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ORIGINAL ARTICLE

The genetic etiology of periodic limb movement in sleep

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Abstract

Study Objectives: Periodic limb movement in sleep is a common sleep phenotype characterized by repetitive leg movements that occur during or before sleep. We conducted a genome-wide association study (GWAS) of periodic limb movements in sleep (PLMS) using a joint analysis (i.e., discovery, replication, and joint meta-analysis) of four cohorts (MrOS, the Wisconsin Sleep Cohort Study, HypnoLaus, and MESA), comprised of 6843 total subjects.

Methods: The MrOS study and Wisconsin Sleep Cohort Study (N = 1745 cases) were used for discovery. Replication in the HypnoLaus and MESA cohorts (1002 cases) preceded joint meta-analysis. We also performed LD score regression, estimated heritability, and computed genetic correlations between potentially associated traits such as restless leg syndrome (RLS) and insomnia. The causality and direction of the relationships between PLMS and RLS was evaluated using Mendelian randomization.

Results: We found 2 independent loci were significantly associated with PLMS: rs113851554 ($p = 3.51 \times 10^{-12}$, $\beta = 0.486$), an SNP located in a putative regulatory element of intron eight of *MEIS1* (2p14); and rs9369062 ($p = 3.06 \times 10^{-22}$, $\beta = 0.2093$), a SNP located in the intron region of *BTBD9* (6p12); both of which were also lead signals in RLS GWAS. PLMS is genetically correlated with insomnia, risk of stroke, and RLS, but not with iron deficiency. Pleiotropy adjusted Mendelian randomization analysis identified a causal effect of RLS on PLMS.

Conclusions: Because PLMS is more common than RLS, PLMS may have multiple causes and additional studies are needed to further validate these findings.

Statement of Significance

This is the first study to explore the genetics of periodic limb movement in sleep (PLMS) and determine whether there is overlapping shared genetic architecture between PLMS and restless leg syndrome (RLS) and other sleep phenotypes. We found two genes, *BTBD9* and *MEIS1*, reached genome-wide significance in their association with PLMS. In addition, we also found a high correlation between PLMS and a genetic predisposition to stroke as well as RLS, and a relatively weak association with insomnia, and finally, using a Mendelian randomization approach, we found that RLS is causally associated with PLMS. Additional studies of this phenotype are warranted considering its high prevalence and the possibility that it could predispose to a variety of clinically significant cardiovascular outcomes.

Key words: periodic limb movements; genome-wide association study; Mendelian randomization; restless leg syndrome

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Introduction

Periodic limb movement in sleep (PLMS) is a common sleep phenotype characterized by repetitive leg movements that occur every 5 to 90 s before falling asleep or during sleep. The identification of periodic limb movement in sleep relies on polysomnography (PSG) using objective scoring criteria defined by the American Academy of Sleep Medicine (AASM) [1].

These leg movements are considered a sleep disorder when they occur excessively during sleep and are accompanied by sleep disturbances [2, 3]. In order to make the diagnosis of PLMS as a disorder (i.e., periodic limb movement disorder), the AASM scoring manual requires these four criteria be met: (1) PSG demonstrating highly stereotyped repetitive limb movements; (2) the number of movements per hour (defined by the periodic limb movement index [PLMI] ≥ 15/h for adults; (3) clinical sleep disturbance of any type and impaired daytime functioning; and (4) not explained by any medical, psychological, or substance abuse disorder [1]. Most often, movements are present independently of any subjective complaint and are an incidental finding of unknown significance.

From an epidemiologic perspective, PLMS increase with age, have no gender predominance, and occur less frequently in individuals of African ancestry compared with European [4, 5]. Prevalence of PLMS ranges between 4% and 11% in the general population [2, 6]. Although the etiology remains unknown, PLMS have also been associated with obstructive sleep apnea (notably after therapy with CPAP), narcolepsy, rapid-eye-movement (REM) behavioral disorder, uremia, spinal cord tumor, and ADHD [7]. It may also be observed as a side effect of certain medications [2, 8]. Most importantly, however, PLMS strongly co-occur with restless leg syndrome (RLS), and these conditions have overlapping risk factors, notably kidney disease and iron deficiency [7, 9–11].

PLMS are very common in middle age and older individuals. Furthermore, most individuals with PLMS do not complain of RLS. In a recent epidemiological study, 28.6% of the adults aged 40 or older (mean age 58.4) had PLMI > 15/h [2, 3, 7]. PLMS are temporally associated with strong increases in heart rate and blood pressure and, as mentioned above, may also be associated with sleep disruption [12–16]. PLMS have also been suggested to contribute to CVD mortality in patients without symptoms of RLS [12, 16–19].

RLS is a sensorimotor disorder characterized by uncomfortable sensations, an urge to move one's limbs that typically only occurs at rest and is more prominent at night [11]. The AAMS criteria for diagnosing RLS is: (1) strong and often overwhelming need or urge to move the legs that is often associated with abnormal, unpleasant, or uncomfortable sensations; (2) urge to move the legs and starts or get worse during rest or inactivity; (3) urge to move the legs is at least temporarily and partially or totally relieved by movements; (4) urge to move the legs starts or is aggravated in the evening or night; and (5) these 4 features are not due to any other medical or behavioral condition [2, 9].

RLS occurs in 7%–10% of the adult population and is twice as common in women. Although PLMS are observed in about 80%–90% of RLS patients, the presence of PLMS during a PSG is not necessary for RLS diagnosis [11, 12]. Evidence from both cross-sectional and longitudinal studies suggests that RLS is associated with hypertension and cardiovascular disease (CVD) [13–17]. RLS is also associated with depression and may occur less frequently in individuals of Asian and African ancestry [11, 18]. Iron deficiency, kidney disease (notably in hemodialysis), and pregnancy are strong predisposing factors for RLS [19].

To date, genome-wide association studies (GWAS) have focused primarily on RLS and less frequently on PLMS because RLS is more easily observable, requiring only a questionnaire or clinical interview, rather than a resource-intensive PSG [15]. Previous PLMS genetic studies had relatively small sample sizes, with the largest cohort of individuals with PLMS included only 2000 subjects, explaining the limitations of the previously identified genetic architecture associated with PLMS [2]. Loci involved in RLS have not shown functional associations with iron metabolism or dopaminergic transmission, two hypothesized pathophysiological factors in the disease [20]. Nonetheless, large scale studies have identified approximately 20 loci with strongest effects found in two transcription factors, *MEIS1* and *BTBD9*, followed by associations in the *MAP2K5/SKOR1* and *TOX3/BC034767* loci [21, 22]. Of note, strong *MEIS1* and *BTBD9* associations are also found in insomnia, though these findings may be confounded by the presence of undiagnosed RLS [23, 24]. Although candidate gene research suggests overlap in genetic associations between RLS and PLMS, there is a need for a systematic analysis of genetic associations of PLMS [25]. The purpose of this study was to examine the genetic etiology of PLMS in 6,843 individuals and determine if there was a genetic correlation between and other phenotypes. We also evaluated whether there was a causal and or directional relationship of RLS and PLMS through Mendelian Randomization (MR).

METHODS

Cohort descriptions

The study procedures were approved by each participating institution's Institutional Review Board and all participants provided informed consent, and all clinical experiments conformed to the principles outlined by the Declaration of Helsinki. The four cohorts included in this GWAS meta-analysis included: (1) the Wisconsin Sleep Cohort (WSC) study [10]; (2) Osteoporotic Fractures in Men Study (MrOS) [26]; (3) the HypnoLaus Study [27], and (4) the Multi-Ethnic Study of Atherosclerosis (MESA) [28]. All cohorts consisted primarily of individuals of European ancestry, with the exception of MESA. All four cohorts also were middle-aged to elderly individuals, with MESA and MrOS consisting of mostly individuals of middle or advanced age (over age 60). A PLMI ≥ 15 events/h of sleep was used as the cut-off criterion for defining a dichotomous phenotype ("PLMS+"), consistent with the International Classification of Sleep Disorders, 3rd edition [29].

