

Low Brain Iron Content in Idiopathic Restless Legs Syndrome Patients Detected by Phase Imaging

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ABSTRACT

Background: The objective of this study was to use phase imaging to evaluate brain iron content in patients with idiopathic restless legs syndrome (RLS).

Methods: Fifteen RLS patients and 15 healthy controls were studied using gradient-echo imaging. Phase analysis was performed on localized brain regions of interest selected on phase maps, sensitive to paramagnetic tissue. Differences between the 2 subject groups were evaluated using ANCOVA including age as a covariate.

Results: Significantly higher phase values were present in the RLS patients compared with healthy controls at the level of the substantia nigra, thalamus, putamen, and pallidum, indicating reduced iron content in several regions of the brain of the patients.

Conclusions: We have used MRI phase analysis to study brain iron content in idiopathic RLS in vivo for the first time. Our results support the hypothesis of reduced brain iron content in RLS patients, which may

have an important role in the pathophysiology of the disorder. © 2013 *Movement Disorder Society*

Key Words: MRI; RLS; iron; phase imaging

Restless legs syndrome (RLS) is characterized by an irresistible urge to move the legs, associated with unpleasant paresthesias in the legs and sometimes arms. These sensations occur at rest, in particular in the evening or at night, and are relieved by movement.^{1,2} The pathophysiology of idiopathic RLS is poorly understood. A number of observations point toward a central role for the dopaminergic system and iron metabolism.³ In 1953 Nils-Brage Nordlander first suggested that iron deficiency might play a primary role in RLS,⁴ a concept emphasized and supported in recent years by Allen and coworkers.³ The potential role of iron metabolism involvement in RLS is indicated primarily by those secondary causes of RLS in which iron insufficiency is clear, but also by some pharmacological studies.⁴⁻⁶ Several studies have shown a relationship between low ferritin concentrations, in particular in the cerebrospinal fluid (CSF), and symptoms of the syndrome.⁷ Studies on CSF have shown decreased ferritin, elevated transferrin, and decreased pro-hepcidin (a prohormone that interacts with the iron transport protein ferroportin on the surface of cells) in patients with RLS.⁸ Neuropathological data have shown alterations of iron regulatory proteins in neuromelanin cells from brains of RLS patients.^{9,10} Altered iron metabolism has been revealed in lymphocytes from subjects with RLS.¹¹ A pathological study showed that compared with control samples, RLS substantia nigra had higher mitochondrial ferritin levels and less cytosolic H-ferritin.¹² Reduced brain iron in RLS patients is also suggested by data from MR studies exploiting the effect of iron on T_2 , T_2^* , and T_2' , although with discrepant results.¹³⁻¹⁷

The recently developed phase-imaging technique, which uses tissue magnetic susceptibility differences to generate a unique contrast different from that of spin density, T_1 , T_2 , and T_2^* , is a sensitive tool to quantify cerebral iron content.¹⁸⁻²⁰ It measures phase shifts in gradient-echo images, which, measured in radians, are inversely correlated with iron content. Tissue-containing (paramagnetic) iron exhibits a negative phase in complex images compared with immediately adjacent tissue, which will have an increased phase. The aim of this

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study was to use phase imaging to assesses brain iron content in patients with idiopathic RLS.

Patients and Methods

Subjects

Seventeen idiopathic RLS patients presenting at the Sleep Medicine Centre and satisfying the International Restless Legs Study Group (IRLSSG)² criteria were consecutively recruited and studied in a 1.5-Tesla GE system. Secondary forms of RLS were excluded by means of detailed history, neurological and physical evaluations, and laboratory analyses. Two patients were excluded because of vascular white matter lesions on MRI. The characteristics of the remaining 15 patients are reported in Table 1.

We also studied 15 healthy volunteers without history suggesting RLS and other disorders and who did not significantly differ in age, sex, and peripheral iron status compared with patients (Table 1). None of the subjects studied showed evidence of gray or white matter abnormalities on conventional MRI. All subjects gave written informed consent, and the study was approved by the local institutional review board.

