Sleep Medicine 16 (2015) 1360–1365

Contents lists available at ScienceDirect

Sleep Medicine

journal homepage: www.elsevier.com/locate/sleep

Original Article

Genetic associations of periodic limb movements of sleep in the elderly for the MrOS sleep study

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ARTICLE INFO

Article history:
Received 17 June 2015
Received in revised form 28 July 2015
Accepted 31 July 2015
Available online 11 August 2015

Keywords:
Periodic limb movements of sleep
Single-nucleotide polymorphism
Restless legs syndrome
BTBD9
MEIS1

ABSTRACT

Objective: The objective of this study was to assess the relationship between single-nucleotide polymorphisms associated with restless legs syndrome and periodic limb movements of sleep in a population cohort of elderly individuals.
Methods: Single-nucleotide polymorphisms previously associated with periodic limb movements of sleep or restless legs syndrome were analyzed in 2356 white male participants in the Osteoporotic Fractures in Men Sleep Study cohort. The associations between single-nucleotide polymorphisms and polysomnographically measured periodic limb movement index ≥15 were examined with logistic regression adjusted for age, ancestry markers, and periodic limb movements of sleep risk factors.
Results: Of the men in this cohort, 61% had a periodic limb movement index ≥15. Significant associations were observed between a periodic limb movement index ≥15 and the number of risk alleles for the two BTBD9 single-nucleotide polymorphisms (rs9357271[T], odds ratio [OR] = 1.38, 95% confidence interval [CI] 1.20–1.58; and rs3923809[A], OR = 1.43, 95% CI 1.26–1.63), one of the MEIS1 single-nucleotide polymorphisms (rs2300478[G], OR = 1.31, 95% CI 1.14–1.51) and the mitogen-activated protein kinase kinase 5 (MAP2K5)/Ski family transcriptional corepressor 1 (SKOR1) single-nucleotide polymorphism (rs1026732[G], OR = 1.16, 95% CI 1.02–1.31). In a multivariable model controlling for each of the two MEIS1 single-nucleotide polymorphisms, the rs6710341[A] single-nucleotide polymorphism became a significant risk allele (OR = 1.59, 95% CI 1.26–2.00).
Conclusions: Our findings confirm an association between the BTBD9, MEIS1, and MAP2K5/SKOR1 single-nucleotide polymorphisms and periodic limb movements of sleep in an elderly cohort not selected for the presence of restless legs syndrome.

Periodic limb movements of sleep (PLMS) are contractions of the lower extremities that occur roughly every 20 s during sleep These movements may produce brief arousals from sleep [1], and they are associated with dramatic increases in blood pressure [2]. They are most prominent in the elderly and in those taking antidepressant medications. In addition, they are observed in roughly 90% of individuals with restless legs syndrome (RLS), which is considered the motor sign of the waking urge to move, a characteristic of this disorder. RLS is associated with a number of specific single-nucleotide polymorphisms (SNPs) based on genome-wide association scans (GWASs) [3,4]. Two studies have also demonstrated that these SNPs are associated with PLMS [3,5]. The present study further attempts to determine the relationship of these SNPs to PLMS in a population cohort of elderly individuals not selected for the presence of RLS.

1. Methods

1.1. Study sample

During the baseline examination of the Osteoporotic Fractures in Men Study (MrOS) from 2000 to 2002, 5994 community-dwelling men 65 years or older were enrolled at six clinical centers in the United States: Birmingham, Alabama; Minneapolis, Minnesota; Palo Alto, California; the Monongahela Valley near Pittsburgh, Pennsylvania; Portland, Oregon; and San Diego, California [6,7]. In

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http://dx.doi.org/10.1016/j.sleep.2015.07.017
1389-9457/

order to participate, men needed to be able to walk without assistance and must not have undergone a bilateral hip replacement.

For a comprehensive sleep assessment, the MrOS Sleep Study, an ancillary study of the parent MrOS cohort, recruited 3135 participants who did not require positive airway pressure or nocturnal oxygen therapy during polysomnography (PSG). Because the candidate SNPs examined were established in Caucasian populations, 319 were excluded because they were nonwhite. A further 175 who did not undergo PSG were excluded. Of these 2641 participants, 285 did not have SNP data, leaving 2356 in this analysis cohort. Compared with this analysis subset, 460 white men who were not included in the analysis were similar in age, body mass index (BMI), PLMS and RLS, and lifestyle covariates. They did have a higher rate of use of dopaminergic medication (2.4% vs. 1.2%, $p = 0.03$).

All men provided written informed consent, and the study was approved by the institutional review board at each site.

1.2. Polysomnography

Sleep testing was conducted with unattended in-home PSG (Safiro, Compumedics, Inc., Melbourne, VIC, Australia). The recording montage included the following: C_3/A_2 and C_4/A_1 electroencephalography, bilateral electrooculography, a bipolar submental electromyography, thoracic/abdominal respiratory inductance plethysmography, naso-oral thermistry, nasal pressure transduction, finger pulse oximetry, lead I electrocardiography (ECG), body position, and bilateral anterior tibialis piezoelectric movement sensing. Centrally trained staff performed home visits for unit setup and impedance value verification for each channel as previously described [8]. Data were downloaded to a central server at the Central Sleep Reading Center (Cleveland, OH, USA) and scored by certified research polysomnologists using standard criteria [9,10]. Apnea was defined as complete or near-complete reduction in thermistor amplitude for at least 10 s; hypopnea was defined as at least 30% reduction in nasal pressure signal or summed inductance bands for at least 10 s. The apnea–hypopnea index (AHI) was calculated as the total number of apneas and hypopneas per hour of sleep, each associated with ≥4% desaturation [10]. Arousals were scored according to the American Academy of Sleep Medicine criteria [9].

