696(G) OR = 1.24] loci were also associated with PLM. Among the markers tested for these loci, the most associated markers were similar to those reported for RLS, except for MEIS1 (Table 4). At this locus, rs12469063 was slightly more associated with PLM than rs2300478. As the two markers are in high linkage disequilibrium ($r^2 = 0.93$), however, this is likely chance variation. These results strongly suggest that RLS and PLM are part of the same

disease continuum, with many more patients having PLMs and no significant RLS.

In contrast to these results, none of the 13 SNPs were found to be associated with "RLS symptoms" in the WSC, whether defined narrowly or more broadly using our questionnaire (Tables S1-S3), a result we believe is due to the poor ascertainment of RLS in this cohort. Indeed, RLS was poorly defined in this study, as we did not ask whether symptoms were worse in the evening, an important criterion of all classifications. The very high percentage of subjects reporting RLS symptoms (21% for likely and 41% for possible) and the lack of difference across gender of prevalence also argue in favor of a poorly defined phenotype, even in this older cohort expected to have higher prevalence of RLS. This problem notwithstanding, we believe that subjects reporting no symptoms are likely to be genuinely RLS free, and thus worth investigation. Further, RLS symptoms in this study were strongly associated with PLM, suggesting some validity (Table S1).

Interestingly, the ranking of these loci in term of effects on PLMs versus previously published effects on RLS somewhat differed, even when the same markers or proxies were used (Table 4), although this comparison suffers from the fact populations are different. This issue notwithstanding, MEIS1 and MAP2K1/SKOR1, for example, may have stronger effects on RLS than other loci, whereas BTBD9, TOX3/BC034767, and PTRD effects on RLS and PLM were similar. In our own data, after stratification of subjects by PLM and RLS symptom categories, we found that in most cases effects were stronger in subjects with PLM plus RLS symptoms, although some effect remained in subjects with PLM without RLS reports (Table S3). An outlier, however, was MEIS1, which surprisingly and opposite to previously published papers, had stronger effects of PLM without RLS. Overall, these results suggest that some loci may have stronger or equivalent effects on sensory versus motor component of the PLM-RLS disease spectrum.

In conclusion, we found that PLMs are strongly associated with loci known to be associated with RLS. Further fine mapping studies and genetic studies in subjects with PLM alone, or of sensory versus motor complaints in RLS patients, may reveal differential effects of these various loci.

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Table S1—Description of WSC participants stratified by RLS symptom category