Phase Imaging Acquisition and Analysis

Anatomical imaging was performed by a T₂-weighted (T₂W) FSE sequence in an axial oblique plane ($\alpha = 90^\circ$; TE, 107 ms; TR, 5080 ms; square FOV, 24 cm; matrix 320 × 256; reconstructed in-plane resolution, 0.938 mm; slice thickness, 4 mm without gap; NEX, 2). Phase-sensitized images were acquired using a gradient-echo sequence, and preserving both real and imaginary channels (TE/TR, 40/60 ms; matrix, 512 × 256; reconstructed in-plane resolution, 0.938 mm; NEX, 2; bandwidth, 15.6 kHz). Slice locations matched those of the anatomical scan,

excluding slices above the central corpus callosum and below the dentate nucleus. Following the published method,¹⁸ data were high-pass-filtered by multiplication with a filter function in k-space, using tools provided by FSL (<http://www.fmrib.ox.ac.uk/fsl>) and AFNI (<http://afni.nimh.nih.gov/afni>), and a phase map was generated using the filtered data (Fig. 1A–E). T₂W data were registered onto the gradient-echo data using FLIRT (FSL). Using both T₂W and phase image data, regions of interest (ROIs) were drawn within the dentate nucleus, red nucleus, substantia nigra, thalamus, globus pallidus, putamen, and caudate head (the darkest portion of each structure on phase image); see Figure 1F. For each ROI considered bilaterally, the median phase value was calculated.

Statistical Analysis

For comparison of phase data, parametric tests were used, as Kolmogorov–Smirnov testing showed that variables were normally distributed. An ANCOVA was used to evaluate differences between the 2 subject groups. Age was included as a covariate, as it is known that in normal subjects there is a correlation¹⁸ between phase values and age, reflecting the progressive increase of brain iron content with aging. Only *P* values < .05 were accepted as statistically significant, after Bonferroni correction for multiple comparisons (for 7 ROIs studied). To investigate the effect of demographic and clinical parameters (sex, age at onset, disease duration, therapy, serum iron, ferritin and transferrin, positive family history and IRLSSG rating scale score²¹) on patient phase data, we used Pearson correlation and multiple regression with a backward stepwise method to obtain the best model in which all included variables had a *P* < .05. We performed correlation analyses separately for each ROI showing a significant difference.

Results

We first evaluated the presence of the expected correlation¹⁸ between phase values and age in the control group. Using a Pearson test, we detected a strong negative correlation in all structures (ranging from $R = -0.53$, $P = .04$, to $R = -0.91$, $P < .0001$) except the dentate nucleus and caudate head, where a trend was still visible. An ANCOVA model with age as a covariate disclosed significantly higher phase values in the RLS patients compared with healthy controls at the level of the substantia nigra, thalamus, putamen, and pallidum also after correction for multiple comparisons (Table 2, Fig. 1F). Mean phase values were also higher for the RLS patient group in the other 3 structures studied (dentate nucleus, red nucleus, and caudate head), although the differences were not significant. No correlation with any clinical parameter was found.

TABLE 1. Demographic and clinical features of healthy controls and RLS patients studied

	Healthy controls (n = 15)	RLS patients (n = 15)
Sex (M/F)	4/11	2/13
Age (y), (mean ± SD)	51 ± 17	51 ± 13
Serum iron (μg/dL), (mean ± SD) ^a	96 ± 33	93 ± 43
Serum ferritin (ng/mL), (mean ± SD) ^b	82 ± 48	71 ± 45
Serum transferrin (g/L), (mean ± SD) ^c	247 ± 73	252 ± 54
Age at onset (y), (mean ± SD)	/	44 ± 14
Disease duration (y), (mean ± SD)	/	7 ± 6
Therapy ^d (yes/no)	/	3/12
Family history (yes/no)	0/9	6/9
IRLSSG rating scale score ²¹ (mean ± SD)	/	21 ± 8

SD, standard deviation; IRLSSG, International Restless Legs Study Group.

^aNormal range of serum iron: F, 35–145; M, 50–160 μg/dL.

^bNormal range of serum ferritin: F, 15–150; M, 30–400 ng/mL.

^cNormal range of serum transferrin, 110–370 g/L.

^dPramipexole 0.18 mg per day (12 patients had never taken therapy).