PLMS were scored according to the 1993 American Academy of Sleep Medicine criteria in which individual leg movements were scored if the duration was between 0.5 and 5 s and there was a clear amplitude increase from baseline in the leg channels [11]. To be considered periodic, at least four movements needed to occur in succession no less than 5 s and no more than 90 s apart. The periodic limb movement index (PLMI) was the total number of periodic leg movements per hour of sleep. Leg movements after respiratory events were excluded unless they were part of a ≥4-movement cluster with at least two movements occurring independently of respiratory events. The PLM parameter was expressed as both a continuous and categorical variable (PLMI ≥ 15 vs. <15). This value was chosen for the categorical threshold, as it is the index above which periodic limb movement disorder (PLMD) can be diagnosed [12]. It is also the threshold at which a recent study found both reduced night-to-night variability and optimal specificity/sensitivity [13]. Similarly, this threshold has been used in previous studies of this cohort [14].

1.3. Genetics

SNPs within the candidate gene regions previously associated with PLMS or RLS were analyzed (MEIS1 SNP, rs2300478; MEIS1 SNP, rs6710341; BTBD9 SNP, rs3923809; BTBD9 SNP, rs9357271; and mitogen-activated protein kinase kinase 5 [MAP2K5]/Ski family transcriptional corepressor 1 [SKOR1] SNP, rs1026732). The investigators at the California Pacific Medical Center and the group from University of California, San Diego, collaborated to develop a custom Illumina GoldenGate assay (Illumina, San Diego, CA, USA) to genotype polymorphisms in sleep-related genes [15]. The genotype concordance rate was >0.99 (8% of MrOS samples were plated in duplicate). Samples with an SNP call rate <90% were excluded. All SNPs summarized here were in Hardy–Weinberg equilibrium ($p > 0.001$).

1.4. Other measures

Other covariates examined included age, BMI, medication use [16], smoking, education, alcohol use, physical activity [17], depression [18], and other PSG-derived parameters (sleep staging, AHI, total sleep time, and time awake after sleep onset). Ancestry markers were calculated using autosomal SNPs in multidimensional scaling (MDS) analysis, as implemented in PLINK. The first two MDS components were used as covariates [19].

1.5. Statistical analysis

The characteristics of participants were compared based on PLMI status using chi-squared tests for categorical variables, *t*-tests for normally distributed continuous variables, and Wilcoxon rank-sum tests for continuous variables with skewed distributions. A linear trend in PLMI across the number of risk alleles for each SNP was examined with a linear regression model. The associations between SNPs and PLMI ≥15 were examined with logistic regression models and presented as odds ratios (ORs) and their 95% confidence intervals (CIs). The ORs were expressed as dose of the risk allele used as a linear variable (0,1,2). The models were first adjusted for age and ancestry markers. Then the multivariable models were further adjusted for covariates shown to be associated with PLMS at $p < 0.10$.

Exploratory analyses of the association between combinations of these SNPs and PLMI were conducted to determine if the associations were independent. Conditional models for the BTBD9 and MEIS1 genes were performed, with the two SNPs in each of these genes as variables in each of the models to determine whether these SNPs were independently associated with the PLMI outcome. The SNPs from each gene with the highest association to PLMI were also combined in one predictor (0–6). In this logistic model, the OR was expressed as the number of risk alleles used as a linear variable.

2. Results

The overall prevalence of PLMI being ≥15 was 61% (Table 1). Compared to those with a PLMI <15, those with a PLMI ≥15 were on average older, were less likely to have an AHI ≥15, had less total sleep time, more likely to be nondrinkers, and had more depressive symptoms. There were no differences in the use of antidepressants, anticonvulsants, dopaminergics, or benzodiazepines between those with and without a PLMI ≥15 (Table 1).

The allele frequencies for each of the five SNPs based on PLMI category (PLMI > 15) are shown in Table 2. PLMI as a continuous measure was significantly associated with the number of risk alleles for four of the five SNPs examined (Fig. 1). After multivariable adjustment, significant associations were observed between the PLMI outcome and the number of risk alleles for the two BTBD9 SNPs (rs9357271[T], OR = 1.38, 95% CI 1.20–1.58; and rs3923809[A], OR = 1.43, 95% CI 1.26–1.63), one of the MEIS1 SNPs (rs2300478[G], OR = 1.31, 95% CI 1.14–1.51), and the MAP2K5/SKOR1 SNP (rs1026732[G], OR = 1.16, 95% CI 1.02–1.31) (Table 3). Analyses with PLMI cutoffs of >10 per hour (which constituted 67% of the cohort) were very similar, with slightly stronger associations between the PLMI outcome and the SNPs.

In further analyses, SNPs were included in the same gene in a multivariable model. In the multivariable model controlling for each of the BTBD9 SNPs, rs3923809[A] continued to be a significant