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Original Article

Association of low ferritin with PLM in the Wisconsin Sleep Cohort

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ABSTRACT

Objective: The origins of periodic leg movements (PLMs), a strong correlate of restless legs syndrome (RLS), are uncertain. This study was performed to assess the relationship between PLMs and peripheral iron deficiency, as measured with ferritin levels corrected for inflammation.
Methods: We included a cross-sectional sample of a cohort study of 801 randomly selected people ($n = 1008$ assays, mean age 58.6 ± 0.3 years) from Wisconsin state employee agencies. A previously validated automatic detector was used to measure PLMs during sleep. The patients were categorized into RLS symptoms-positive and RLS symptoms-negative based on a mailed survey response and prior analysis. Analyses were performed using a linear model with PLM category above and below 15 PLM/h (periodic leg movement index, PLMI) as the dependent variable, and adjusting for known covariates, including previously associated single-nucleotide polymorphisms (SNPs) within BTBD9, TOX3/BC034767, MEIS1, MAP2K5/SKOR1, and PTPRD. Ferritin and C-reactive protein (CRP) levels were measured in serum, and ferritin levels corrected for inflammation using CRP levels.
Results: After controlling for cofactors, PLMI ≥ 15 was associated with low (≤50 ng/mL) ferritin levels (OR = 1.55, $p = 0.020$). The best model was found using quasi-least squares regression of ferritin as a function of PLMI, with an increase of 0.0034 PLM/h predicted by a decrease of 1 ng/mL ferritin ($p = 0.00447$).
Conclusions: An association was found between low ferritin and greater PLMs in a general population of older adults, independent of genetic polymorphisms, suggesting a role of low iron stores in the expression of these phenotypes. Patients with high PLMI may require to be checked for iron deficiency.

1. Introduction

Periodic leg movements (PLMs) are repetitive, involuntary movements that occur during sleep. PLMs are found to be highly associated with restless legs syndrome (RLS), with 80–90% occurring in RLS patients [1,2]. Much research has been done in order to understand the physiology of PLMs in RLS. In many cases, however, PLMs are observed to be in isolation of RLS symptoms, notably in older adults, and there is current debate in the community on whether these can have health or behavioral consequences, such as cardiovascular problems [3] and daytime sleepiness [4,5]. For example, in the Wisconsin Sleep Cohort (WSC), a sample of older adults of mean age 59.6 ± 8.0 years, a prior study found that one-third had a periodic leg movement index (PLMI) of above 15/h [6]. Similar figures are found in Osteoporotic Fractures in Men (MrOs), a sample of older males [7]. PLMs are also known to be associated with many other disorders and pathologies, such as depression, rapid eye movement behavior disorder (RBD), narcolepsy, and Parkinson's disease [8–10].

RLS is a sensorimotor disorder affecting up to 10% of the population [5,11]. It is characterized by an urge to move the legs accompanied by uncomfortable sensations in them, worsening at night and times of rest [11]. RLS is typically categorized into primary (idiopathic) and secondary cases. Secondary RLS is typically associated with low iron stores, anemia, or kidney disease [12,13]. Low levels of peripheral iron stores likely play a role in causing RLS in these pathologies, with ferritin levels below 50 ng/mL (in the absence of associated inflammation that increases ferritin levels) used as a common cutoff to recommend iron supplementation. Iron supplementation may reduce RLS symptoms in patients with ferritin levels below 45 ng/mL [13,14].

Reduced brain iron level is believed to play a role in idiopathic RLS, as low levels of iron in the substantia nigra are reported in these patients [12]. Further, reduced ferritin levels in cerebrospinal fluid (CSF) have been found in idiopathic RLS [13,14]. In these subjects, serum ferritin and CSF ferritin levels are correlated in both RLS and

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non-RLS patients, although the correlation is stronger in non-RLS patients [14].

