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Original Article

Iron deficiency anemia in infancy exerts long-term effects on the tibialis anterior motor activity during sleep in childhood

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ABSTRACT

Objectives: To explore the eventual connection between iron deficiency anemia (IDA) in infancy and altered leg movements during sleep in a 10-year follow-up study in children who did or did not have IDA in infancy.
Subjects and methods: Polysomnographic studies were performed in 32 10-year-old children (13 females and 19 males) who had IDA in infancy and 26 peers (10 females and 16 males) who were nonanemic controls. The time structure of their polysomnographically recorded leg movements (LM) was analyzed by means of an approach particularly able to consider their quantity, periodicity, and distribution during the night.
Results: All LM indexes and those related to periodic LM during sleep (PLMS) were slightly higher in the former IDA group than in the control group, but not always significant. The Periodicity index during NREM sleep was higher and was reflected by a small but significant increase in PLMS separated by 10–50 s intervals. PLMS index tended to be higher in former IDA children than in controls throughout the whole night.
Conclusion: The association between IDA in infancy, despite iron therapy, and PLMS in childhood could lead to new research in this area. Indeed, transient infantile IDA, a common nutritional problem among human infants, may turn out to be important for understanding the mechanisms of PLMS or restless legs syndrome, which are common in adulthood.

1. Introduction

Iron deficiency (ID) is prevalent in human infants, especially in the late infancy/toddler period (between the ages of six and 24 months) [1–5]. There is mounting evidence that ID affects motor activity, not only in animal studies [6–12], but also in humans. In the adult human, maximal physical performance, submaximal endurance, and work productivity have been shown to be reduced in iron deficiency anemia (IDA) and, in some cases, in ID without anemia [13–17]. The few published studies of ID and motor activity in school-aged children report decreased activity while awake [18]. In the human infant Lozoff et al. [19] found decreased activity in a study of play behavior and mother-infant interaction. In another study of 12- to 18-month-old undernourished infants with IDA, Harahap et al. [20] found reduced Bayley motor scores and motor activity scores, in comparison to controls, before iron therapy. Following iron treatment, the activity of IDA infants increased to a larger extent than controls. Using actigraphs, in a laboratory setting, Angulo-Kinzler et al. [21] found decreased frequency of motor activity at 12 and 18 months in infants who were anemic (IDA) at six months and received iron treatment beginning at that age. These results were limited to a short-term period of observation (activity during the waking time immediately preceding and following an afternoon nap in the laboratory). Using 24-h actigraphic recordings in the home, Angulo-Kinzler et al. [22] reported that six-month-old infants with IDA showed an overall increase in motor activity compared to controls, with actigraphs worn on the ankle; differences were no longer observed at 12 and 18 months of age after effective iron supplementation treatment. We hypothesized that increased leg activity during the period of IDA might indicate a shared underlying mechanism with restless legs syndrome (RLS). RLS is a sensorimotor dysfunction where ID is suspected to play an important pathogenic role. ID might trigger RLS symptoms and might worsen a pre-existing RLS form, and iron

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supplementation can ameliorate them [23], but its relation to IDA in infancy has not been investigated.

We had a chance to explore a connection between IDA in infancy and altered leg movements in a 10-year follow-up performed at INTA, University of Chile, within an ongoing collaborative research project with University of Michigan. We studied motor activity during sleep as recorded from the tibialis anterior muscles of 10-year-old children who did or did not have IDA in infancy. We focused on the so-called periodic leg movements during sleep (PLMS), a typical sleep-related motor phenomenon usually found in more than 80% of affected individuals with RLS [24]. We analyzed PLMS by means of a new approach that has been shown to be particularly able to describe the periodicity of this motor pattern [25].

2. Subjects and methods

2.1. Subjects

The children in this study are participants in ongoing research in Chile on the behavioral, developmental, neurosensory, cognitive, and sleep–wake patterns effects of IDA in infancy. Detailed descriptions of the population and findings during infancy have been published elsewhere [26,27]. In brief, study participants were healthy full-term infants (birth weights $\geqslant$3.0 kg, no perinatal complications, and no acute or chronic illnesses). Infants were assessed for IDA at 6, 12, and 18 months. Anemia was defined as venous hemoglobin $\leqslant$100 g/L at six months and <110 g/L at 12 and 18 months (for details see Lozoff et al. [26]). ID was defined as two of three iron measures in the iron-deficient range (mean cell volume <70 fl, erythrocyte protoporphyrin >100 μg/dL red blood cells [1.77 μmol/L], serum ferritin <12 μg/L). For each IDA infant, an infant of the same age who was clearly nonanemic (venous hemoglobin $\geqslant$115 g/L) was randomly selected. They constituted the "control" group. Six-month-old infants were treated for one year with 15 mg/day of elemental iron as oral ferrous sulphate; infants identified at 12 or 18 months were treated with oral iron (30 mg/day) for a minimum of six months. Given that IDA in infancy was very common in the population at the time, infants from the control group underwent the same iron treatment in order to assure they did not become anemic with advancing age.