	RLS(A) (n = 188, o = 417)	RLS(B) (n = 192, o = 443)	RLS(AB) (n = 380, o = 860)	RLS(C) (n = 535, o = 1,169)
Demographics/Clinical				
Age	58.8 ± 0.62 (188)	58.3 ± 0.64 (191)	58.6 ± 0.44 (379)	59.0 ± 0.34 (535)
Sex, Male (%)	47.9% (188)	51.8% (191)	49.9% (379)	55.2% (534)
BMI (kg/m²)	33.7 ± 0.57 (186)	30.3 ± 0.44 (191)	32.0 ± 0.37 (377)	31.3 ± 0.30 (532)
AHI (events/h)	15.6 ± 1.36 (179)	13.2 ± 1.15 (187)	14.4 ± 0.89 (366)	12.4 ± 0.59 (516)
AHI > 15 (%)	33.5% (179)	28.9% (187)	31.1% (366)	27.5% (516)
PLMI (PLM/h)	18.7 ± 1.79 (188)	15.3 ± 1.20 (191)	17.0 ± 1.07 (379)	10.8 ± 0.64 (535)
Polysomnogram				
TST (h)	5.98 ± 0.07 (188)	6.13 ± 0.07 (191)	6.06 ± 0.05 (379)	6.11 ± 0.04 (535)
Stage 1 (%)	11.4 ± 0.5 (188)	9.88 ± 0.35 (191)	10.6 ± 0.3 (379)	10.6 ± 0.3 (535)
Stage 2 (%)	66.3 ± 0.5 (188)	66.5 ± 0.5 (191)	66.4 ± 0.4 (379)	66.6 ± 0.3 (535)
SWS (%)	7.25 ± 0.45 (173)	8.10 ± 0.47 (181)	7.69 ± 0.33 (354)	7.23 ± 0.27 (492)
REMS (%)	16.0 ± 0.4 (186)	16.2 ± 0.4 (190)	16.1 ± 0.3 (376)	16.3 ± 0.2 (533)
WASO (h)	1.35 ± 0.06 (188)	1.19 ± 0.04 (191)	1.27 ± 0.04 (379)	1.22 ± 0.03 (535)
Medication				
RLS symptom aggravators	40.00%	37.50%	38.70%	32.40%
RLS symptom inhibitors	15.60%	11.10%	13.30%	8.60%
SNP*				
BTBD9(6)				
rs9357271(T)	0.793 (179)	0.798 (178)	0.796 (357)	0.765 (493)
rs9296249(T)	0.787 (167)	0.796 (169)	0.792 (336)	0.761 (464)
rs3923809(A)	0.723 (177)	0.718 (179)	0.721 (356)	0.680 (490)
TOX3/BC034767(16)				
rs3104767(G)	0.581 (179)	0.592 (180)	0.586 (359)	0.569 (495)
rs3104774(G)	0.584 (179)	0.592 (180)	0.588 (359)	0.575 (481)
rs3104788(T)	0.586 (180)	0.589 (180)	0.588 (360)	0.575 (495)
MEIS1(2)				
rs6710341(G)	0.120 (179)	0.143 (178)	0.132 (357)	0.148 (495)
rs12469063(G)	0.237 (179)	0.219 (180)	0.228 (359)	0.250 (494)
rs2300478(G)	0.236 (174)	0.230 (172)	0.233 (346)	0.247 (476)
Intergenic region (2)				
rs6747972(A)	0.444 (180)	0.475 (179)	0.460 (359)	0.436 (494)
MAP2K5/SKOR1(15)				
rs6494696(G)	0.737 (177)	0.648 (179)	0.692 (356)	0.693 (492)
PTPRD(9)				
rs4626664(A)	0.173 (179)	0.137 (179)	0.155 (358)	0.163 (489)
rs1975197(A)	0.161 (177)	0.154 (179)	0.157 (356)	0.161 (492)

See text for full description of each RLS category. RLS(A), subject likely have RLS symptoms; RLS(B), subjects probably have RLS symptoms; and RLS(C), subjects have no RLS symptoms. RLS categories are compared using *t*-test or χ^2 with statistical significance set at 0.05 (bold). Data are shown as mean ± standard error of mean with patient count (n) show in parenthesis. *SNP are listed according to gene (chromosome in parenthesis) and with the risk allele in parenthesis odd ratios are calculated using the risk allele for PLM phenotype. Definition of terms: Total sleep time (TST); Body mass index (BMI); Apnea hypopnea index (AHI); Slow wave sleep (SWS); Rapid eye movement sleep (REMS); Wake after sleep onset (WASO); Patient count (n); Observation count (o); Restless legs syndrome (RLS); Periodic leg movement index (PLMI); Single nucleotide polymorphism (SNP).

Table S1 continues on the following page

Table S1 (*continued*)—Description of WSC participants stratified by RLS symptom category