Wisconsin Sleep Cohort (WSC) Study —The WSC is a longitudinal study of sleep habits and sleep disorders in the general population [30]. All employees of 5 Wisconsin state agencies, ages 30–60 years at study initiation, were mailed a survey on sleep habits, health, and demographics in 1988. Of the 6947 state employees who received the survey, 5091 (73%) completed and returned it. From these respondents, a sampling frame was constructed from which a stratified sample of 2884 individuals was recruited for an initial overnight protocol including polysomnography. There were 1545 individuals (53% of those invited) who agreed to come in for a baseline sleep study (the primary reason for non-participation was the burden of being away from home overnight). After baseline studies, participants were invited to return for repeat visits at approximate 4-year intervals.

Despite the availability of multiple polysomnograms per individual, in this study we tested association with the first polysomnogram for each individual. In contrast to other included studies, WSC included an in-lab continuous polygraphic recording (Polygraph model 78, Grass Instruments, Quincy, Mass.), in which leg movements were recorded from surface electromyography, along with other standard polysomnographic leads [30]. Due to the lack of manual leg movement annotations, this study utilized a previously validated, automated periodic limb movement detection algorithm, which relied on the 2007 AASM scoring criteria: a burst of electromyographic muscle activity exceeding 8 μV above baseline and then falling below 2 μV above baseline; duration between 0.5 and 10 s; inter-movement interval of 5-90 s; a series of four or more leg movements; movements are unrelated to sleep-disordered breathing respiratory events [31]. This algorithm has been applied and validated in multiple other studies of PLM clinical outcomes and genetics [2, 25].

Osteoporotic Fractures in Men Study (MrOS) : MrOS was a prospective, observational study originally designed to determine risk factors for osteoporosis and fractures. The study recruited 5994 men who were 65-year-old (or older) between 2000 and 2002, residing in six U.S. communities [32]. From 2003 through 2005, 3135 MrOS subjects participated in an ancillary sleep study (MrOS Sleep Study), among whom 2436 individuals were used in this analysis. While detailed elsewhere, the MrOS Sleep Study included a comprehensive sleep assessment in individuals who did not require positive airway pressure or nocturnal oxygen therapy during polysomnography [33]. Leg movements were monitored with bilateral anterior tibialis piezoelectric movement sensors as part of an unattended in-home polysomnogram that included standard polysomnographic sensors and signals (Safiro, Compumedics, Inc., Melbourne, Australia). Similar to MESA, PLMS were scored by a central Sleep Reading Center, blinded to other data, based on a train of at least 4 leg movements, of 0.5-5 s in duration each, with a periodicity of 5-90 s were noted [34]. Leg movements following respiratory events were excluded only if they were not part of a 4-leg-movement train, in which at least 2 of the leg movements were not associated with respiratory events [34]. With this definition high levels of agreement were found with scoring of PLMS using traditional, in-lab electromyography ($r = 0.81$) [35].

HypnoLaus —The CoLaus/PsyCoLaus is a population-based cohort study conducted in Lausanne, Switzerland between 2003 and 2006. The study recruited a random sample of 6734 participants between the ages of 35 and 75 years [27]. Five years after the initial evaluation, during the first follow-up, a random subset of the participants was included in the nested HypnoLaus cohort and received an evaluation of subjective and objective sleep characteristics, including a polysomnogram [36]. Details of the polysomnographic methods are described elsewhere [27, 37]. PLMS were scored according to the official standards of the World Association of Sleep Medicine [38, 39]. Leg movements occurring within 0.5 s following the end of a respiratory event were not scored as PLMS.

Multi-Ethnic Study of Atherosclerosis (MESA) —MESA was initiated in 2000 and recruited participants until 2002, to investigate the prevalence, correlates, and progression of subclinical cardiovascular disease [40]. It included 6814 men and women aged 45–84 years old from six U.S. communities. A sleep ancillary study was conducted between 2010 and2013, which included type II, unattended, in-home polysomnography (Compumedics Somte Systems; Compumedics Ltd., Abbostville, Australia) and standardized sleep questionnaires. Of the 2261 participants who were eligible and completed the sleep exam 2057 had the required data (including a record of PLMS on the sleep study) to be included in this analysis. Leg movements were scored by the same Sleep Reading Center as for MrOS, using the same criteria.

Statistical Analysis of PLMS+

The statistical effects of cohorts and age on PLMI were examined using linear regression in a combined sample from the WSC, MrOS, HypnoLaus, and MESA cohorts. A cohort adjusted PLMI was then calculated as the regression residuals plus the average effect of cohorts across the sample. The cohort adjusted PLMI was used to visualize the association between age and PLMI.

Genotyping and GWAS Methods

GWAS data from the four studies were imputed to > 10 million SNPs with the IMPUTE2 (v2.3) software using the 1000 Genomes Project (phase release 3, March 2012) reference panel [41, 42]. Genotypes were aligned to the positive strand in both imputation and genotyping. Imputation was conducted separately for each study, and each of the datasets was filtered to high quality common variants shared between cases and controls after imputation and quality control (QC). Thresholds for imputation quality were set to retain potential risk variants with MAF > 0.05. Poorly imputed SNPs, defined by an information measure < 0.90 with IMPUTE2, were excluded, as were SNPs exhibiting significant deviation from Hardy–Weinberg equilibrium ($p < 5 \times 10^{-8}$). Tests of association between imputed SNPs, PLMS+, and $\sqrt[3]{\text{PLMI}}$ (i.e., PLMS per hour as a continuous measure, which is then cube rooted to reduce the skewed distribution in order to satisfy the assumption of linear models) were performed using SNPTEST (v2.5) under an additive frequentist model [43]. Genotype uncertainty was considered using a missing data likelihood score test. The same procedure for testing association was performed for PLMS+ as a dichotomous phenotype (i.e., PLMI ≥ 15/h). The adequacy of the case–control matching and the possibility of differential genotyping of cases and controls were formally evaluated using Q-Q plots of test statistics (Supplementary Fig. 1). To reduce confounding due to population stratification, the first five dimensions of a multidimensional scaling (MDS) analysis, generated using common SNPs, were included in the analysis to limit the effects of cryptic population stratification that otherwise might cause inflation of test statistics. MDS adjustment was performed for each cohort and each major racial/ethnic group independently using PLINK [44]. In addition to the first five dimensions, sex, age, and BMI were also included as covariates in this model.

We conducted quality-control (QC) analyses on each cohort separately. SNPs with call rates < 95% were removed as part of QC in PLINK, followed by removal of subjects with call rates <95%. Concordance of replicate samples was assessed, and the sample with the higher call rate was retained. Subject's sex was

verified using the sex check option in PLINK. To address potential bias due to cryptic semi-relatedness, relationship checking was performed by estimating the proportion of alleles shared identical by descent (IBD) for all pairs of subjects in PLINK. Subjects indicated to be of non-European ancestry were excluded. SNP associations at $p < 5 \times 10^{-8}$ in the meta-analyses are considered genome-wide significant [45].

Post-imputation, there were 37,905,187 variants in the MrOS cohort, 37,732,761 variants in the Wisconsin sleep cohort 27,534,370 in the MESA European cohort and 20,278,837 variants, in the HypnoLAUS cohort. After post-imputation QC procedures, there were 8,248,889 variants present and 7,921,998 variants when restricted only to individuals of European ancestry. Prior to the LD Score regression, the primary joint meta-analysis results for PLMI and PLMS+ were filtered to HapMap3 variants and independently LD-pruned pairwise to 931,397 variants, and 777,526 variants when restricted to individuals only of European ancestry.