As part of the 10-year-old follow-up, we compared tibialis anterior activity patterns during sleep between 32 children (13 females and 19 males) who had IDA in infancy (former IDA) and 26 children (10 females and 16 males) who were nonanemic controls. All were healthy and not taking any medications at the time of polysomnographic recording. The sex distribution was not different between the two groups (Chi-square = 0.001, *d.f.* = 1, $p = 0.963$). Table 1 shows the background characteristics of the groups. There were no statistically significant differences in child or family factors, including attention deficit and hyperactivity disorder (ADHD) subtype (aggressive, impulsive, hyperactive) score and apnea/hypopnea index (AHI) in children, as well as the prevalence of a positive family history of RLS in one or both biologic parents.

The research protocol was approved by the Institutional Review Boards of the University of Michigan Medical Center, Ann Arbor, of INTA, University of Chile, Santiago, and of the Office of Protection from Research Risks, NIH. Parental signed informed consent and child assent was obtained.

2.2. Nocturnal polysomnography

Subjects were allowed to sleep until they spontaneously woke up in the morning. Lights-out time was based on individual habitual bed time and ranged between 9:30 and 11:30 p.m. The following signals were recorded during polysomnography: EEG (six channels, F4, C4, O2, F3, C3, O1, referred to the contralateral earlobe); electrooculogram (electrodes placed 1 cm above the right outer cantus and 1 cm below the left outer cantus and referred to A1), electromyogram (EMG) of the submentalis muscle, ECG (one derivation), body position, and temperature. EMG of the right and left tibialis anterior muscles (bipolar derivations with two electrodes placed 3 cm apart on the belly of the anterior tibialis muscle of each leg, impedance was kept less than 10 KΩ), according to the WASM-IRLSSG scoring criteria [28]. Sleep signals were sampled at 200 Hz and stored on hard disk in European data format (EDF, see Kemp et al. [29] for details) for further analysis. EMG signals, in particular, were digitally band-pass filtered at 10–100 Hz, with a notch filter at 50 Hz. The sleep respiratory pattern of each patient was also monitored using nasal pressure cannula and oral airflow thermistor, abdominal respiratory effort strain gauge, snoring by microphone, and by monitoring oxygen saturation (pulse-oxymetry). Subjects with an AHI $\geqslant$5 were not included. All sleep measures were acquired and processed without knowledge of whether a given child was former IDA or control.

2.3. Sleep scoring and detection of leg movements

Prior to any recording we verified that the EMG amplitude recorded from the two tibialis anterior muscles was below 2 μV at rest and exceeded 7–10 μV for small voluntary flexions of the foot. EMG amplitude at maximal deflection was also measured for the application of the WASM-IRLSSG scoring criteria [28]. In all subjects, sleep was subdivided into 30-s epochs, and sleep stages were

Table 1
Background characteristics of the two groups of subjects studied.

	Former IDA (n = 32)	Controls (n = 26)	p Value
Males, n (%)	19 (59.4%)	16 (61.5%)	NS
Age at 10 year testing (years)	10.08 (0.11)	10.05 (0.06)	NS
BMI (kg/m^2)	19.00 (3.52)	19.70 (3.43)	NS
Birth weight (g)	3467.8 (362.2)	3558.5 (411.8)	NS
Gestational age (weeks)	39.34 (0.94)	39.38 (0.94)	NS
Cow milk/formula consumption[a] (ml/day)	301.1 (138.0)	235.3 (167.2)	NS
Iron sufficient at 10 years, n (%)	29 (93.6%)	23 (92.0%)	NS
Parental RLS[b] (%)	14.8	10.8	NS
Apnea/hypopnea index	0.58 (0.62)	0.54 (0.43)	NS
ADHD score[c]	11.2 (4.1)	12.7 (3.5)	NS