	RLS(A) vs RLS(C)	RLS(B) vs RLS(C)	RLS(AB) vs RLS(C)	P (ANOVA)
Demographics				
Age	P = 0.700	P = 0.330	P = 0.382	P = 0.592
Sex, Male (%)	OR = 0.74, P = 0.081	OR = 0.87, P = 0.417	OR = 0.81, P = 0.109	χ^2 = 3.17, P = 0.075
BMI (kg/m²)	$P < 10^{-3}$	P = 0.064	P = 0.144	**$P < 10^{-5}$**
AHI (events/h)	P = 0.032	P = 0.535	P = 0.065	P = 0.050
AHI > 15 (%)	OR = 1.33, P = 0.128	OR = 1.07, P = 0.723	OR = 1.19, P = 0.242	χ^2 = 2.33, P = 0.127
PLMI (PLM/h)	$P < 10^{-4}$	$P < 10^{-3}$	**$P < 10^{-6}$**	**$P < 10^{-6}$**
Polysomnogram				
TST (h)	P = 0.126	P = 0.819	P = 0.391	P = 0.214
Stage 1 (%)	P = 0.112	P = 0.132	P = 0.818	P = 0.049
Stage 2 (%)	P = 0.601	P = 0.791	P = 0.622	P = 0.868
SWS (%)	P = 0.968	P = 0.105	P = 0.279	P = 0.229
REM (%)	P = 0.467	P = 0.805	P = 0.529	P = 0.741
WASO (h)	P = 0.028	P = 0.621	P = 0.231	P = 0.030
Medication				
RLS symptom aggravators	**OR = 1.39, P = 0.005**	OR = 1.25, P = 0.056	**OR = 1.32, P = 0.003**	**χ^2 = 9.26, P = 0.002**
RLS symptom inhibitors	**OR = 1.97, $P < 10^{-4}$**	OR = 1.33, P = 0.121	**OR = 1.63, $P < 10^{-3}$**	**χ^2 = 16.27, $P < 10^{-4}$**
SNP*				
BTBD9(6)				
rs9357271(T)	OR = 1.18, P = 0.269	OR = 1.21, P = 0.202	OR = 1.20, P = 0.132	χ^2 = 2.29, P = 0.130
rs9296249(T)	OR = 1.16, P = 0.323	OR = 1.23, P = 0.189	OR = 1.19, P = 0.145	χ^2 = 2.19, P = 0.139
rs3923809(A)	OR = 1.23, P = 0.128	OR = 1.20, P = 0.180	OR = 1.22, P = 0.071	χ^2 = 3.29, P = 0.070
TOX3/BC034767(16)				
rs3104767(G)	OR = 1.05, P = 0.686	OR = 1.10, P = 0.450	OR = 1.08, P = 0.466	χ^2 = 0.62, P = 0.433
rs3104774(G)	OR = 1.04, P = 0.770	OR = 1.07, P = 0.581	OR = 1.05, P = 0.596	χ^2 = 0.33, P = 0.568
rs3104788(T)	OR = 1.05, P = 0.709	OR = 1.06, P = 0.642	OR = 1.05, P = 0.598	χ^2 = 0.28, P = 0.594
MEIS1(2)				
rs6710341(G)	OR = 0.78, P = 0.186	OR = 0.96, P = 0.811	OR = 0.87, P = 0.325	χ^2 = 1.76, P = 0.185
rs12469063(G)	OR = 0.93, P = 0.636	OR = 0.84, P = 0.246	OR = 0.89, P = 0.303	χ^2 = 1.38, P = 0.241
rs2300478(G)	OR = 0.94, P = 0.677	OR = 0.91, P = 0.523	OR = 0.93, P = 0.507	χ^2 = 0.48, P = 0.491
Intergenic region(2)				
rs6747972(A)	OR = 1.03, P = 0.788	OR = 1.17, P = 0.208	OR = 1.10, P = 0.338	χ^2 = 1.59, P = 0.207
MAP2K5/SKOR1(15)				
rs6494696(G)	OR = 1.24, P = 0.118	OR = 0.82, P = 0.117	OR = 1.00, P = 0.976	χ^2 = 6.66, P = 0.010
PTPRD(9)				
rs4626664(A)	OR = 1.08, P = 0.644	OR = 0.82, P = 0.251	OR = 0.95, P = 0.675	χ^2 = 1.94, P = 0.164
rs1975197(A)	OR = 1.00, P = 0.984	OR = 0.95, P = 0.758	OR = 0.98, P = 0.856	χ^2 = 0.11, P = 0.745

See text for full description of each RLS category. RLS(A), subject likely have RLS symptoms; RLS(B), subjects probably have RLS symptoms; and RLS(C), subjects have no RLS symptoms. RLS categories are compared using *t*-test or χ^2 with statistical significance set at 0.05 (bold). Data are shown as mean ± standard error of mean with patient count (n) show in parenthesis. *SNP are listed according to gene (chromosome in parenthesis) and with the risk allele in parenthesis odd ratios are calculated using the risk allele for PLM phenotype. Definition of terms: Total sleep time (TST); Body mass index (BMI); Apnea hypopnea index (AHI); Slow wave sleep (SWS); Rapid eye movement sleep (REMS); Wake after sleep onset (WASO); Patient count (n); Observation count (o); Restless legs syndrome (RLS); Periodic leg movement index (PLMI); Single nucleotide polymorphism (SNP).

Table S2A—SNP odds ratios (OR) for RLS(A) versus RLS(C) and for PLM+ versus PLM− with two model adjustments each.