Data sets included in the study. Demographics and clinical information regarding the four cohorts included in the GWAS meta-analysis are described in Table 1. We defined the discovery set as the samples from WSC and MrOS, in which individual level genotype and phenotype data were available and the replication/validation set as the samples from HypnoLaus and MESA, in which only summary statistics were provided from submitted analysis requests. Briefly, the four cohorts comprise 8319 individuals, with cases defined as PLMS positive (PLMS+ or PLMI ≥ 15), prior to genotyping QC. After QC, the final cohort included 6843 individuals of which there were 1745 cases in the discovery set, and 1002 cases in the replication set. The meta-analysis included individuals of all ancestry groups, while the Mendelian randomization was restricted to individuals of only European ancestry (n = 5479).

Meta-analysis and additional statistical analyses

Given that joint analyses increase power in GWAS, following the discovery-replication analysis, joint meta-analysis was performed using all four cohorts: two discovery cohorts (WSC and MrOS) with raw genotypes available and two replication/validation cohorts (HypnoLaus and MESA) that provided summary statistics from requested analyses. We performed meta-analyses of the primary and replication cohorts using METAL [46, 47]. Briefly, the P-values and direction of effect were combined, weighted by effect sizes using the inverse of the corresponding standard errors. A correction for genomic inflation was applied to the P-values in cohorts exhibiting a median test statistic greater than that expected by chance by comparing the median test statistic to Cochran's Q-statistic was used to test for heterogeneity, and the I^2 statistic was used to quantify the proportion of the total variation due to heterogeneity taking I^2 values > 75 to indicate significant heterogeneity [48].

We used the meta-analysis summary statistics and linkage disequilibrium (LD) correlations from a reference panel of the 1000 Genomes Project. (PLMI). The discovery cohorts included the MrOS and the WSC (N = 3398) because the individual-level data was available, and the replication cohort included the MESA and the HypnoLaus Cohorts (N = 3618), where only the summary statistics were provided. All four cohorts were included in the joint meta-analysis (N = 6843).

LD score regression, estimation of heritability and genetic correlation between traits

Linkage disequilibrium (LD) score regression is a statistical methodology that uses genome-wide SNP association data and patterns of LD to estimate heritability and correlations between traits, while minimizing the effect of confounding and population stratification [49]. Briefly, the method regresses summary statistics from GWAS on the LD score, in which each individual variant tags other variants in the genome. We used LDSC v1.0.1 to estimate genetic correlation between traits (R_g), the 95% confidence intervals (95% CI), and P-values for each pairing, and heritability for each trait [48]. The LD score regression intercept, estimated lambda (λ, an estimate of genetic inflation), maximum χ2 statistic, and intercept of genetic covariance were also calculated. The total number of SNPs used in each pairwise analysis varied due to variation in array used by study and study-specific imputation quality scores. After merging the shared SNPS for

Table 1. Demographic and clinical information from included cohorts

	Discovery (n = 3398)		**Replication (n = 3445)**	
	WiSC, n = 962	**MrOS, n = 2436**	**HypnoLaus, n = 1388**	**MESA, n = 2057**
Age (year)	55.9 ± 7.7	76.5 ± 5.6	52.6 ± 10.5	69.6 ± 9.2
Sex, male (n (%))	512 (53%)	2436 (100%)	776 (48.4%)	1036 (46%)
Ethnicity	European	European	European	(573 African, 743 European, 250 East Asian, 491 Native American)
BMI (kg/m^2)	31.6 ± 7.2	27.2 ± 3.8	26.0 ± 4.3	28.8 ± 5.3
PLMI (PLMS/h)	6.4 [2.0, 16.9]	24.3 [3.4, 56.5]	2.4 [0.0, 19.8]	2.6 [0.0, 18.3]
PLMS+ (n (%))	275 (29%)	1470 (60%)	425 (31%)	577 (26%)
Platform	Affymetrix 6.0/500k	Illumina	Affymetrix	Illumina
Study type	Population based	Population based	Clinical sample	Population based
PLMS ascertainment	EMG; validated PLM detector algorithm [2, 25]	Piezoelectric; manual annotation	EMG; WASM criteria	Piezoelectric; manual annotation

Note: BMI, body mass index; EMG, electromyography; PLMI, periodic limb movement index; PLMS, periodic limb movements in sleep; PLMS+PLMI ≥ 15 events per hour; WASM, World Association of Sleep Medicine. Values for age and BMI are presented as mean ± standard deviation. Values for PLMI are presented as a mean with a 95% confidence interval.

each trait based on a pairwise intersection, the variants were then filtered to a HapMap3 LD-reference panel. P-values were corrected using the Benjamini–Hochberg procedure, and associations were considered significant at adjusted $p < 0.05$.

Estimations of causal interaction between phenotypes using Mendelian randomization (MR)

We conducted Mendelian randomization (MR) analysis to infer causal relationships and directionality between the main phenotypes studied here: PLMS+ (i.e., PLMI ≥ 15), RLS, insomnia, and stroke. MR uses genetic variants as instrumental variables (IVs) to assess causal relevance of exposures to a disease outcome [50]. MR is based on the assumptions that the genetic variants that are used as instruments are: (1) linked to the outcome only through the exposure, which is also called the no horizontal pleiotropy assumption, and (2) not influenced by reverse causation. In the absence of pleiotropy, MR can provide unbiased estimates for the causal link from the exposure to the outcome [51]. For each SNP, causal effect estimates were generated for PLMS+, and RLS as ORs per one standard deviation unit increase in the putative exposure (OR_{SD}), with 95% confidence intervals (CIs), using the Wald ratio [52]. For this analysis we restricted our sample to individuals with European ancestry (i.e., sub-setting MESA to individuals of European-descent, and keeping the other cohorts as is). To account for potential horizontal and correlated pleiotropy, we performed Causal Analysis Using Summary Effect Estimates (CAUSE v1.2.0), as it directly models pleiotropy by assuming that such variants with pleiotropic correlation do not form the majority of the variants of the exposure, and thus the bias caused by horizontal pleiotropy can be corrected while analyzing all variants jointly [52]. In short, CAUSE tests the posteriors under a causal model fit to assess if there is a better fit than the posteriors under a shared genetics model, concluding that the data is consistent with a causal effect rather than due to pleiotropy. It is important to note that the CAUSE method is very conservative and so a null causal association should be interpreted with care. To determine which causal variants were valid instruments, we set a p-value threshold at 0.0001. To further assess the strength of the relationship between our instrumental variables and phenotype, as well as check for bias, the F-statistic was computed and considered unbiased when greater than 10 [53].

For our main results we also tested additional MR methods using the MendelianRandomization R package [54]. This was added to examine if the causal estimates tended to be robust across the different methods. We also examined the results of the MR-Egger regression, specifically to check for the effect size and significance of the intercept.

Results

Age and periodic leg movements

We analyzed the effects of age and cohort on PLMI in all four cohorts using linear regression (Supplementary Table S1). Subjects from the MrOS cohort had an elevated $\sqrt[3]{\text{PLMI}}$/per hour of 0.70 ($p = 2.28 \times 10^{-31}$) independent of age, most likely the result of using piezoelectric sensors instead of EMG electrodes. The cohort-adjusted PLMI is visualized for 5-year age intervals in Figure 1. As can be seen in the figure, PLMI increases progressively with age (B = 0.49 increase in PLMI per year increase in age, $p = 2.01 \times 10^{-44}$).