Means (S.D.) are shown, unless otherwise noted; p values are from t-tests for continuous variables, and from chi-squared test for categorical variables; the number of subjects vary slightly due to occasional missing data in some measures. BMI = body mass-index.
NS = not significant differences.
[a] This is the average daily intake throughout the supplementation study, that is, from six to 12 months; RLS = Restless legs syndrome.
[b] In one or both biologic parents; ADHD = Attention-deficit hyperactivity disorder.
[c] Subtype aggressive, impulsive, hyperactive.

scored according to the standard criteria by Rechtschaffen and Kales [30] using the sleep analysis software Hypnolab 1.2 (SWS Soft, Italy). Leg movements (LMs) during sleep were first detected by the same software that allows their computer-assisted detection. With this software, the detection is performed using a human-supervised automatic approach controlled by the scorer that uses the WASM-IRLSSG scoring criteria [28]. The performance of this system has been evaluated and validated [31]. In this study one scorer also visually edited the detections proposed by the automatic analysis before computing a final result. The total LM index was calculated as the total number of LMs per hour of sleep. The PLMS index was calculated as the number of LMs in a series of four or more, separated by more than 5 and less than 90 s, per hour of sleep [28].

2.4. Further analysis of leg movement architecture

In this analysis we used the newly described approach to study LM periodicity [25]. These parameters are in line with those proposed by the WASM-IRLSSG [28] and include, in particular, the Periodicity Index computed as the number of sequences of three inter-LM intervals $10 < i \leqslant 90$ s/total number of inter-LM intervals [25]. This index can vary between zero (absence of periodicity) to one (all intervals with length $10 < i \leqslant 90$ s). Periodicity index is independent of the absolute number of LMs recorded.

2.5. Statistical analysis

The comparison between the different sleep scoring and LM parameters obtained from children with or without IDA in infancy was carried out by means of the Student's *t*-test for independent data sets after controlling for the normality of distribution of data. The comparison between groups in the number of PLMS during each hour of the night was performed using a zero-inflated Poisson regression model [32]; this approach was chosen due to the excess number of zero PLMS for individual hours of the night. Statistical analyses were conducted using SAS and STATISTICA data analysis software system version 6. (StatSoft Inc., 2001. www.statsoft.com).

3. Results

3.1. Sleep architecture

Table 2 reports the sleep architecture parameters in the two groups of children. Former IDA children showed a longer sleep latency and lower number of stage shifts and awakenings than controls. However, these differences did not pass the conservative Bonferroni correction.

3.2. Leg motor activity

Table 3 reports the parameters derived from the analysis of the tibialis anterior EMG activity. All LM indexes and those related to PLMS were slightly higher in former IDA subjects than in controls. However, only the differences in total and NREM sleep PLMS index reached statistical significance. Former IDA subjects showed significantly longer LM than controls in both REM and NREM sleep; however, none of these differences remained significant after the Bonferroni correction. The total sleep Periodicity index and that calculated for NREM were significantly higher in former IDA children than in normal controls; also, these differences did not pass the conservative Bonferroni correction for multiple comparisons.

3.3. Analysis of inter-LM intervals

Fig. 1 shows the distribution of inter-LM intervals in former IDA children and controls. Former IDA children showed higher values than normal controls for several interval classes depicted in this figure, mostly for those in the range between 10 and 50 s. Consistent with the results obtained for the Periodicity index (Table 3), statistically significant differences were found for some of these interval classes, as indicated by asterisks.

3.4. Night distribution of LMs

Fig. 2 compares the distribution of the number of PLMS and isolated LM and total LM per hour of sleep (first eight hours shown) between former IDA and control children. PLMS tended to be higher in former IDA children than in controls throughout the whole night. The differences were statistically significant for the first, third, sixth, seventh, and eighth hour of recording and marginally significant for the fourth hour ($p = 0.071$). In general, the differences between the two groups seemed to be more evident during the last three hours of the night. Both groups showed a trend for the number of PLMS to decrease throughout the night. There were no group differences for the distribution of isolated LMs, which showed a similar number in all hours of the night.

4. Discussion

The main finding of this study is that 10-year-old children who experienced IDA in infancy showed a mild but significant increase of tibialis anterior EMG activity during sleep when compared to

Table 2
Comparison between sleep architecture parameters obtained in the two groups of children.