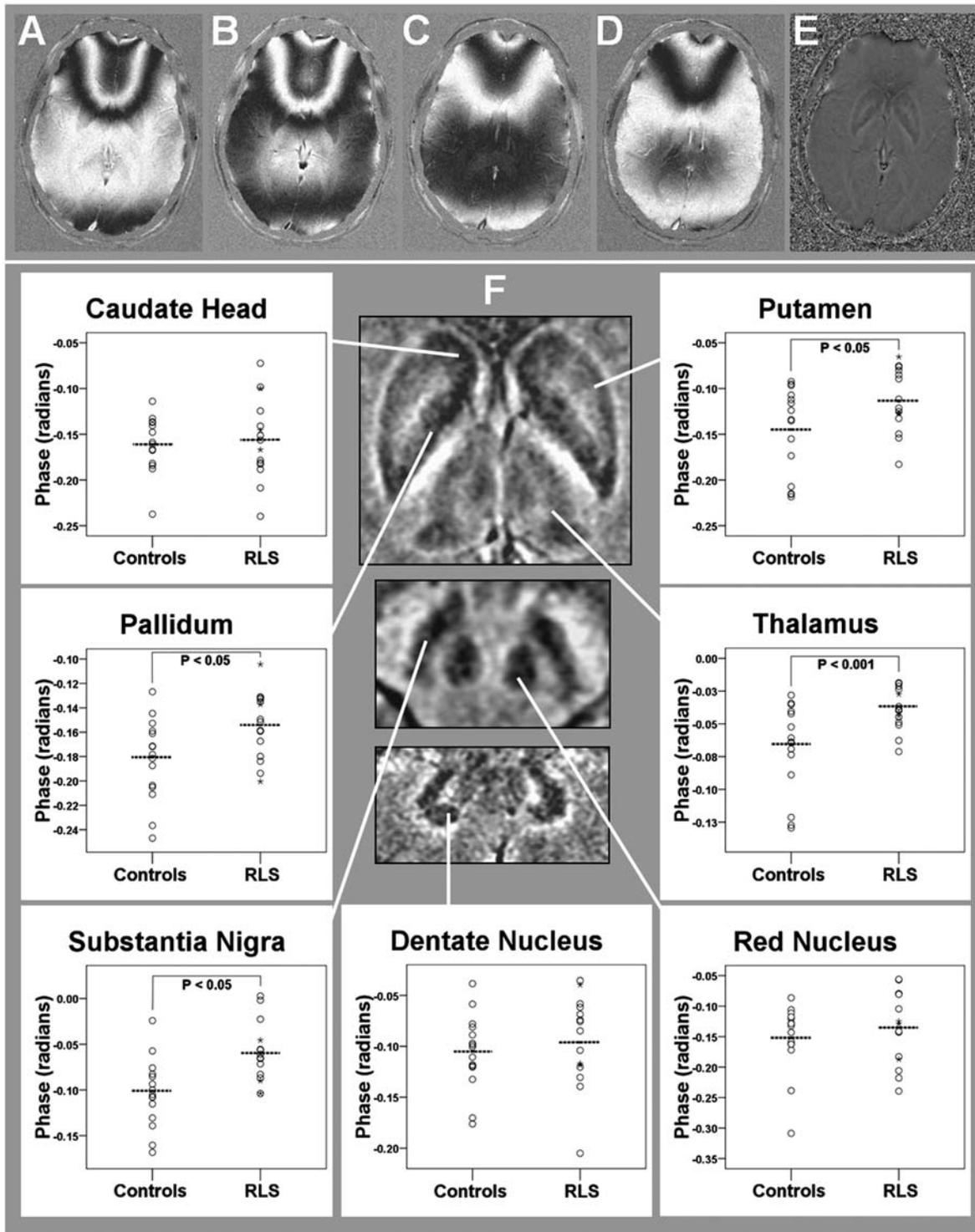


FIG. 1. A–E: Schematic illustration of preprocessing of phase images. **A, B:** Real and imaginary images. **C, D:** Low-pass filtered images. **E:** Phase map. **F:** Details of iron-rich structures selected as regions of interest and corresponding scatter plots of phase values in healthy controls and RLS patients (horizontal dotted lines indicate mean values; those RLS patients who received dopaminergic agents are represented by asterisks and those who were never treated by circles).