Genome-wide association study: discovery, replication, and joint meta-analysis

For both PLMS as a continuous ($\sqrt[3]{\text{PLMI}}$) and dichotomous phenotype (PLMS + or PLMI ≥ 15/h), we analyzed genome-wide SNP genotypes on the discovery set and replication sets separately, and then performed a joint meta-analysis of all four cohorts. Q-Q plots for the SNPs with the MAF > 5% post imputation did not show evidence of substantive overdispersion (λ between 0.99 and 1.04; Supplementary Figure S1). *MEIS1* did not reach genome-wide significance in the discovery set ($p = 2.0137 \times 10^{-06}$, OR = 1.51, SE 0.1) and only in the replication set ($p = 9.49 \times 10^{-09}$, OR = 1.74, SE = 0.1), presumably because of the relatively smaller individual sample

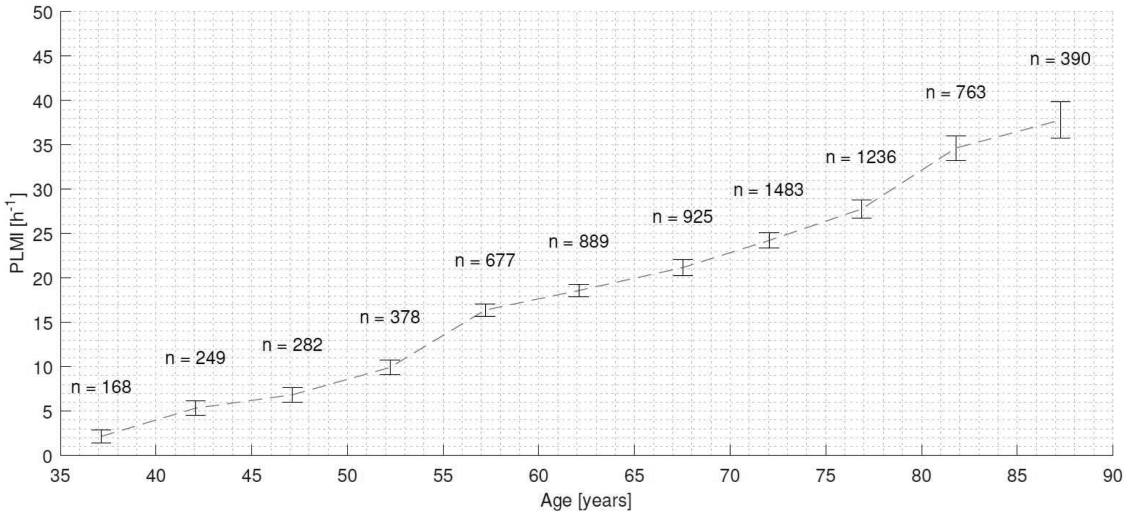

Figure 1. Average PLMI in 5-year age intervals adjusted by cohort effect. The effects of cohorts have been averaged between all samples ($n = 7440$) according to effects of the multiple linear regression model in Supplementary Table S1. The error bars indicate ±the standard error of the mean (SEM) calculated as $\sigma/\sqrt{n}$, where σ is the standard deviation. PLMI: periodic leg movement index.

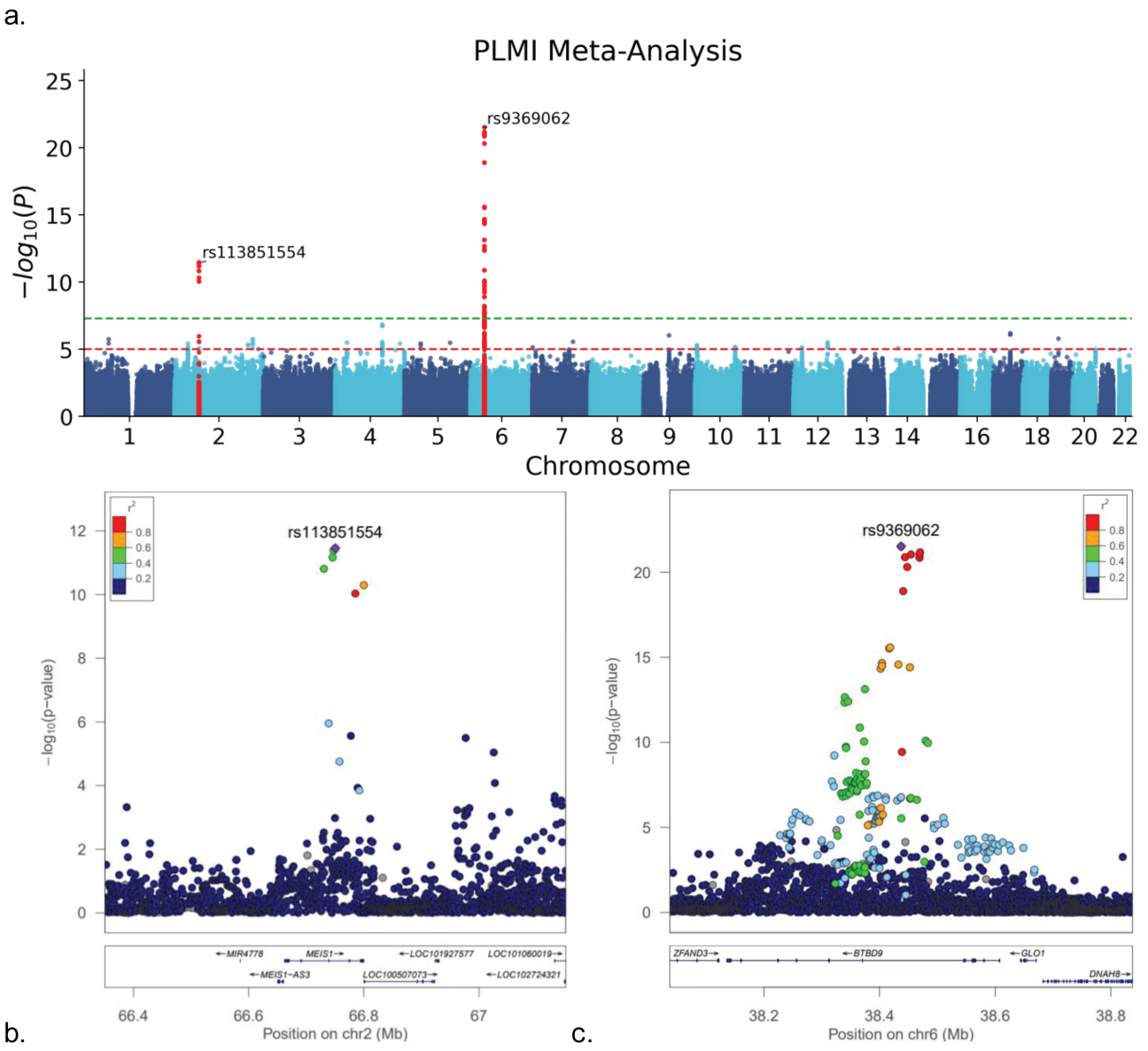

Figure 2. Manhattan plot for PMLI genome-wide meta-analysis and regional association of significant loci. (a) Manhattan plot of combined cohorts meta-analysis. MEIS1 and BTBD9 are the only genes to achieve genome-wide significance (N = 7,016 PLMI cases from the four cohorts). Along the X-axis SNPS are plotted by position in ascending order, labels are shown for chromosome number. The Y-axis corresponds to the $-\log_{10}$(P-value) of the association for each SNP with PLMI. Genome-wide significance of 5.0 × 10E−08 is shown as a dashed line. (b) A regional plot produced by locus zoom of the MEIS1 gene on chromosome 2 showing the top LD-independent SNP to be rs13851554 with a P-value of 3.51E−12, which is well surrounded by correlated SNPs. (c) A locus zoom plot of the BTBD9 gene on chromosome 6 showing the top LD independent SNP to be rs9369062 with a P-value of 3.058E−22.

sizes in the discovery cohorts (Supplementary Figure 3). From the joint meta-analysis of the 4 cohorts, Figure 2 shows the entire Manhattan plot and the regional plots for chromosomes 2 and 6, where *MEIS1* (rs113851554) and *BTBD9* (rs9369062) are the genes most significantly associated with PLMI.