	Former IDA ($n = 32$)		Controls ($n = 26$)		Student's *t*-test	
	Mean	S.D.	Mean	S.D.	*t*	*p*<
Time in bed, min	524.6	67.56	533.8	64.14	−0.521	NS
Sleep period time, min	466.6	60.57	492.4	62.03	−1.593	NS
Total sleep time, min	435.3	72.25	444.4	67.55	−0.488	NS
Sleep latency, min	37.2	35.13	19.3	18.19	2.320	0.024
REM sleep latency, min	161.7	67.67	136.2	61.35	1.477	NS
Stage shift/hour	4.9	1.79	6.5	3.02	−2.491	0.016
Awakenings/hour	1.0	1.25	2.3	2.78	−2.422	0.019
Sleep efficiency, %	83.3	11.07	83.5	9.75	−0.056	NS
Wakefulness after sleep onset, %	6.8	9.15	9.6	9.19	−1.171	NS
Sleep stage 1, %	7.7	3.88	7.7	3.29	0.028	NS
Sleep stage 2, %	47.8	8.23	43.6	9.28	1.816	NS
Slow-wave sleep, %	21.6	5.76	22.7	6.64	−0.687	NS
REM sleep, %	16.1	5.24	16.3	5.48	−0.165	NS

NS = not significant.

Table 3
Comparison between the LM activity parameters obtained in the two groups of children.

	Former IDA (n = 32)		Controls (n = 26)		Student's t-test	
	Mean	S.D.	Mean	S.D.	t	$p<$
NREM sleep						
Total LM, index	15.4	8.48	11.4	6.08	1.982	NS
PLMS, index	8.8	7.60	5.0	4.50	2.214	0.03
Isolated LM, index	6.6	1.91	6.4	2.55	0.297	NS
REM sleep						
Total LM, index	13.9	9.00	10.7	8.56	1.366	NS
PLMS, index	4.4	7.16	3.3	4.92	0.682	NS
Isolated LM, index	9.5	5.14	7.4	4.95	1.531	NS
Total sleep						
Total LM, index	15.1	7.96	11.4	6.00	1.941	NS
PLMS, index	8.0	6.84	4.7	4.22	2.105	0.04
Isolated LM, index	7.1	2.11	6.6	2.66	0.683	NS
PLMS sequence, number	8.5	7.16	5.7	4.61	1.708	NS
PLMS sequence duration, s	17.2	23.54	12.4	18.77	0.836	NS
PLMS duration (REM), s	1.6	1.36	1.3	1.29	0.817	NS
PLMS duration (NREM), s	2.7	1.22	2.0	1.00	2.113	0.039
Isolated LM duration (REM), s	2.5	1.24	1.8	1.27	2.041	0.046
Isolated LM duration (NREM), s	2.8	0.99	2.1	1.00	2.410	0.019
Periodicity Index, total sleep	0.251	0.186	0.132	0.136	2.676	0.0097
Periodicity Index, NREM	0.242	0.187	0.132	0.143	2.433	0.018
Periodicity Index, REM	0.143	0.238	0.042	0.085	1.992	NS

NS = not significant.

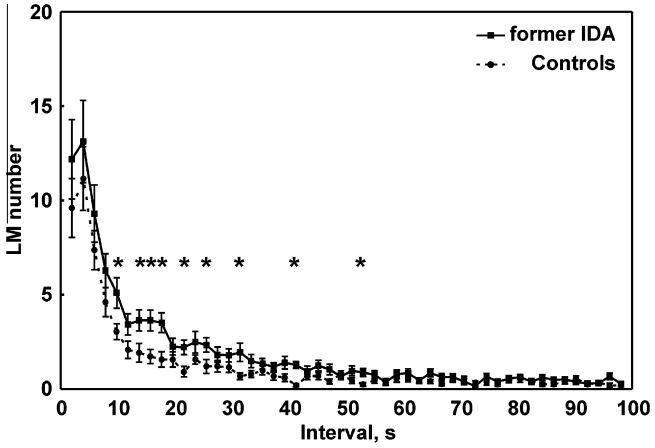

Fig. 1. Distribution histograms of inter-LM intervals during sleep of the two subject groups. All values are shown as mean and SEM (whiskers). Asterisks indicate the points for which a significant difference was found between the graphs at statistical analysis (Student's t-test; $p < 0.05$).

age-matched normal controls. The activity is characterized by a slightly but significantly higher periodicity due to a selective increase of muscle activations separated by an interval ranging approximately 10–50 s. Taken all together, these findings point to the possibility that long-term consequences of IDA in infancy, despite iron therapy, can be detected during sleep [27].