In spite of these findings, it has been difficult to reconcile known genetic association results with the iron-deficiency hypothesis, which involves mostly developmental genes [15]. Polymorphisms associated with abnormal ferritin levels in genome-wide association studies do not correlate with RLS [16]. Therefore, low iron levels are universally involved in the pathology of both primary and secondary RLS, although this is debated, and may involve effects in the central nervous system.

The gold standard for determining iron deficiency is examination of the bone marrow for absent iron stores. Because of the invasiveness of the procedure and the unavailability of the patients, blood tests are used instead. Determining serum ferritin level in blood has been shown to be the best screening test for evaluating iron deficiency in older people [17]. Iron deficiency, in the context of RLS, is usually defined as serum ferritin <50 ng/mL, although in a broader clinical context it is defined as below 15–30 ng/mL. Serum ferritin is an acute phase reactant, and in patients with acute or chronic inflammation, it may be artificially elevated [18], which obscures iron deficiency. For example, a patient with inflammation or liver disease can be iron deficient when ferritin is 70 ng/mL, as opposed to 45 ng/mL in the general population [19]. As a result, many studies account for elevated ferritin levels by assaying transferrin saturation, which stays relatively constant throughout the inflammatory response, or C-reactive protein (CRP), another acute phase reactant.

Whereas much is known in the area of clinical RLS and iron regulation, virtually nothing is known regarding the relationship of iron status with PLMs, and even less so in a population-based sample. There has been only one study showing a negative correlation between iron levels and PLMs, that too in only a small sample of children [20]. In this study, we used the WSC, a cohort of older adults, to explore the possible relationship of low ferritin levels with PLM events and RLS symptoms.

2. Methods

2.1. Demographics

The WSC study is an ongoing longitudinal community-based study designed to investigate sleep patterns and problems. The University of Wisconsin-Madison Health Sciences Institutional Review Board approved WSC protocols and informed consent documents. A random sample of people aged 30–60 years was taken in 1988 from a sampling frame of Wisconsin state employees. They were mailed questionnaires every five years inquiring their sleep and medical history. A subsample was studied every four years by in-laboratory polysomnography, which coincided with a blood draw. In this study, we included a cross-sectional sample from the WSC of 801 people, of which a maximum of two observations (separated by no more than four years) were allowed for a total of 1008 observations. The mean age of the people in this subsample at mid-observation was 58.6 ± 0.3 years.

2.2. Periodic leg movement index

PLMs occur most frequently in the lower limbs. Typically they involve extension of the big toe and partial flexion of the ankle, knee, and hip. Current sleep scoring standards define leg movements as muscle contractions lasting 0.5–10.0 s as measured via electrodes placed on the left and right and anterior tibialis [21,22]. *Although not ideal*, separate leg signal recordings may be combined to form a single channel for leg movement analysis in some clinical settings [21], as is the case with the WSC. PLMs are evident when four or more leg movements occur within a span of 5–90 s, provided each leg movement is separated by a minimum of 5 s from the next and is not the result of respiratory effort (eg, apnea) [21,22].

These movements may be associated with an autonomic arousal, a cortical arousal, or an awakening. PLMs are highly associated with RLS. Five or more PLMs occur per hour in 80–90% of RLS patients. PLMI is calculated by dividing the total number of PLMs by sleep time in hours.

In this study, we used the Stanford PLM Automatic Detector (S-PLMAD), an automatic detector of PLMs that was validated in the WSC and a clinical sample in relation to a gold standard [23]. PLM indices derived from the detector, which do not include arousal-associated PLM events, correlated with self-reports of RLS symptoms in the cohort and with most SNPs previously shown to be associated with RLS [6]. The detector was optimized to remove false signals from leg channels, such as ECG contamination and fragmentary myoclonus-like patterns. Furthermore, respiratory exclusion rules for removing leg movements associated with apneas and hypopneas were modified after discovering that those given in current standards exhibited poor boundary definitions: overly excluded leg movements during respiratory events and under-excluded leg movements before or after the event [23]. A similar finding was also recently reported by Manconi et al. [20].