The lead genome-wide significant SNP for *MEIS1*, rs113851554 ($p = 3.51 \times 10^{-12}$, $\beta = 0.486$, se = 0.0699), is located in a putative regulatory element in intron 8 and maps to 2p14. The lead SNP for *BTBD9*, rs9369062 ($p = 3.06 \times 10^{-22}$, $\beta = 0.2093$, se = 0.0216), is located in an intronic region and maps to 6p12. Table 2 shows the results for these two loci in all three analyses: discovery, replication, and combined meta-analysis. All analyses of these two genetic loci, except for *MEIS1* in the discovery set, result in genome-wide-significant associations. However, it is important to note, that despite a lack of genome-wide significance in the discovery cohorts, the direction of effect is consistent with the final joint analysis and the p-values are below the frequently used "exploratory" threshold of significance ($p < 10^{-5}$).

Given the aforementioned analysis demonstrating a positive correlation between PLMI and age, we sought to explore the relationship between these identified SNPs and PLMI adjusted for 5 MDs, sex, age, and BMI using multiple linear regressions (Supplementary Figure S2). The cohort-adjusted PLMI was grouped by the number of copies *MEIS1* and *BTBD9* and visualized in 10 years age intervals. While the sample sizes of individuals with higher doses of either of the effect alleles from *MEIS1* and *BTBD9* were too small to draw meaningful statistical inference, it appears that dose of the effect alleles doesn't change the correlation between age and PLMI.

Table 2. Genome-wide meta-analysis of SNPs identified in the PLMS+ and PLMI discovery, validation, and combined cohorts

Meta-analysis	Odds ratio	1.63	1.22	2.97	0.58	1.69
	SE	0.07	0.02	<0.01	0.08	0.08
	P-value	3.51E–12	1.42E–21	2.91E–09	4.00E–11	5.58E–11
	HetP-Val	0.04	0.95	0.14	0.38	0.27
Validation	Odds ratio	1.74	1.22	1.0894	0.77	1.28
	SE	0.11	0.03	0.14	0.04	0.04
	P-value	9.94E–09	2.73E–14	3.13E–10	1.71E–12	4.67E–12
Discovery	Odds ratio	1.51	1.23	1.27	0.54	1.85
	SE	0.1	0.04	0.27	0.11	0.11
	P-value	4.56E–05	8.63E–09	1.88E–06	1.66E–08	1.13E–08
Marker information	Gene	MEIS1	BTDB9	MEIS1	BTBD9	BTBD9
	Freq SE	0.0156	0.1051	0.0072	0.0088	0.008
	EAF	0.05	0.67	0.06	0.28	0.7
	Allele2	G	C	G	C	T
	Allele1	T	A	A	T	C
	Marker name	rs113851554	rs10947738	rs11679120	rs4236060	rs4714163
	Phenotype	PLMI	PLMI	PLMS+(PLMI > 15)	PLMS+(PLMI > 15)	PLMS+(PLMI > 15)

Note: EAF, effect allele frequency; SE, standard error; FreqSE, standard error of frequency; HetPVal, heterogeneity of P-value.

PLMS+, insomnia, and RLS GWAS comparison

For these comparisons, we used the dichotomous PLMS + phenotype to facilitate comparisons with GWAS associations reported for RLS as a dichotomous phenotype. The two genes identified above, *BTBD9* and *MEIS1*, have been previously associated with RLS [22]. Odds ratios (ORs) identified in our PLMS + meta-analysis were in the same direction and reached genome-wide significance as reported in the RLS studies of Schormair et al. and Didriksen et al. [21, 22]. PLMS + OR for *BTBD9* rs4714163 was 0.83; $p = 3.70 \times 10^{-10}$ while for RLS study of Didriksen et al. [22]. OR was 0.76 ($p = 3.11 \times 10^{-50}$). For *MEIS1* rs113851554, PLMS + OR was 1.63; $p = 3.51 \times 10^{-12}$ compared to OR = 1.89; $p = 4.5 \times 10^{-100}$ for RLS (Table 3).

A similar analysis comparing PLMS + SNPs from our study with insomnia-associated SNPs from Posthuma (2020, unpublished) was performed (Table 4). In this analysis, although SNPs in the *BTBD9* and *MEIS1* genes were also significant for both PLMS+ and insomnia, lead SNPs were different from those found for RLS and PLMS+. The beta coefficient for *BTBD9* (rs9394502) in the insomnia study was in the negative direction similar to our PLMI results with the same SNP (rs9394502), although both reached genome-wide significance ($p = 3.92 \times 10^{-15}$ for PLMI and $p = 3.53 \times 10^{-20}$ for insomnia) effect sizes were much weaker in insomnia. MEIS1 (rs62144053) is significantly associated with both PLMI and insomnia ($p = 6.81 \times 10^{-12}$ for PLMI and $p = 2.56 \times 10^{-53}$ for insomnia).

LD score regression

We evaluated genome-wide correlations between the genetic architecture of RLS, PLMI, and known risk-related phenotypes, such as doctor diagnosed insomnia, iron-deficiency anemia, and risk of stroke (as defined by the UK Biobank data field 6150). Table 5 shows the genome-wide pairwise correlations of all phenotype pairs tested for correlation, as well as the resulting Z-scores. Genetic correlation was determined for PLMI with RLS, and PLMI with insomnia, and iron deficiency anemia with RLS and insomnia. PLMI was genetically correlated with stroke, insomnia and RLS, but not iron deficiency. PLMI and stroke were highly correlated and marginally significant at $p < 0.05$ after correcting for multiple hypothesis testing ($R_g = 0.913$, se = 0.3996, $p = 0.0256$). The LD score regression analysis is an indication that these phenotypes may be correlated and likely candidates to examine using causal inference using Mendelian randomization (MR). The genetic architecture of the LD score regression of the shared phenotypes is also illustrated as a correlogram (Figure 3) and shows the genetic correlation (R_g) between the phenotypes also described in Table 5, and show the correlations are strongest for stroke and PLMI followed by stroke and insomnia, and RLS and insomnia. However, only the correlation between PLMI and RLS suggested a sufficiently strong heritability due to genetics with an observed heritability for these two traits of 0.4175

Mendelian randomization analysis

We performed MR causal inference analysis using the CAUSE algorithm (see Methods for a description of the analysis). From RLS to PLMS+, we used 271 variants to estimate the posteriors for the causal relationship using the CAUSE test statistic. This analysis indicated that the pleiotropy adjusted causal model is a better fit than the shared genetic model ($p = 0.0066$, Figure 4). In this analysis, the strength of the instrumental variables was computed to be sufficiently strong, with an F-statistic of 18.629 [55]. To further explore a causal relationship of RLS on PLMS+, we also examined other methods including inverse-variance weighting (IVW), median MR, and MR-Egger. Table 6 shows that the causal estimates are similar across the eleven different methods. All methods found a statistically significant causal test statistic estimate for the relationship between RLS and PLMS+($p < 0.001$) with estimates ranging between 0.558 using the inverse variance weighted method (IVW) to 0.777 using the penalized robust MR-Egger estimate. Moreover, the MR-Egger analysis suggested a limited significance for the intercept.

In summary, our analysis identified a main finding that RLS is causal in PLMS+. That is, that the presence of RLS symptoms increases the likelihood of having PLMS+(i.e. PLMI ≥ 15). This finding further supports the conclusion that there is a likely causal relationship between RLS and PLMS+, extending beyond the shared genetic architecture.