Since groups were similar in several child or family factors, including ADHD score and AHI in children, and the prevalence of a positive family history of RLS, differences between groups do not appear to be influenced by these potential confounding variables. Also, given that only three subjects were included in this study and in the infancy one [21], it is difficult to have a hypothesis regarding the fact that there were no differences in leg activity between groups at 1–1.5 years of age and the difference in PLMS index several years later. Further, the methodology used for leg movement detection in infancy (actigraphy) and childhood (polysomnography, tibialis anterior EMG) studies might help in

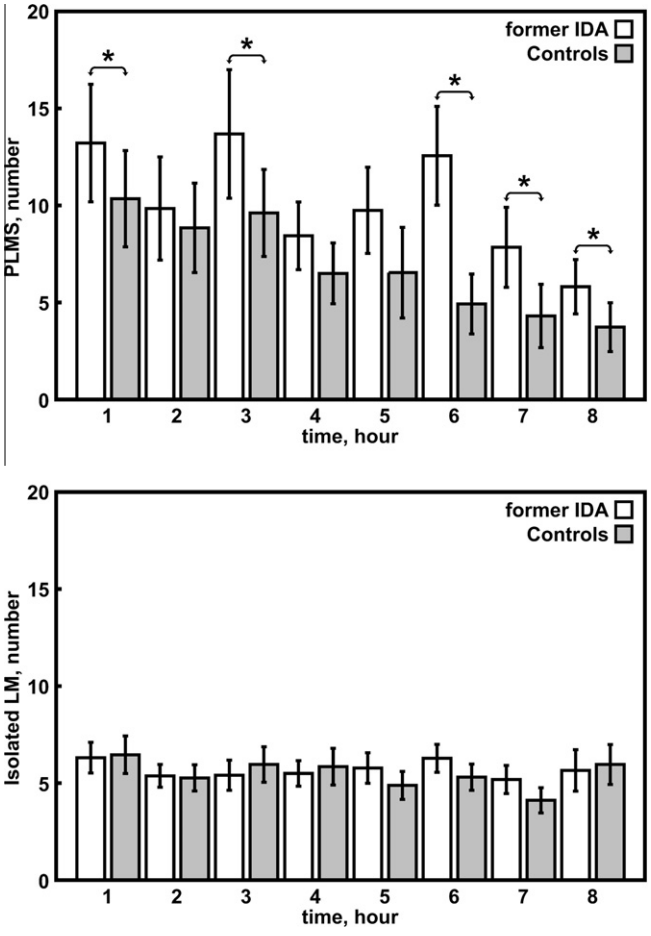

Fig. 2. Comparison between the distribution of number of PLMS (top panel) and isolated LMs (bottom panel) per hour of sleep (first 8 h shown) between subject groups. All values are shown as mean and SEM (whiskers). Asterisks indicate the points for which a significant difference was found between the graphs in each panel at statistical analysis.

understanding the absence in infancy and the presence of leg activity differences in childhood.

Considering that the diagnosis of definite RLS in children and adolescents could be preceded by many years of persistent sleep disturbances, periodic limb movement disorder (PLMD), or possible/probable RLS [33], a developmental approach deserves special attention. In this context, children with PLMD have been characterized as having important clinical and polysomnographic correlates, including sleep onset and maintenance problems, leg pain/discomfort at night, parasomnias, getting out of bed at night, and family history of RLS [34]. On PSG recordings they present more awakenings, stage 1 sleep, stage shifts, and spontaneous arousals. In our study, compared to controls, former IDA children show similar criteria prevalence for RLS [35], but have longer sleep latency in PSG recordings that could relate to sleep onset problems seen in PLMD children. However, they also have other characteristics that differ from the PLMD patterns. For instance, based on parental report, former IDA children were less frequently identified as restless sleepers (data not shown) and presented less awakenings and stage shifts throughout nighttime PSG recordings relative to controls.