	Adjust for use of RLS affecting medication		Adjust for RLS effecting medication and PLMI	
RLS(A) (n = 417) versus RLS(C) (n = 1,169)	**OR**	**P**	**OR**	**P**
BTBD9(6)				
rs9357271(T)	1.17 (0.90, 1.44)	0.265	1.12 (0.85, 1.39)	0.420
rs9296249(T)	1.16 (0.89, 1.43)	0.273	1.10 (0.83, 1.37)	0.476
rs3923809(A)	1.28 (1.04, 1.52)	0.047	1.22 (0.98, 1.46)	0.103
TOX3/BC034767(16)				
rs3104767(G)	1.03 (0.83, 1.23)	0.77	1.01 (0.80, 1.21)	0.954
rs3104774(G)	1.00 (0.80, 1.21)	0.967	0.98 (0.77, 1.19)	0.847
rs3104788(T)	1.01 (0.81, 1.21)	0.927	0.98 (0.78, 1.19)	0.872
MEIS1(2)				
rs6710341(G)	0.77 (0.45, 1.09)	0.104	0.78 (0.46, 1.09)	0.115
rs12469063(G)	0.89 (0.64, 1.15)	0.383	0.87 (0.61, 1.12)	0.266
rs2300478(G)	0.90 (0.64, 1.16)	0.415	0.87 (0.61, 1.13)	0.292
Intergenic region(2)				
rs6747972(A)	1.00 (0.78, 1.22)	0.992	0.97 (0.75, 1.20)	0.822
MAP2K5/SKOR1(15)				
rs6494696(G)	1.32 (1.08, 1.57)	0.0263	1.28 (1.03, 1.53)	0.0489
PTPRD(9)				
rs4626664(A)	1.09 (0.81, 1.37)	0.547	1.10 (0.81, 1.38)	0.526
rs1975197(A)	0.96 (0.66, 1.25)	0.76	0.94 (0.65, 1.24)	0.702

RLS(A) versus RLS(C) is shown first adjusting for RLS effecting medication and then also adjusting for PLMI. Nothing reaches statistical significance, which is set to 0.0083 on account of the multiple testing of six genes.

Table S2B—SNP odds ratios (OR) are shown for RLS(AB) versus RLS(C) using two adjusted models each.

	Adjusted for use of RLS affecting medication		Adjusted for RLS effecting medication and PLMI	
RLS(AB) (n = 860) versus RLS(C) (n = 1,169)	**OR**	**P**	**OR**	**P**
BTBD9(6)				
rs9357271(T)	1.19 (0.99, 1.39)	0.0926	1.15 (0.94, 1.35)	0.194
rs9296249(T)	1.17 (0.97, 1.37)	0.121	1.13 (0.93, 1.33)	0.243
rs3923809(A)	1.22 (1.04, 1.41)	0.0339	1.17 (0.99, 1.36)	0.0907
TOX3/BC034767(16)				
rs3104767(G)	1.00 (0.84, 1.16)	1	0.98 (0.82, 1.14)	0.815
rs3104774(G)	0.98 (0.82, 1.14)	0.839	0.96 (0.80, 1.12)	0.651
rs3104788(T)	0.98 (0.82, 1.14)	0.802	0.96 (0.80, 1.12)	0.606
MEIS1(2)				
rs6710341(G)	0.92 (0.69, 1.16)	0.511	0.93 (0.69, 1.16)	0.526
rs12469063(G)	0.89 (0.70, 1.08)	0.218	0.85 (0.66, 1.05)	0.106
rs2300478(G)	0.91 (0.71, 1.10)	0.329	0.88 (0.68, 1.07)	0.193
Intergenic region(2)				
rs6747972(A)	1.08 (0.91, 1.25)	0.364	1.07 (0.90, 1.24)	0.426
MAP2K5/SKOR1(15)				
rs6494696(G)	0.99 (0.81, 1.17)	0.912	0.96 (0.78, 1.15)	0.689
PTPRD(9)				
rs4626664(A)	0.96 (0.74, 1.19)	0.74	0.97 (0.75, 1.20)	0.797
rs1975197(A)	0.92 (0.70, 1.14)	0.436	0.89 (0.67, 1.11)	0.312

RLS(AB) versus RLS(C) is shown first adjusting for RLS effecting medication and then also adjusting for PLMI. Nothing reaches the 0.0083 Bonferroni corrected threshold for statistical significance.