2.3. RLS symptoms

Patients in the WSC were identified as having RLS symptoms by an existing questionnaire response from them [24]. The questionnaire did not address all required diagnostic criteria [11]; notably, it did not ask if the symptoms were worse at night. For this reason, patients were designated as positive for these questions as having "RLS symptoms" and data used as such in prior published studies. The questionnaire inquired patients to provide the frequency with which they felt (a) repeated urge to move legs, (b) strange and uncomfortable feelings in the legs, and (c) the duration of several leg jumps or jerks. The choice of frequency included never, less than once a month, monthly, weekly, and nightly. If the patient answered "never" for these questions or they skipped ahead, two more questions were presented: (d) Do these feelings get better when you get and start walking? (e) Do these feelings disrupt your sleep? On the basis of extensive prior analysis, we defined four categories from these responses. Category A ($n = 184$), definite RLS symptoms, was defined by responses (a) weekly or more often, (d) yes, and (e) yes. Category B ($n = 185$), possible RLS symptoms, was defined by responses (a) monthly or more frequent and (d) yes. Category B could not include members in Category A. Category C ($n = 515$), no RLS symptoms, was defined by responses (a) less than monthly and (b) missing or less than monthly. Category D ($n = 171$), unknown or uncertain, included the remaining response possibilities and cases with missing responses. Because all correlations were similar in patients with RLS Category A or B, patients in these groups were considered to have "possible RLS symptoms." Category C, which did not endorse any symptom, was considered to have patients with "negative RLS symptoms." Patients from Category D were excluded from any analysis where RLS symptoms were used as covariates. Because we believe that RLS symptoms are poorly characterized in this cohort, our work with the cohort focuses on PLMs.

2.4. Other parameters

Additional parameters of interest were included in the analysis when they were known correlates of RLS, PLMs, or ferritin based on the results of prior studies, including those from this cohort. Drugs that exacerbated RLS symptoms were grouped together: antidepressants, antipsychotics, tricyclic antidepressants, selective serotonin reuptake inhibitors (SSRI), SSRI antagonists, and antihistamines. Drugs that inhibited RLS symptoms were benzodiazepines, opioids,

Parkinson's medication, and anticonvulsives/gabapentin. Blood pressure medication was its own category.

2.5. RLS-associated polymorphisms

A total of 14 SNPs from six loci, BTBD9 (chromosome 6), TOX3/BC034767 (chromosome 16), MEIS 1 (chromosome 2, two loci), MAP2K5/SKOR1 (chromosome 15), and PTPRD (chromosome 9) were used. Genotyping for 13 of these SNPs was performed on the MassARRAY system using Matrix-Assisted Laser Desorption/Ionization Time-of-Flight (MALDI-TOF) mass spectrometry with the iPLEX Gold chemistry (Sequenom Inc., San Diego, CA, USA), as reported by Winkelmann et al. [15]. The 14th SNP, rs11693221(T), is a MEIS1 polymorphism recently shown to be more strongly associated with RLS than previously reported rs6710341(G) or rs2300478(G) [25]. Genotypes from this SNP were derived from imputing Genome-Wide Data from the cohort using Affymetrix 6.0 arrays.

2.6. Biochemical assays

All ferritin measurements were taken within four years of response to the RLS questionnaire ($n = 1191$). Furthermore, repeated ferritin measurements (when taken) occurred within four years of each other, coinciding with repeated sleep studies. Blood sampling was performed the morning after the patient's sleep study. A ferritin enzyme-linked immunosorbent assay (ELISA) kit (Eagle Biosciences, Nashua, NH, USA) was used for the quantitative determination of ferritin in serum. A human CRP ELISA kit (Alpha Diagnostic International, San Antonio, TX, USA) was used to quantitatively measure CRP in serum. Both assays were performed in duplicate. Ferritin and CRP are both acute phase reactants, and elevated levels of CRP are an indication of inflated levels of ferritin. In order to completely remove the influence of inflammation, ferritin values with high CRP levels (i.e., >5000 μg/L) were excluded ($n = 300$ observations in 228 patients).

