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Brief Communication

MRI-determined regional brain iron concentrations in early- and late-onset restless legs syndrome

Christopher J. Earley [a,*], Peter B. Barker [b], Alena Horská [b], Richard P. Allen [a]

[a] *Department of Neurology, Johns Hopkins School of Medicine, Baltimore, MD 21224, USA*
[b] *Department of Radiology, Johns Hopkins School of Medicine, Baltimore, MD, USA*

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Abstract

Objective: Cerebrospinal fluid (CSF), magnetic resonance imaging (MRI) and autopsy studies have suggested that brain iron may be reduced in restless legs syndrome (RLS). Further analysis of the data also suggests that diminished brain iron may selectively be for early-onset RLS. This study was designed to replicate and extend our previous findings, specifically with regard to early-onset RLS. In this study our primary hypothesis was that substantia nigra (SN) iron index would be decreased in early-onset RLS compared to controls.

Methods: The iron concentration or 'iron index' in 10 brain regions was determined using MRI in 39 controls and in 22 early-onset and 19 late-onset RLS subjects. The Johns Hopkins RLS severity (JHRLSS) scale was used to define disease severity.

Results: The mean iron index from the SN was significantly lower in the early-onset RLS compared to controls ($t=2.5$, $P=0.016$), while late-onset RLS and controls did not differ. There was a significant negative Spearman rank correlation between SN iron index and JHRLSS scale for the control-early-onset-RLS cohort ($\rho=-0.32$, $P=0.016$).

Conclusions: The current MRI results in combination with previous autopsy data support the role of low brain iron in the SN in at least those with early-onset RLS symptoms.

Keywords: Restless legs syndrome; Early-onset; Late-onset; Iron; MRI; Substantia nigra; Age

1. Introduction

Cerebrospinal fluid (CSF) [1], magnetic resonance imaging (MRI) [2] and autopsy studies have supported the hypothesis of decreased brain iron in RLS [3,4]. The next challenge is to understand how different phenotypes may play into this hypothesis. The best-explored phenotype is the age at which symptoms first appear [5]. The first family history study found a bimodal distribution of the reported age at which RLS symptoms first started, with the best age for defining the two subgroups being 45 years. Thus, early-onset (<45 years) and late-onset (>45 years) RLS were defined. The phenotypic differences between an early- and late-onset subgroups have been established primarily for the degree of genetic susceptibility, with greater rates of familial occurrence of RLS for probands whose RLS started before age 45 years. Furthermore, the patients with early-onset phenotype have a slow progression of symptoms over about 10–20 years, while patients with late-onset have a more rapid transition from mild to severe symptoms. Therefore, this phenotype may define two pathologically distinct RLS populations.

Our post-hoc assessment of the CSF [1] and MRI [2] data suggested that the majority of the changes in iron status were attributable to those with early-onset RLS. Furthermore, the autopsy data showing diminished iron in the SN were all performed on brains in patients with early-onset RLS [3]. This led to an a priori evaluation of CSF ferritin that specifically appraised early-onset RLS in comparison to controls. This study demonstrated that CSF ferritin was diminished in early-onset but not late-onset RLS [6]. Late-onset RLS patients may include many for whom decreased brain iron is not involved and therefore would contribute to the variance or 'noise' in any study designed to evaluate the role of brain iron in RLS. This

* Corresponding author. Address: Johns Hopkins Bayview Medical Center, 5501 Hopkins Bayview circle, Rm 1B-82, Baltimore, MD 21224, USA. Tel.: +1 410 550 550 1044; fax: +1 410 550 3364.
E-mail address: cearley@jhmi.edu (C.J. Earley).

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study, therefore, was designed to replicate and extend the previous MRI findings but specifically with regard to early-onset RLS. In this study, our primary hypothesis was that SN iron index would be decreased in early-onset RLS compared to controls.