Table 3. Meta-analysis of PLMS+ combined cohorts compared with restless leg syndrome (RLS) (Didriksen et al. [22])

Marker information (Didricksen)						RLS (Didriksen et al. [22])		PLMS+ (this study)	
rsName	Band	EAF Europe	EA	OA	Closest gene	P-value	Odds ratio (95% CI)	P-value	Odds ratio (95% CI)
rs10177089	2p25.3	0.38882	C	T		8.49e–12	0.89 (0.86–0.93)	2.10E–04	0.92 (0.88–0.97)
rs3784709	15q23	0.30409	T	C	MAP2K5	4.91e–28	0.82 (0.79–0.86)	7.16E–03	0.95 (0.91–0.99)
rs4714163	6p21.2	0.29530	C	T	BTBD9	3.11e–50	0.76 (0.73–0.8)	3.70E–10	0.83 (0.77–0.89)
rs7034030	9p24.1	0.16055	G	C	PTPRD	1.47e–11	1.15 (1.11–1.19)	1.94E–03	1.08 (1.03–1.14)
rs10208712	2p25.3	0.35111	G	A		2.34e–09	0.91 (0.88–0.94)	2.35E–04	0.92 (0.88–0.97)
rs10952927	7q21.13	0.13149	G	A		1.9e–09	1.13 (1.09–1.17)	1.12E–03	1.08 (1.04–1.13)
rs111652004	15q21.1	0.10322	T	G		2.2e–11	0.83 (0.77–0.88)	1.59E–04	0.88 (0.82–0.95)
rs113851554	2p14	0.05670	T	G	MEIS1	4.5e–100	1.89 (1.83–1.94)	3.51E–12	1.63 (1.49–1.76)
rs12046503	1p21.1	0.41907	C	T		1.09e–17	1.15 (1.11–1.18)	2.97E–03	1.06 (1.02–1.1)
rs12450895	17q21.32	0.20817	A	G		5.69e–06	1.09 (1.05–1.13)	1.28E–03	1.08 (1.03–1.12)
rs12962305	18q12.3	0.25833	T	C		0.0113	1.03 (1.01–1.05)	7.90E–01	1.01 (0.96–1.05)
rs17636328	6p21.2	0.17932	G	A		7.63e–08	0.90 (0.86–0.94)	3.63E–02	0.95 (0.9–1)
rs1820989	2p14	0.45302	A	C		2.86e–13	0.90 (0.87–0.93)	5.33E–02	1.06 (1–1.12)
rs1836229	9p24.1	0.48598	G	A	PTPRD	3.68e–08	0.92 (0.89–0.95)	8.40E–03	0.95 (0.91–0.99)
rs1848460	3p26.2	0.24450	T	A		7.3e–05	0.92 (0.87–0.96)	1.56E–01	0.97 (0.93–1.01)
rs340561	13q21.33	0.18514	T	G		0.001	1.07 (1.03–1.1)	1.01E–03	1.08 (1.03–1.13)
rs35987657	3q22.1	0.33340	G	A		1.45e–09	0.9 (0.87–0.94)	1.88E–03	0.94 (0.9–0.98)
rs365032	20q13.33	0.26694	G	A	MYT1	2.13e–06	1.09 (1.05–1.12)	3.98E–05	1.09 (1.05–1.14)
rs45544231	16q12.1	0.42204	G	C		5.71e–34	0.82 (0.79–0.85)	9.38E–05	0.93 (0.89–0.97)
rs61192259	6p21.2	0.40881	C	A	BTBD9	4.71e–30	0.83 (0.8–0.86)	1.87E–07	0.89 (0.85–0.93)
rs62535767	9p23	0.32098	T	C	PTPRD	2.2e–05	0.93 (0.89–0.96)	1.40E–01	0.97 (0.93–1.01)
rs80319144	2q24.1	0.24907	T	C	CCDC148	2.11e–07	0.91 (0.87–0.95)	6.60E–02	0.96 (0.91–1)
rs868036	15q23	0.31107	T	A	MAP2K5	4.67e–28	0.83 (0.79–0.86)	1.24E–03	0.94 (0.9–0.98)
rs996064	15q14	0.06880	T	A		2.8e–08	1.21 (1.14–1.27)	3.01E–04	1.2 (1.1–1.31)
rs10068599	5q35.1	0.32681	T	C	RANBP17	4.29e–08	1.10 (1.06–1.13)	5.66E–01	1.01 (0.97–1.05)
rs10188680	2q32.2	0.41022	T	A	SLC40A1	4.28e–08	1.09 (1.06–1.13)	4.85E–01	1.01 (0.98–1.05)
rs10769894	11p15.4	0.44845	A	G		6.62e–10	0.90 (0.87–0.93)	5.18E–02	0.96 (0.92–1)
rs112716420	7p22.3	0.07505	G	C		4.49e–14	1.25 (1.19–1.31)	2.62E–02	1.09 (1.01–1.16)
rs58127855	18q21.32	0.0053	T	C		5.06e–09	4.72 (4.2–5.24)	7.21E–01	0.97 (0.83–1.12)
rs112716420	7p22.3	0.07505	G	C	MICALL2	1.5e–18	1.25 (1.19–1.31)	2.62E–02	1.09 (1.01–1.16)
rs10769894	11p15.4	0.44845	A	G	LMO1	9.4e–14	0.90 (0.88–0.93)	5.18E–02	0.96 (0.92–1)
rs10068599	5q35.1	0.32681	T	C	RANBP17	6.9e–10	1.09 (1.06–1.12)	5.66E–01	1.01 (0.97–1.05)
rs10188680	2q32.2	0.41022	T	A	SLC40A1	5.4e–08	1.07 (1.05–1.11)	4.85E–01	1.01 (0.98–1.05)
rs58127855	18q21.32	0.0053	T	C	PMAIP1	6.3e–07	3.03 (2.01–4.97)	7.21E–01	0.97 (0.83–1.12)

Note: rsName, reference SNP ID; EAF, effect allele frequency in European ancestry; EA, effect allele; OA, other allele. Odds ratios are presented with 95% confidence intervals in parentheses. Statistics for restless leg syndrome were obtained from Didriksen et al. [22].

Discussion

Using four cohorts, totaling 6843 individuals with polysomnographic measurements and genetic data, periodic limb movements were evaluated as both a continuous (PLMI) and dichotomous phenotype (PLMS+). We found strong associations between the genetic architecture of PLMI and RLS ($R_g = 0.42$) with top SNPs in the *MEIS1* (*rs113851554*) and *BTBD9* (*rs9369062*) genes being most strongly associated with both phenotypes (Table 2). In addition, 27 of the 34 SNPs associated with RLS by Didriksen et al. [22] were nominally significant and associated with RLS, with the same direction of effects (Table 3), illustrating the clinical and genetic overlap of both phenotypes. In contrast, although SNPs in similar genes were present by Posthuma et al. (2020, unpublished) insomnia associations in some common genes such as *BTBD9*, *MEIS1*, and *PTRD*, top SNPs were different between the two phenotypes (insomnia and PLMI). In fact, in the case of *BTBD9*, the top PLMI-associated SNP that was found in our analysis was weakly negatively associated with insomnia, suggesting that even if these genes are involved in both PLMI and RLS on one side and insomnia on the other, the situation is likely complex, reflecting both pleiotropy and RLS cases misdiagnosed as insomnia in the UK Biobank sample (Posthuma, 2020; unpublished). This perspective was supported by our genetic correlation analysis, which did not suggest a strong shared heritability between PLMI and insomnia (although the phenotypes were significantly correlated at $R_g = 0.26$), but a strong shared heritability in RLS and PLMS+. The strong association also fits with the concept that both phenotypes are strongly associated with kidney disease, notably among individuals undergoing hemodialysis [7].