2.7. Statistical analysis

All statistical analyses were performed using SAS 9.3 (SAS Institute, Cary, NC, USA) and MATLAB (MathWorks, Natick, MA, USA). Generalized estimating equations (GEE) were used to optimize power in the presence of patients with multiple observations, and mixed models were used to study association between ferritin, RLS, and PLMI, with a robust covariance matrix for estimating significance and effect sizes. We used GENMOD procedure in SAS and GEEQBOX toolbox [26] in MATLAB. GEE with a Markov correlation structure assumes that the correlation of repeated measurements depends on their timing, which was patient age in this study. GEEQBOX estimates the correlation parameter for this structure using quasi-least squares (QLS) [26]. A two-tailed $p \leq 0.05$ was considered statistically significant.

PLMI was studied as the outcome as both a continuous and a dichotomous variable (with categories of PLMI ≤ 15 and PLMI > 15). A 15 PLM/h cutoff was selected because it is commonly used in clinical studies, especially considering the older age of our population. Prior studies in the cohort have explored other cutoffs for PLM and RLS or SNP association with similar results [6]. In order to examine the effect of ferritin on PLMI, it was used as either a continuous or a categorical variable (levels ≥50 vs. <50 ng/mL). GEE models and χ^2 tests were used to analyze categorical data. Associations of categorical variables were expressed as odds ratios (OR) with 95% confidence interval (CI). Sex, age, body mass index (BMI), drugs exacerbating and inhibiting RLS symptoms, depression, RLS category, and genetic factors significant for RLS in this cohort were examined as potential confounders. On the basis of prior studies, RLS symptoms were split into RLS A + B (definitely RLS and RLS possible) and RLS C (no RLS) [6]. BMI was calculated as kilograms per meter squared.

3. Results

3.1. Demographic and clinical data

In order to determine the factors associated with low ferritin levels, we compared patients with ferritin <50 ng/mL and patients with ferritin >50 ng/mL, as this value is often used clinically (Table 1). As mentioned earlier, samples with high CRP were removed. A total of 81 patients with two time measurements had ferritin values above 50 ng/mL at one time point and below 50 ng/mL at the other time point; these were removed from Table 1, leading to 801 patients at one or two time points per patient or a total of 1008 observations (Table 1). Mean age, apnea–hypopnea index (AHI), BMI, and PLMI were used for patients with two observations while depression or use of relevant medication was defined by the presence of depression or use of medication at either time point.

Patients with low ferritin were younger, more frequently female in this cohort, and had slightly less sleep-disordered breathing. They also scored higher on the Zung depression scale, and took more medication known to exacerbate RLS (including antidepressants). In unadjusted comparisons, PLMI and RLS were comparable in patients with low ferritin and high ferritin (Table 1). Patients with low ferritin also took less blood pressure medication, although they did not differ much in percentage of patients with high blood pressure. RLS polymorphisms known to affect RLS and PLMI in this cohort were also examined by ferritin category (Table 1), with no significant findings.

We next classified our subsample into those with PLMI <15 ($n = 551$ patients totaling 717 observations) and ≥15 ($n = 189$ patients totaling 246 observations) (Supplementary Table S1). Similar to what was done earlier for patients switching ferritin categories, patients with PLMI switching from above to below 15/h at either time points were excluded from this table. Using this observation, we found that people in the high PLMI category were older and more male (Supplementary Table S1) as found in the entire cohort [24]. Examining cardiovascular health factors, there was higher usage of blood pressure medication with high PLMI. All other factors were not found to differ significantly, including BMI (Supplementary Table S1). As reported earlier in this sample with a larger number, many RLS SNPs were associated with high PLMI (Supplementary Table S1) as reported in the entire cohort [6]. The most significant SNPs at each loci were BTBD9, rs3923809(A); MEIS1, rs12469063(G); and TOX3/BC034767, rs3104788(T). These covariates were used in subsequent models exploring the effects of ferritin on PLMI. Factors that differed significantly in cases with and without low ferritin levels, such as AHI, were also considered as covariates, but did not affect results (data not shown). Of note, we also explored whether high PLMI was associated with high CRP, but did not find any relation (data not shown), including for patients with PLMI ≥ 45, in contrast to a smaller prior study [3].