Discussion

We have used MRI phase analysis to study brain iron content in idiopathic RLS in vivo for the first time. We found significantly higher phase values in the

RLS patients compared with healthy controls at the level of the substantia nigra, thalamus, putamen, and pallidum. These results support the presence of reduced iron content in several regions of the brains

TABLE 2. Comparison of phase values between healthy controls and RLS patients

	Phase (radians)		<i>P</i> ^a
	Healthy controls (n = 15)	RLS patients (n = 15)	
Dentate nucleus	-0.106 ± 0.036	-0.096 ± 0.044	n.s.
Red nucleus	-0.152 ± 0.056	-0.135 ± 0.060	n.s.
Substantia nigra	-0.103 ± 0.037	-0.060 ± 0.033	.011
Thalamus	-0.069 ± 0.034	-0.039 ± 0.016	.0007
Putamen	-0.146 ± 0.048	-0.114 ± 0.034	.045
Pallidum	-0.182 ± 0.033	-0.154 ± 0.027	.014
Caudate head	-0.161 ± 0.031	-0.156 ± 0.044	n.s.

^aANCOVA using age as covariate: *P* < .05 corrected for multiple comparisons was considered significant (*x*7). Values are reported as mean and standard deviation.

n.s., Not significant. Each region was considered bilaterally.

of RLS patients. Previous MR studies^{13–17} used T_2 , T_2^* , or T_2' ($1/T_2' = 1/T_2^* - 1/T_2$) sequences to quantify iron level in RLS patients, and almost all^{13–16} results were in line with ours, although with some discrepancies. In 2 studies by the same group, regional brain iron concentration was assessed in RLS patients by R_2' measurement ($R_2' = 1/T_2'$), and the mean iron content of the substantia nigra (SN) was significantly lower only in the early-onset RLS patients (<45 years).^{13,14} In a study performed in patients with late-onset RLS patients, the T_2 relaxation time was assessed separately for the 2 components of the SN, and low iron content was found in the pars compacta but not in the pars reticulata.¹⁵ The same authors subsequently showed an unexpectedly decreased T_2 relaxation time in the right globus pallidus internal and the subthalamic nuclei of untreated patients with early-onset RLS, indicating increased iron content without any SN change.¹⁶ Another group, without differentiating between early onset and late onset, disclosed that mean T_2 values of multiple regions were higher in RLS patients, although significantly increased in 4 regions (caudate head and medial, dorsal, and ventral thalamus).¹⁷

In addition, transcranial B-mode sonography was used in RLS patients that exhibited SN hypoechogenicity^{17,22} inversely correlated with T_2 values¹⁷ and interpreted as secondary to iron deficiency.

Both our data and the results summarized above are consistent with a diffuse but regionally variable low brain iron content in idiopathic RLS patients, possibly because of impairment in brain iron homeostasis.^{8–10} Some discordant results in the previous studies could be explained, other than by clinical inhomogeneity, by technical considerations, as whereas T_1 and T_2 also depend on water content and other local structural changes that can affect relaxation times, this is not true for T_2' or phase.¹⁹

All the clinical,^{4–6} biochemical,^{7,8,11} pathological,^{9,10,12} and radiological^{13–17} observations favor a more general iron-dopamine model of RLS, and it has been suggested that dopaminergic dysfunction can be mediated by low brain iron levels because iron is needed as a cofactor for tyrosine hydroxylase, the D2 receptor is an iron-containing protein, and the dopaminergic synaptic protein Thy-1 requires iron for its activity.³

In support of this hypothesis some animal models have shown increased wakefulness in the 4 hours preceding the resting phase of iron-deficient mice²³ and decreased striatal D2 receptors in iron-deficient rats.²⁴ Using a translational approach, Connor et al disclosed decreased D2 receptors in the putamen that correlated with severity of RLS and increased phosphorylated tyrosine hydroxylase (pTH) in the substantia nigra and the putamen from RLS autopsy tissues and a similar increased pTH in animal and cell models of iron insufficiency.²⁵ These data support the hypothesis of basal ganglia involvement in RLS and are in line with the reduced basal ganglia iron content detected in our study. In addition, we also found reduced iron content in the thalamus, a structure that has been shown to have impaired metabolism,²⁶ activation,¹⁵ and dopaminergic receptor density²⁷ and fewer beta-endorphin and met-enkephalin positive cells²⁸ in RLS patients.

In conclusion, our data add further support to the hypothesis of reduced brain iron content in RLS patients, which could have an important role in the pathophysiology of the disorder, by mediating abnormal regulation, dopaminergic or otherwise, of sensorimotor integration and nociception. ■

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