2. Methods

2.1. Subjects

A consecutive series of subjects had MRI determination of brain iron concentration as part of their involvement in one of several different clinical studies. All RLS subjects met the four basic features required for the diagnosis of RLS [7]. Patients developing RLS in relationship to other specific causes like anemia, iron deficiency (serum ferritin <18 mcg/l; percent iron saturation $<16\%$), renal dialysis, peripheral neuropathy (based on clinical findings), neurodegenerative disorder or other obvious medical problems or medications were excluded from involvement in the study. Control subjects had no symptoms to indicate the presence of RLS and had documented (by activity meter or polysomnogram) PLMS <20 per hour. Details of the other specific inclusion or exclusion criteria have been published elsewhere [6].

Subjects in whom RLS symptoms began before 45 years of age were placed in the early-onset group. All other subjects were placed in the late-onset group. The age of symptom onset was established by the subject's reported earliest experience with RLS symptoms. All subjects had discontinued RLS medication for at least seven days (most had been off for 14 days) prior to the assessment of the subject's score on the Johns Hopkins RLS Severity (JHRLSS) scale (controls are given zero). The JHRLSS scale is a validated, four-point severity scale (0–3 for none to severe RLS symptoms) based on the time of day that RLS symptoms usually occur [8].

2.2. MRI techniques

The MRI-determined brain iron concentration was performed as described previously [2]. The R_2' (a mathematical derivative of the transverse relaxation time) was used as the primary measure of tissue iron concentration ('iron index') [9]. The 10 regions of interest were SN, dentate nucleus, pons, red nucleus, globus pallidus, putamen, caudate nucleus, thalamus, frontal white matter, and dorsolateral prefrontal cortex. All MRI measurements were made without knowledge of the subject's diagnosis or other characteristics.

2.3. Statistical analysis

All subjects were given a random five-digit number that served as the only source of identity on all data sets. All data sets were processed blind to the status of the subjects. All decisions about potential errors in the data set were made prior to breaking the blind.

The JHRLSS was the only measure of disease severity used in this study. The primary region of interest was the SN, as this region showed reduced iron concentration in the previous MRI, which correlated significantly with the JHRLSS [2], and autopsy studies of early-onset RLS patients [3]. Secondary measures of interest include the other nine brain regions.

The two-tailed *t*-test was used to test our primary hypotheses of lower SN iron index for early-onset RLS compared to controls and our secondary hypothesis of lower SN iron index for early-onset compared to late-onset RLS. We used Spearman rank-order correlation corrected for ties to evaluate the relationship between the RLS severity and the brain iron regions for the SN. This ordinal variable allowed us to assign 0 to the severity of the control subjects for these correlations. Our exploratory analyses, which did not involve specific hypotheses, included two-tailed *t*-tests for nine other brain regions for comparisons of early-onset RLS with controls and for all 10 regions for late-onset RLS compared to controls, using a Bonferonni correction for the 19 tests. Any significant difference found for any region in these exploratory analyses was further investigated for correlation with RLS severity using the Spearman rank correlation corrected for ties.

While the ages of the early-onset and controls were well matched, the late-onset RLS sample was older. In order to correct for potential age-related confounds, we performed a separate analyses on age-restricted subgroups. The age limits were set to be the lowest age of the late-onset RLS patients (53-years-old), eliminating the younger subjects seen only in the controls and early-onset RLS group.

3. Results

The clinical characteristics of the study population are given in Table 1. The control ($N=39$) and early-onset RLS