Finally, we also grouped patients into RLS-likely ($n = 328$) and RLS-negative ($n = 428$), and excluded patients who had "unknown" RLS status (Supplementary Table S2). Medication that aggravates and inhibits RLS factors was more common in the RLS-likely group. There were no SNPs that were significantly increased in patients with RLS [6]. These covariates were used in subsequent models exploring the effects of ferritin on RLS symptoms. As discussed in prior publications [6,23], we realize that RLS symptoms were poorly ascertained in this cohort; thus, these analyses were only exploratory and are reported as supplementary material.

Table 1
Descriptive data by ferritin category.

	Ferritin ≤50 (*o* = 248, *n* = 201)	Ferritin >50 (*o* = 760, *n* = 600)	All (*o* = 1008, *n* = 801)	Ferritin ≤50 vs. Ferritin >50
Demographics				
Age	56.6 ± 0.5	59.2 ± 0.3	58.6 ± 0.3	***p* < 1e–4**
Sex, Male (*n*)	35.30%	61.20%	54.70%	**OR = 0.35, *p* < 1e–9**
Clinical Data				
Body Mass Index (kg/m^2)	32.0 ± 0.6	31.5 ± 0.3	31.6 ± 0.2	*p* = 0.398
Apnea–Hypopnea Index	11.0 ± 1.0 (189)	14.3 ± 0.7 (564)	13.4 ± 0.6 (753)	***p* = 0.011**
PLMI	11.8 ± 1.2	10.4 ± 0.6	10.8 ± 0.5	*p* = 0.294
RLS (AB)	34.80%	37.20%	36.60%	OR = 0.90, *p* = 0.551
Depression	31.80%	20.80%	23.60%	**OR = 1.78, *p* = 0.001**
Medication				
RLS symptom aggravators	44.30%	29.00%	32.80%	**OR = 1.95, *p* < 1e–4**
RLS symptom inhibitors	11.40%	10.30%	10.60%	OR = 1.12, *p* = 0.658
Blood pressure	33.80%	40.20%	38.60%	OR = 0.76, *p* = 0.110
SNP				
BTBD9(6)				
rs9357271(T)	0.775 (109)	0.812 (312)	0.803 (421)	OR = 0.80, *p* = 0.234
rs9296249(T)	0.774 (106)	0.808 (295)	0.799 (401)	OR = 0.81, *p* = 0.277
rs3923809(A)	0.712 (111)	0.734 (312)	0.728 (423)	OR = 0.89, *p* = 0.522
MAP2K5/SKOR1(15)				
rs6494696(G)	0.691 (110)	0.700 (312)	0.698 (422)	OR = 0.96, *p* = 0.794
MEIS1(2)				
rs2300478(G)	0.248 (107)	0.238 (305)	0.240 (412)	OR = 1.06, *p* = 0.769
rs12469063(G)	0.252 (111)	0.230 (313)	0.236 (424)	OR = 1.13, *p* = 0.503
rs6710341(G)	0.140 (111)	0.139 (314)	0.139 (425)	OR = 1.01, *p* = 0.967
rs11693221(T)	0.058 (104)	0.044 (282)	0.048 (386)	OR = 1.32, *p* = 0.441
no gene(2)				
rs6747972(A)	0.450 (110)	0.446 (313)	0.447 (423)	OR = 1.02, *p* = 0.912
PTPRD(9)				
rs1975197(A)	0.177 (110)	0.151 (311)	0.158 (421)	OR = 1.21, *p* = 0.361
rs4626664(A)	0.186 (110)	0.129 (311)	0.144 (421)	OR = 1.55, *p* = 0.036
TOX3/BC034767(16)				
rs3104788(T)	0.568 (110)	0.562 (314)	0.564 (424)	OR = 1.03, *p* = 0.876
rs3104774(G)	0.575 (107)	0.564 (306)	0.567 (413)	OR = 1.05, *p* = 0.779
rs3104767(G)	0.568 (110)	0.557 (314)	0.560 (424)	OR = 1.05, *p* = 0.780