There are several possibilities that might explain a causal relationship of RLS on PLMS. Both RLS and PLMI increase in frequency with age, but only RLS is more frequent in women than men. Given the incomplete overlap between RLS and PLMS noted clinically, it is likely that RLS is one of multiple contributors to PLMS [2]. One possibility may be that because RLS is a sensorimotor disorder, PLMS generated in the context of RLS are secondary to disturbed sensory inputs, while PLMS in the absence of RLS is a purely motor phenomenon. Increased PLMS are often observed in the context of other CNS pathologies. For example, PLMS are known to be more prevalent in HLA-associated narcolepsy Type 1, with a slightly different periodicity, suggesting

Table 4. Meta-analysis of PLMI SNPs compared with the loci associated with insomnia (Posthuma et al. 2020)

Marker information							PLMI (this study)			Insomnia (Posthuma et al. 2020)			
Marker name	Band	MAF	ref	alt	Gene	Top SNP	Beta	SE	P-value	#SNPs in LD	Beta	SE	P-value
rs9394502	6p21.2	0.3918	T	C	BTBD9	1	−0.1587	0.0202	3.92E−15	23	-0.009	0.001	3.53E-20
rs62144053	2p14	0.0901	A	G	MEIS1	1	0.3626	0.0528	6.81E−12	4	0.025	0.002	2.56E−53
rs72826719	2p14	0.0512	A	G	MEIS1	0	0.3422	0.0735	3.19E−06	2	0.022	0.002	3.85E−23
rs11126082	2p14	0.4722	C	G	MEIS1	0	−0.107	0.0278	0.0001179	2	−0.009	0.001	3.36E−20
rs77614227	15q14	0.9486	G	T	AC021351.1	0	−0.1793	0.0517	0.0005218	2	−0.012	0.002	4.74E−09
rs4238749	16q12.2	0.4459	A	C	CASC16	0	−0.0624	0.0193	0.001253	103	−0.007	0.001	9.34E−16
rs11980428	7q21.13	0.2607	A	G	AC002127.2	1	0.0798	0.0249	0.001325	84	0.009	0.001	1.06E−11
rs68030046	6p21.2	0.0606	A	G	BTBD9	0	−0.137	0.0433	0.001548	2	−0.01	0.002	1.06E−08
rs8025163	15q23	0.1117	T	C	IQCH	1	−0.0988	0.032	0.002026	2	−0.008	0.001	1.32E−08
rs118166957	9p24.1	0.1667	T	C	PTPRD	1	0.0834	0.0272	0.002133	25	0.012	0.001	2.46E−20

Note: MAF, minor allele frequency; ref, reference allele; alt, alternative allele; TopSNP is the most significant (smallest P-value) SNP within LD block, binarized as yes (1) or no (0); #SNPs in LD number of significant SNPs in linkage disequilibrium with SNP; SE, standard error.

Table 5. Genome-wide correlations with PLMI, RLS, insomnia, iron deficiency anemia, and stroke

Phenotype 1	Phenotype 2	R_g	se	z	P	h2_obs	h2_obs_se	N (p2 cases)
PLMI	Restless legs syndrome	0.4229	0.20	2.15	0.0317	0.4175	0.0586	6228
PLMI	Sleeplessness/insomnia	0.2645	0.11	2.36	0.0182	0.0642	0.0027	360,738
PLMI	Iron-deficiency anemia	0.0038	0.30	0.01	0.9900	0.0046	0.0013	2557
PLMI	Stroke*	0.9308	0.42	2.24	0.0250	0.0041	0.0013	8178
Restless legs syndrome	Stroke*	0	0.14	0.21	0.8350	0.0037	0.0013	8178
Restless legs syndrome	Sleeplessness/insomnia	0.2752	0.05	5.31	1.13E-07	0.0654	0.003	360,738
Restless leg syndrome	Iron-deficiency anemia	0.2619	0.13	2.00	0.0450	0.0045	0.0015	2557
Stroke*	Sleeplessness/insomnia	0.3755	0.09	4.11	3.88E-05	0.0638	0.0028	360,738
Stroke*	Iron-deficiency anemia	0.4113	0.24	1.73	0.0829	0.0048	0.0013	2557
Iron-deficiency anemia	Sleeplessness/Insomnia	0.2358	0.08	3.12	0.0018	0.0638	0.0028	360,738

*Vascular/heart problems diagnosed by doctor: stroke.

Note: R_g, correlation between phenotypes (Pairwise); se, standard error; *h2_obs*, observed heritability of phenotype pair (on the liability scale); *h2_obs_se*, standard error of the observed heritability; *N (p2 cases)*, sample size of the cases in the phenotype 2 study.

a different pathophysiology and modulation by hypocretin/orexin [56]. Finally, antidepressants such as selective serotonin reuptake inhibitors (SSRIs) and Mirtazapine increase PLMS, suggesting serotonergic effects [8, 57–61]. As such, it may be that RLS along with other pathologies, but not insomnia, contribute to PLMS.

The genesis of PLMS is largely understudied, as noted by many authors, leg movements frequently occur after the beginning of a rise in heart rate that is consistently found in association with a PLM [55]. Associations with electroencephalographic (EEG) K complexes, waves resembling evoked potentials, suggest that PLMS are part of a complex subcortical autonomic arousal event only rarely leading to a full-blown cortical arousal [62, 63]. Of note, however, PLMS are not increased with experimentally induced sleep disruptions that are known to precipitate K complexes, suggesting a subcortical origin of the PLM, not the other way around [75]. Finally, PLMS are more common in stage N2 sleep (where K complexes predominate) than during slow wave sleep or REM sleep [64]. Furthermore, the presence of sleep apnea often obfuscates identification of PLMS as sleep apnea events may have a periodic occurrence, and PLMS may synchronize to these respiratory events [65].

An unexpected finding of our analysis was a genetic correlation ($R_g = 0.93$, SE = 0.42, p = 0.0250) found between PLMI and stroke that was not a causal relationship based on our MR study. This result, limited by the small sample size, had a borderline p-value that will need confirmation in future studies. Stroke is known to be associated with increased prevalence of many sleep disorders, including PLMS [66, 67]. Similarly, multiple studies have demonstrated associations between cardiovascular disease and elevated PLMI, where PLMI was associated with increased risk of coronary heart disease, myocardial infarction, but not stroke [68], in addition to variable associations with overall cardiovascular risk and atrial fibrillation [18, 65–70]. These results may be complicated by the fact that the *MEIS1* and, more importantly, *MEIS2*, genes associated with RLS are also known heart transcription factors coordinating septation of outflow tracts [71].

This study has several limitations. First, the sample size is modest, and as a result we could only detect two genome-wide significant loci and had limited power for MR studies. Nevertheless, the *MEIS1* results in the discovery set can still be regarded as significant for replication with $p = 5 \times 10^{-5}$, as many studies use greater p-value cutoffs for replication [48]. Second, while MR has been shown to be a useful technique in many studies, it cannot replace proper randomized trials for identifying unbiased causal effects. In our analysis we investigated the causal connection between two diseases/syndromes, and thus no proper trial can be done. We were therefore limited to analyzing observational data. As a result, our analysis is limited by MR's reliance on causal assumptions. Future data from additional cohorts and ethnicities can be used to further corroborate our finding. We used CAUSE

(v1.2.0) because it is a state-of-the-art method that utilizes relatively weak causal assumptions to account for pleiotropy, and it has been shown to perform well in many situations in practice while outperforming other methods [72]. We also report the results using other methods. Specifically, MR-Egger suggested a significant pleiotropic effect ($p < 0.001$). Similarly to CAUSE, the MR-Egger model was developed to correct for pleiotropy bias. A significant p-value here means that the bias was reduced under the assumptions of the model [72]. However, while we provide MR-Egger results as an additional analysis, our main results and discovery rely on CAUSE as MR-Egger has been shown to be very sensitive to deviations from its model and has inflated type 1 errors in practice [73, 74].