In order to understand how transient infantile IDA might influence PLMS approximately 10 years later, it is important to consider the neurophysiology of PLMS. The link could be via dopamine; dopamine D3 subtype receptors are probably directly involved in the pathogenesis of PLMS and RLS symptoms [36–38]. These receptors appear to be concentrated in the limbic system and in the superficial layers of the dorsal horns of the cervical and lumbar regions and intermedio-lateralis gray matter of the spinal cord [39–42]. Spinal D3 subtype receptors receive dopaminergic projections from the A11 nuclei, located within the lateral hypothalamus, which are suspected to play a role in the pathogenesis of RLS and PLM and are closely related to the central circadian pacemaker of the suprachiasmatic nuclei [43]. In the spinal cord, dopaminergic actions from the A11 exert direct inhibitory actions on dorsal neurons and on the sympathetic preganglionic neurons in the intermediolateral nucleus. It is thought that A11 similarly limits activity in the raphe-spinal projections and that a compromise in A11 inhibitory function might be able to shift the balance of descending control of the sympathetic preganglionics neurons toward excitation [44]. This view is supported by the well-known and marked reduction of PLMS induced by dopamine-agonist therapy [45,46] (the first-choice treatment for RLS [47–49]), which selectively reduces a sub-population of the whole LM activity during sleep, with intermovement interval of 6–46 s and ranging 2–4 s in duration [50]. This category of LM is especially sensitive to dopaminergic substances targeting the D3 receptor subtype [51].

Even though it is not clearly established whether dopamine-antagonists induce PLMS, centrally active dopaminergic antagonists exacerbate existing PLMS and clinically worsen RLS, but this was not the case for a peripherally active dopamine-antagonist [52]. These findings emphasize the involvement of central nervous system dopaminergic function (presumably at the subcortical levels of the brain) in the pathophysiology of PLMS.

Neurotransmitter systems are maturing during key periods of high risk for ID, and iron is essential for a number of enzymes involved in neurotransmitter synthesis, including tryptophan hydroxylase (serotonin) and tyrosine hydroxylase (norepinephrine and dopamine) [53]. Relevant to the issue of long-lasting effects are findings in rodent studies showing that only some of the alterations in dopamine, serotonin, and noradrenergic transporters and receptors were reversible by iron supplementation at weaning [12,54,55]. Therefore, reduced iron in the brain might cause reduced dopaminergic function by decreasing the activity of tyrosine hydroxylase or the expression of transporters or receptors for dopamine [56]. The ID hypothesis for RLS is based on autopsy and MRI studies reporting lower iron levels in the substantia nigra of RLS patients [57,58], on the lower cerebral spinal fluid ferritin and iron concentrations found in RLS patients with normal serum iron stores [59,60], and on the impaired capacity to store iron in the brain of patients with early onset RLS compared to patients with late-onset RLS [59]. Finally, iron therapy has been demonstrated to be effective on RLS as well as on PLMS in adult patients [23,61] and to be associated with clinical improvement in children with PLMS [62,63].

Our findings show that transient IDA in infancy is associated with a neurophysiological "scar," as shown by our detailed analysis of PLMS. The changes may be connected to a subtle but polysomnographically detectable dopaminergic dysfunction, even at 10 years of age. This is a period of life when clinically relevant RLS is uncommon [64] and PLMS are not usually observed in normal conditions [65]. PLMS do not seem to show particular periodicity, even in children of this age with clinical conditions characterized by increased leg motor activity during sleep [66,67]. The statistically significant difference observed in this study for PLMS index and periodicity index cannot be translated into a clinical significance at this stage and only the long-term follow-up of these children will be able to provide information on its eventual predictive value of an eventual clinical consequence. There are no population-based or longitudinal data that assess what proportion of individuals with PLMS eventually exhibit some or all of the diagnostic criteria for RLS. However, it is now accepted that PLMS are an endophenotype of RLS [68]. Asymptomatic PLMS are often a precursor to RLS [33,69], and they are more common in relatives of RLS patients [70,71] and more prevalent and abundant in ethnic groups that have the highest frequencies of RLS/PLMS risk alleles [72]. The possibility that a history of a transient ID might induce a late-onset RLS, even after the re-establishment of normal values of ferritin, recalls to some extent the symptomatic RLS form associated with pregnancy. Pregnancy is a strong risk factor for both ID and a transient form of RLS, which usually disappears after delivery; however, women who experience RLS symptoms during pregnancy are more likely to develop a chronic form of RLS later in their life compared to women who do not [73,74]. Moreover, there is a positive correlation between the number of pregnancies and the risk to be affected by RLS [75].

It is not possible to know, at this stage, if IDA in infancy is a risk factor for the development of RLS later in life. However, our findings warrant continued follow-up. If this sleep motor phenomenon is further enhanced with advancing age, transient infantile IDA, a common nutritional problem among human infants [1–5], may turn out to be important for understanding the mechanisms of PLMS and, maybe, of RLS, a common sleep disturbance of adulthood [64,76].

Conflict 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.2012.05.011.

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