Subject descriptions are presented ferritin level (≤50 ng/mL as cutoff); observations where CRP levels above 5000 μg/L have been excluded. Subjects may have at most two observations. In the case of two observations, the mean value is used to group the subject. In cases of medication use and depression for multiple observations, the subject's value is taken as the positive occurrence of depression or medication use in either observation. See text for further description. Values are mean ± SEM for continuous variables and percentage for categorical variables.
Abbreviations: OR, odds ratio; PLMI, periodic leg movement index; RLS, restless legs syndrome; SNP, single-nucleotide polymorphism; n, subject count; o, observation count.

3.2. Influence of low ferritin on RLS symptoms and PLMI

Supplementary Table S3 reports on the effect of ferritin as a dichotomous variable (with a cutoff of 50 ng/mL) on the presence of possible RLS symptoms (dependent variable). Average ferritin levels did not differ significantly between the RLS-likely and RLS-negative categories, even after correction for covariates (data not shown).

Table 2 reports on the influence of ferritin on PLMI as a dependent, dichotomous variable (PLMI cutoff of 15/h), after controlling for covariates identified in Supplementary Table S1. As a dichotomous variable, PLMI ≥ 15 was associated with low ferritin (≤50 ng/mL) both with (OR = 1.53, *p* = 0.009) and without (OR = 1.35, *p* = 0.04) adjustment for RLS symptoms. Table 3 shows the relationship between PLMI and ferritin as continuous variables. QLS regression indicated a significant association between PLMI and ferritin ($\beta = -1.68$, *p* = 0.0192), with an increase of 1 PLM/h resulted in a decrease of 0.44 ng/mL in ferritin when controlling for covariates. The association was also significant when the model was further adjusted for RLS symptoms ($\beta = -1.50$, *p* = 0.0431), with an increase of 1 PLMI per 0.49 ng/mL decrease in ferritin.

Considering the longitudinal nature of the study, we finally investigated whether decreases in ferritin within individuals resulted in changes in PLMI. In addition to the patients with multiple

Table 2
GEE model results of high PLMI (15 PLM/h) as predicted by low ferritin (50 ng/mL) with and without removing observations due to inflammation† and then adjusting for covariates* and RLS symptoms.

	Observations	Beta	OR	*p*
Ferritin†	1191	0.25	1.28 (0.99,1.57)	0.094
Ferritin	891	0.31	1.36 (1.02,1.70)	0.077
Ferritin after adjusting for covariates*	854	0.44	1.55 (1.18,1.91)	0.020
Ferritin after adjusting for covariates* and presence of RLS symptoms	726	0.47	1.60 (1.19,2.01)	0.025

General estimating equations (GEE) model with high PLMI as the dependent variable. Odds ratios (OR) are shown with 95% confidence interval in parentheses.
Abbreviations: PLMI, periodic leg movement index; CRP, C-reactive protein; OR, odds ratio; RLS, restless legs syndrome. See text for definition of RLS symptoms.
† Observations include cases where CRP levels exceed 5000 μg/L.
* Covariates include age, sex, medications (blood pressure), and single-nucleotide polymorphisms (rs3923809, rs3104788, and rs12469063).

Table 3
Quasi-least squares regression of PLMI as a function of ferritin and covariates.