Table S3—Effects of SNPs on PLM stratified by RLS status

		RLS+ and PLM+ (n = 353) vs Controls (n = 848)		RLS+ and PLM− (n = 507) vs Controls (n = 848)		RLS− and PLM+ (n = 321) vs Controls (n = 848)	
		OR	P	OR	P	OR	P
BTBD9(6)							
rs9357271(T)		1.79 (1.50, 2.07)	6.04×10^{-5}	0.96 (0.73, 1.20)	0.742	1.04 (0.79, 1.29)	0.778
rs9296249(T)		1.77 (1.48, 2.06)	9.43×10^{-5}	0.98 (0.75, 1.21)	0.861	1.15 (0.90, 1.40)	0.273
rs3923809(A)		1.75 (1.49, 2.00)	2.16×10^{-5}	1.09 (0.87, 1.31)	0.448	1.32 (1.08, 1.56)	0.0254
TOX3/BC034767(16)							
rs3104767(G)		1.36 (1.15, 1.57)	0.00401	0.84 (0.65, 1.04)	0.0815	1.07 (0.85, 1.29)	0.534
rs3104774(G)		1.38 (1.17, 1.59)	0.0029	0.82 (0.63, 1.01)	0.0451	1.11 (0.89, 1.33)	0.347
rs3104788(T)		1.35 (1.14, 1.55)	0.00514	0.84 (0.65, 1.03)	0.0704	1.14 (0.92, 1.36)	0.233
MEIS1(2)							
rs6710341(G)		0.99 (0.69, 1.30)	0.969	0.87 (0.58, 1.16)	0.333	1.00 (0.70, 1.31)	0.987
rs12469063(G)		1.09 (0.84, 1.35)	0.484	0.89 (0.65, 1.13)	0.355	1.40 (1.15, 1.66)	0.00953
rs2300478(G)		1.07 (0.82, 1.33)	0.586	0.92 (0.68, 1.16)	0.51	1.34 (1.08, 1.61)	0.0288
Intergenic region(2)							
rs6747972(A)		1.17 (0.94, 1.39)	0.18	1.00 (0.79, 1.20)	0.973	0.87 (0.64, 1.11)	0.254
MAP2K5/SKOR1(15)							
rs6494696(G)		1.12 (0.88, 1.37)	0.349	0.91 (0.69, 1.13)	0.402	1.04 (0.79, 1.29)	0.733
PTPRD(9)							
rs4626664(A)		0.83 (0.54, 1.13)	0.22	0.96 (0.69, 1.22)	0.752	0.94 (0.64, 1.24)	0.701
rs1975197(A)		1.09 (0.80, 1.38)	0.552	0.82 (0.53, 1.10)	0.157	1.20 (0.91, 1.49)	0.219
Multivariate Model							
BTBD9	rs3923809(A)	1.78 (1.52, 2.05)	1.45×10^{-5}	1.06 (0.84, 1.28)	0.598	1.39 (1.14, 1.64)	0.00915
TOX3/BC034767	rs3104788(T)	1.34 (1.13, 1.56)	0.008	0.82 (0.62, 1.02)	0.0484	1.13 (0.91, 1.36)	0.284
MEIS1	rs12469063(G)	1.17 (0.91, 1.43)	0.245	0.87 (0.62, 1.12)	0.266	1.44 (1.17, 1.71)	0.0076
MAP2K5/SKOR1	rs6494696(G)	1.12 (0.87, 1.38)	0.358	0.91 (0.69, 1.14)	0.422	1.07 (0.81, 1.32)	0.63
PRPRD	rs1975197(A)	1.13 (0.82, 1.44)	0.428	0.84 (0.56, 1.12)	0.219	1.22 (0.93, 1.51)	0.173

This table shows SNP odds ratios (OR) for presence of RLS symptoms, RLS+ or RLS(AB), with PLMI ≥ 15 (PLM+) vs controls, RLS+ with PLMI ≤ 15 (PLM−) vs controls, and no RLS symptoms, RLS− or RLS(C), with PLM+ vs controls. The control group consists of subjects with RLS− and PLM− phenotypes for each analysis. BTBD9, TOX3/BCO34767 and MEIS1 genes show statistical significance that overlaps slightly between models. Covariates used in each case include RLS effecting medication, age, gender, TST, and WASO duration. Statistical significance is set to 0.0083 after Bonferroni correction for multiple testing.