Table 1
The mean $\pm$ the standard error for subject age and for the iron index from each of the 10 brain regions for each diagnostic group

	n	Age	Substantia Nigra	Red nucleus	Globus pallidus	Putamen	Caudate nucleus	Frontal WM	Dentate nucleus	Pons	Thalamus	Prefrontal cortex
Control	39	60.5±1.7	5.6±0.31	5.1±0.29	4.7±0.41	3.2±0.22	2.6±0.21	1.6±0.14	4.2±0.24	1.8±0.15	1.2±0.09	1.4±0.09
Early-onset	22	57.1±2.0	4.4±0.31	5.0±0.34	4.3±0.47	3.4±0.30	2.8±0.24	1.6±0.22	4.0±0.44	1.9±0.22	1.4±0.21	1.2±0.13
Late-onset	19	67.4±1.8	5.2±0.43	5.4±0.52	5.0±0.33	4.3±0.36	3.3±0.38	2.0±0.24	5.0±0.36	2.6±0.30	1.3±0.19	1.3±0.21

Abbreviations: *n*, number of subjects per group; WM, white matter.

($N=22$) groups were well balanced for age ($t=1.24$, $P=0.2$) and gender (51 and 50% females respectively, chi-square $=0.009$, $P=0.95$). The late-onset RLS group was significantly older than either control ($t=2.6$, $P<0.02$) or early-onset RLS ($t=3.7$, $P<0.01$) groups but was gender-balanced. The age-restricted sub-groups did not differ significantly for age (mean age $\pm$SD(sample size): controls 65.3 ± 8.2 (28); early-onset RLS 63.4 ± 6.4 (13); late-onset RLS 67.4 ± 8.1 (19)).

The mean SN iron index (Table 1) was significantly lower in the early-onset RLS compared to controls ($t=2.5$, $P=0.016$). The difference between early- and late-onset RLS was not significant ($P=0.12$), though early-onset RLS had a relatively lower SN iron index than late-onset RLS group. There was a significant negative Spearman rank correlation between SN iron index and JHRLSS scale for the control-early-onset-RLS cohort ($\rho=-0.32$, $P=0.016$).

Exploratory analyses using individual t-test comparisons did not show significant differences ($P<0.05$) in iron index between early-onset RLS and controls in any of the other nine brain regions. The late-onset RLS compared to controls showed significant differences for the putamen ($t=2.7$, $P=0.009$) and the pons ($t=2.9$, $P=0.005$) with higher iron index for late-onset RLS in both of these areas (Table 1). The same comparisons using the age-restricted sub-groups showed the same results; that is, the only significant differences were increased iron in the putamen and pons. However, when the Bonferonni correction for the 19 different comparisons was performed, these two regional differences were not statistically significant ($P>0.05$).

Owing to the older age of the late-onset RLS group, we explored for any relationship between current age and SN iron index using the Spearman correlation. The age-iron-index correlation was not significant for any of the groups: control ($r=0.14$, $P=0.40$), early-onset RLS ($r=0.08$, $P=0.73$) and late-onset RLS ($r=0.13$, $P=0.60$).

4. Discussion

A decrease in the SN iron index in RLS subjects, although previously reported [2], was based on only five RLS patients and five control subjects. At minimum, those results needed to be replicated, and specifically for early-onset RLS, as both CSF [6] and autopsy [3] studies would predict such a finding. Therefore, the primary question for this study was whether early-onset RLS would show a diminished SN iron index compared to controls, which it did.

A secondary question was whether early- and late-onset RLS would differ with regard to the SN iron index. Although showing relatively lower SN iron index, early-onset did not show a significant difference from late-onset RLS group, nor did late-onset RLS differ from controls. The late-onset RLS group did in fact show a moderate increase in the pontine and putamenal iron index, but the statistical significance was lost with Bonferonni correction. The late-onset RLS group was significantly older than either the control or early-onset group. In addition, autopsy studies have shown that iron increases in the brain with aging [10]. Could, then, the older age of the late-onset group constitute a significant confounding variable? We found no correlation between brain iron and the current age of the subjects for any of the groups. An age-restricted-group analysis also failed to find a difference between late-onset and control groups. Finally the age-related increases in brain iron found at autopsy were near maximal by 40–50 years of age [10], and thus unlikely to be a confounding variable in this study where 97% of the subjects were over age 45. Future MRI studies need to re-evaluate specifically late-onset RLS with the primary hypothesis of increased iron index in the putamen and pons. If the find proves to be true, it would suggest at least a different pathology for late-onset RLS group.