Response variable and covariates	Observations	Beta	*p*
PLMI adjusted for covariates*	891	–0.0034	0.00447
PLMI adjusted for covariates* and presence of RLS symptoms	760	–0.0029	0.00850

Quasi-Least Squares (QLS) regression with ferritin as an independent variable covaried with confounding factors and PLMI as the dependent variable.
Abbreviations: PLMI, periodic leg movement index; CRP, C-reactive protein; OR, odds ratio; RLS, restless legs syndrome. See text for definition of RLS symptoms.
* Covariates include age, sex, use of blood pressure medication, and single-nucleotide polymorphisms (rs3923809, rs3104788, and rs12469063).

observations listed in Table 1 ($n = 207$), we also included the 81 patients who changed ferritin levels between repeated measurements (see Section 3.1), but found no significant association ($n = 288$, $r = 0.046$, $p = 0.434$). The fact that PLMI levels across time points are highly correlated ($n = 288$, $r = 0.664$, $p < 10^{-37}$), as are those of ferritin across 4 years ($n = 288$, $r = 0.258$, $p < 10^{-5}$), likely explains the lack of significance in the longitudinal model.

4. Discussion

This study is the first evidence for the presence of a significant association between iron deficiency, as measured by low ferritin levels, and increased PLMI in the general population, independent of genetic polymorphisms. In agreement with this finding, a prior study in children found a significant association of PLMI with low serum iron, and nonsignificantly lower ferritin levels [20]. In our study, the effect was statistically significant but the effect size was small, predicting an increase of 0.0034 PLM/h for the decrease of every 1 ng/mL of ferritin. This effect was observed in the GEE model using ferritin as a dichotomous variable. It is likely that separating ferritin into ≤50 ng/mL and >50 ng/mL categories is more meaningful than using the entire spectrum of ferritin values with the linear model. This cutoff is used clinically, so it has heuristic value.

We could not find a significant association between iron deficiency and RLS symptoms, with or without control of covariates. Iron deficiency has long been established as a secondary cause of RLS, although evidence for population-based association of iron deficiency with RLS is lacking. The likely explanation for this unanticipated result is measurement error for RLS symptoms, as our questionnaire did not address all RLS symptoms, and hence ascertainment was poor [6,23]. By contrast, measuring PLMI (a partial correlate of RLS), an objective PSG parameter, is likely more accurate than a subjective, nonclinical-based assessment of RLS, explaining why we found a clear association of PLMI with low ferritin.

As reported in the genome-wide association study (GWAS) of ferritin levels, we did not find strong genetic associations between low ferritin and any RLS-associated SNPs, suggesting these genes do not have an impact on ferritin directly [27]. Although Stefansson et al. found lower ferritin in RLS patients with BTBD9 rs3923809 (A) [28], the association was only within patients with RLS, suggesting that maybe the low ferritin interacts with the genetic polymorphism to exacerbate RLS symptoms, raising them to the level of severity consistent with a clinical diagnosis. Overall, RLS-associated polymorphisms in this study were most strongly associated with PLMI, not ferritin or RLS symptoms.

In summary, we found an association between low ferritin and increased PLMI in the WSC. A cutoff of 50 ng/mL for ferritin level is appropriate to define the low iron levels that may have an impact on PLMI in patients with a specific genetic background. Measuring CRP is however needed to exclude high or normal ferritin value in the presence of iron deficiency because of inflammation. By contrast, we were still unable to find a relationship between iron deficiency and RLS symptoms, probably because this phenotype was poorly ascertained. Overall, our results support the concept that RLS–PLMI is associated with low ferritin level independent of genetic factors. Patients with high PLMI may require ferritin screening to prevent iron deficiency.

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Conflict of interest

The authors have indicated no financial conflicts of interest.

The ICMJE Uniform Disclosure Form for Potential Conflicts of Interest associated with this article can be viewed by clicking on the following link: http://dx.doi.org/10.1016/j.sleep.2015.05.015.

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Appendix: Supplementary material

Supplementary data to this article can be found online at doi:10.1016/j.sleep.2015.05.015.

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