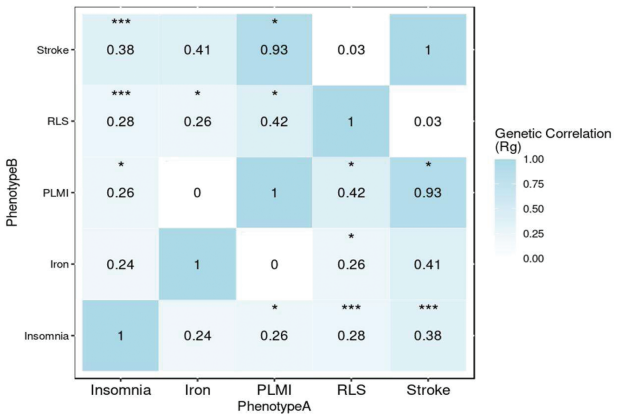

Figure 3. Correlogram of genetic architecture shared between phenotypes. A matrix of the genetic correlations (R_g) between phenotypes. Correlation is displayed numerically and as a color scheme. Asterisks are shown for pair-wise correlation reaching significance at P-values of 0.05(*), 0.01(**), 0.001(***). Iron deficiency Anemia labeled as iron.

However, given the costs and logistical difficulties in developing large datasets that contain both polysomnographic and genetic data, this is a relatively robust sample size that benefits from a more objective phenotype (PLMI) to study genetically. Second, the cohorts included were all middle aged or older adults, limiting our ability to explore early-onset PLMS, which may have a stronger genetic basis. However, our supplementary analyses, stratifying the age-PLMI association by dose of the 2 main alleles (rs113851554 in *MEIS1* and rs9369062 in *BTBD9*), suggested that the correlation between age and PLMI was not modified by the exposure to the risk alleles (albeit shifted higher). Finally, PLMI was recorded using slightly different methodologies between studies, which could have contributed to differential findings across cohorts. However, using random effects meta-analysis attempts to mitigate the between-study variability in data collection, as well as other occult confounders that may have existed.

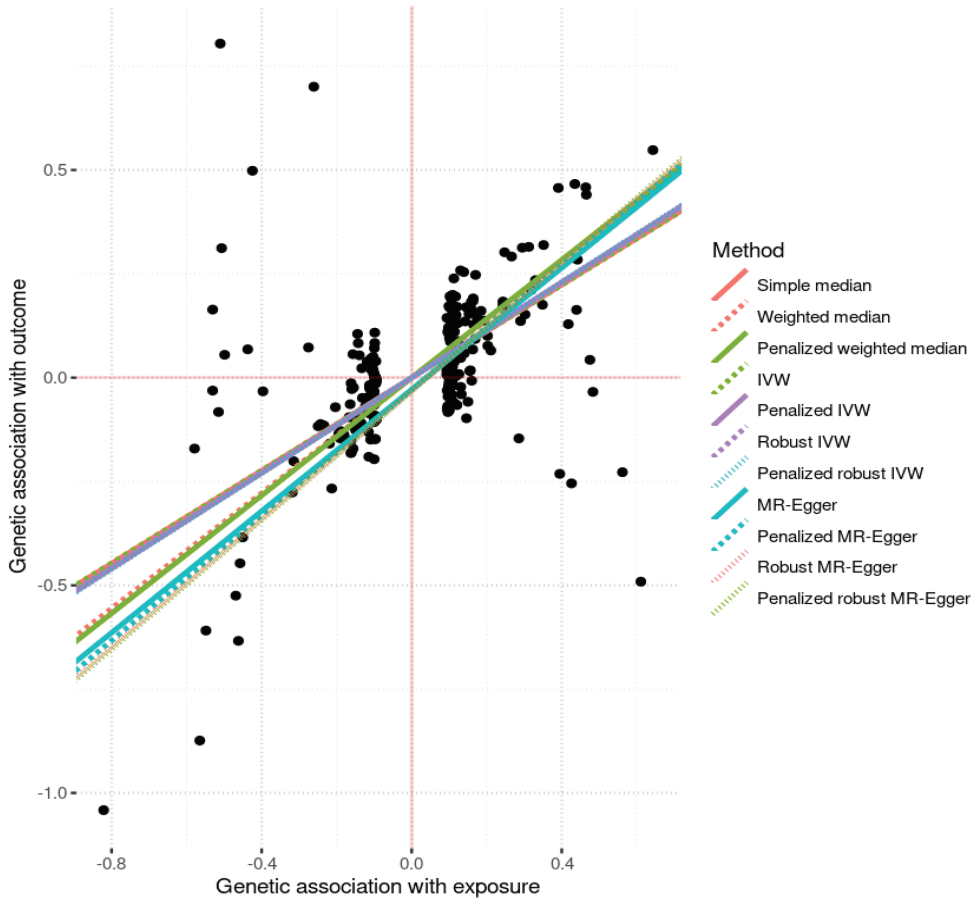

Figure 4. Mendelian randomization models of RLS (exposure) with PLMS+ (outcome). Causal relationship of restless leg syndrome (RLS) with PLMS+. Association between single-nucleotide polymorphisms (SNP) associated with restless leg syndrome (exposure) and PLMS+ (outcome) All methods identify a causal relationship indicated by an increasing positive slope. The lines are fitted on the odds ratio of outcome over the exposure for the 11 methods tested using the CAUSE R package. IVW-inverse variance weighted.

Table 6. MR methods testing the causal relationship between RLS as an exposure and PLMS+ as an outcome

Method	Estimate	Std error	95% CI upper	95% CI lower	P-value
Simple median	0.559	0.049	0.464	0.654	<0.001
Weighted median	0.694	0.048	0.601	0.788	<0.001
Penalized weighted median	0.710	0.048	0.616	0.803	<0.001
IVW	0.558	0.033	0.494	0.623	<0.001
Penalized IVW	0.575	0.031	0.513	0.636	<0.001
Robust IVW	0.570	0.035	0.501	0.639	<0.001
Penalized robust IVW	0.577	0.033	0.513	0.641	<0.001
MR-Egger	0.732	0.085	0.565	0.899	<0.001
(intercept)	–0.028	0.013	–0.054	–0.003	0.027
Penalized MR-Egger	0.755	0.081	0.596	0.914	<0.001
++(intercept)	–0.029	0.012	–0.053	–0.005	0.018
Robust MR-Egger	0.769	0.102	0.570	0.969	<0.001
(intercept)	–0.032	0.016	–0.063	–0.002	0.037
Penalized robust MR-Egger	0.777	0.099	0.583	0.970	<0.001
(intercept)	–0.032	0.015	–0.062	–0.003	0.033

Notes: IVW—inverse-variance weighted, MR-Egger—Mendelian randomization Egger method.

In summary, this first attempt at exploring the genetic etiology of PLMS yielded interesting insights, including the *BTBD9* and *MEIS1* associations that reached genome-wide significance in their associations with PLMS. In addition, we found a high correlation between PLMS and genetic predisposition of stroke as well as RLS and a relatively weak association with insomnia, and finally we found that RLS is causal in PLMS. Additional studies of this phenotype are warranted considering its high prevalence.

Supplementary material

Supplementary material is available at *SLEEP* online.

Acknowledgments

Dr. Schomair (personal communication) provided the GWAS summary statistics (including betas, standard errors, and p-values) from the EU-RLS-GENE consortium [21] and we obtained the additional GWAS summary statistics from the UKBIOBANK [71]. The EU-RLS-GENE study included 6228 cases and 10 992 controls RLS. We were unable to obtain summary statistics from Didriksen et al. [22]. Summary statistics for PLMS+ and PLMI will be made publicly available upon publication and submitted to the GWAS catalog for discovery use in further studies. Further documentation for the MrOS study can be found using the following link https://mrosonline.ucsf.edu.

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Disclosure Statement

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