The iron index reflects only the ferritin-bound iron but not the total tissue iron [9,11]. It also does not discern the relative amounts of H- and L-ferritin in a tissue. The autopsy studies in RLS have shown that H-ferritin is decreased, but L-ferritin is unchanged even though the tissue concentration of iron was low and transferrin was increased [4]. The persistence of normal L-ferritin levels reduces the sensitivity of the MRI-determined measures to the disease severity. Therefore, the failure to find MRI changes in many of the brain regions in early- or late-onset RLS does not exclude the possibility of there being altered iron metabolism in those regions. The iron index also does not provide information about the site of iron storage or its accessibility. The autopsy studies showed that L-ferritin in the SN of RLS patients, although normal in quantity, was concentrated primarily in glial cells (where the iron may be less accessible), rather than the oligodendrocytes, which are the usual cells for iron storage [4]. Therefore, an increased iron index (as noted in late-onset RLS) does not necessarily mean increased iron availability. At minimum, it reflects altered iron metabolism, the significance of which is uncertain.

Despite the limitations in the MRI measurements of brain iron, the results are consistent with both the autopsy results and CSF studies, providing strong support for brain iron deficiency in early-onset RLS patients. The MRI results, however, fail to clarify the role of altered brain iron metabolism in late-onset RLS but do give pause to speculate that late-onset RLS compared to early-onset may differ in this regard. These differences may be relevant in defining for whom intravenous iron therapy may or may not be a viable therapeutic option [12].

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References

[1] Earley CJ, Connor JR, Beard JL, et al. Abnormalities in CSF concentrations of ferritin and transferrin in restless legs syndrome. Neurology 2000;54(8):1698–700.
[2] Allen RP, Barker PB, Wehrl F, et al. MRI measurement of brain iron in patients with restless legs syndrome. Neurology 2001;56(2):263–5.
[3] Connor JR, Boyer PJ, Menzies SL, et al. Neuropathological examination suggests impaired brain iron acquisition in restless legs syndrome. Neurology 2003;61:304–9.
[4] Connor JR, Wang XS, Patton SM, et al. Decreased transferrin receptor expression by neuromelanin cells in restless legs syndrome. Neurology 2004;62(9):1563–7.
[5] Allen RP, La Buda MC, Becker P, Earley CJ. Family history study of the restless legs syndrome. Sleep Med 2002;3(Suppl.):S3–S7.
[6] Earley CJ, Connor JR, Beard JL, et al. Ferritin levels in the cerebrospinal fluid and restless legs syndrome: effects of different clinical phenotypes. Sleep 2005;28(9):1069–75.
[7] Allen RP, Picchietti D, Hening WA, et al. Restless legs syndrome: diagnostic criteria, special considerations, and epidemiology. A report from the restless legs syndrome diagnosis and epidemiology workshop at the National Institutes of Health. Sleep Med 2003;4(2):101–19.
[8] Allen RP, Earley CJ. Validation of the Johns Hopkins restless legs severity scale. Sleep Med 2001;2:239–42.
[9] Gelman N, Gorell JM, Barker PB, et al. MR Imaging of human brain at 3.0 T: preliminary report on transverse relaxation rates and relation to estimated iron content. Radiology 1999;210:759–67.
[10] Hallgren B, Sourander P. The effect of age on the non-haemin iron in the human brain. J Neurochem 1958;3:41–51.
[11] Ma J, Wehrli FE. Method for image-based measurement of the reversible and irreversible contribution to the transverse relaxation rate. J Magn Reson B 1996;111(1):61–9.
[12] Earley CJ, Heckler D, Horská A, et al. The treatment of restless legs syndrome with intravenous iron dextran. Sleep Med 2004;5(3):231–5.