Movement Disorders
CLINICAL PRACTICE
REVIEW

Iron in Restless Legs Syndrome

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Abstract: A link between restless legs syndrome (RLS) and iron has been recognized for several decades. Yet, the precise role that iron or other components of iron metabolism play in bringing about RLS is still a matter of debate. During the last few years, many new pieces of evidence from genetics, pathology, imaging, and clinical studies have surfaced. However, the way this evidence fits into the larger picture of RLS as a disease is not always easily understood. To provide a better understanding of the complex interplay between iron metabolism and RLS and highlight areas that need further elucidation, we systematically and critically review the current literature on the role of iron in RLS pathophysiology and treatment with a special emphasis on genetics, neuropathology, cell and animal models, imaging studies, and therapy.

Karl Ekbom, the Swedish neurologist, who, in 1945, rendered the first "modern" description of restless legs syndrome (RLS), was the first to describe a link between iron and the disease.[1] His contemporary, Nils Nordlander, subsequently reported on the use of intravenous (IV) iron in the treatment of RLS in 1953.[2] In the current clinical setting, one still encounters many individuals with RLS with low serum ferritin levels who appear to benefit from iron therapy. Moreover, although imaging studies have not always yielded results congruent to each other, the large majority points to dysregulation of brain-iron status in individuals with RLS. Yet, the precise role that iron or other components of iron metabolism play in bringing about the disease is still a matter of debate. It is, for instance, known that dopaminergic treatment efficiently improves RLS symptoms. Though the exact mechanism of dopaminergic action in RLS remains to be elucidated, it is interesting to note that iron is a cofactor in the rate-limiting step of the conversion of tyrosine to levodopa by the enzyme, tyrosine hydroxylase, which is subsequently decarboxylated to form dopamine (DA). Iron deficiency could thus be postulated to reduce functional DA levels and worsen RLS symptoms. Yet, the situation is not as simple as one might assume because, for example, other components of iron pathways have also been shown to be altered in patients with RLS; some genetic factors implicated in RLS suggest a yet largely unexplored influence on iron metabolism, and individuals with hemochromatosis-induced systemic iron overload are in no way immune to RLS.

During the last few years, many new pieces of evidence from genetics, pathology, imaging, and clinical studies have surfaced regarding the involvement of iron in RLS. However, the way this evidence fits into the larger picture of RLS as a disease is not always easily understood. Here, we summarize the current understanding of the influence of iron and different components of iron metabolism on the development and treatment of RLS.

Search Strategy

We systematically reviewed research reports, clinical studies, case series, and review articles published in English between January 1981 and June 2013 and indexed in Medline. The literature search was performed using "restless legs syndrome," "RLS," "iron," and "ferritin" as query terms. A total of 414 publications were assessed. Where relevant, basic science research on iron metabolism without direct relation to RLS as well as singular studies published before 1981 were also included.

Iron Pathway

Overall, in humans, iron metabolism consists of two regulatory systems controlling systemic and cellular iron homeostasis independently either at the transcriptional (systemic) or post-tran-

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scriptional level (systemic and cellular).[3] On average, approximately 4 g of iron are present in the human body of adult males. Half of this is bound to hemoglobin in erythrocytes. The other half can be found bound intracellularly to ferritin in complexes used to store iron in all cells of the human body that require it.[4] The main storage sites include, first and foremost, the liver, but also the spleen and the bone marrow. At any given time, only approximately 3 to 4 mg of iron are found in the plasma bound to the iron transport protein, transferrin.[4] Most iron is amassed and continuously recycled in the mononuclear phagocyte system.[4] However, because there is also enteral or hemorrhagic loss of iron, which cannot be regulated,[3] the system necessitates a constant—though low-level—influx of iron from external sources. Dietary iron is absorbed by duodenal enterocytes either directly if it is bound to proteins such as heme[5] or through a mechanism dependent upon iron reductase duodenal cytochrome b[6] and divalent metal transporter 1 (DMT1).[7] Further transport throughout the body relies on basolateral export from the enterocytes through the transmembranal iron transporter, ferroportin, and oxidation by the iron oxidase, hephaestin, before loading onto iron transport protein transferrin.[3] Uptake by peripheral hepatic, myeloic, and splenic cells with prominent iron storage function occurs through ferroportin (SLC40A1). Ferroportin expression is controlled by the hepatic peptide hormone, hepcidin, a presumed key regulator of systemic iron metabolism.[8] Noniron storage cells, such as erythrocytes, and all other cells requiring iron in their function take up iron by receptor-mediated endocytosis dependent upon binding of iron-loaded transferrin to the transferrin receptor present on the cell surface.[4] On the cellular level, iron-regulatory proteins (IRP1 and IRP2) bind to iron-responsive elements (IREs) in the 5′ and 3′ untranslated regions (UTRs) of regulated messenger RNAs (mRNAs), thus inhibiting translation initiation or inducing ubiquitination and proteasomal degradation.[3] Some points of cross-talk between the systemic and cellular pathways are known, such as, for instance, in the fact that ferroportin on a systemic level is regulated by hepcidin and on an intracellular level by the IRP/IRE system through an IRE in its 5′ UTR.[3] Yet, it seems likely that there would need to exist many additional points of interaction that are not fully understood to date in order to account for the intricate balance between the two systems in human iron homeostasis.

Conditions Compromising Iron Status and RLS

A first line of evidence, that iron is of importance to the pathophysiology of RLS, stems from the observation that RLS occurs more frequently in individuals with compromised iron status than in the general population. Conditions such as, but not limited to, pregnancy,[9–11] primary nutritional iron deficiency, and iron deficiency anemia,[12,13] as well as—in some, but not all, populations—repeated blood donations[14–20] are known to precipitate RLS.

RLS during pregnancy is a common phenomenon reported by 28.6% of the total collective of 1,225 women in four large studies from Norway, France, Italy, and Turkey.[9–11,21] Depending on the specific study, this included up to 33.3% of women who had experienced symptoms of RLS before the given pregnancy. Though a correlation between the occurrence of RLS and low hemoglobin levels was reported in the Italian and the Turkish study, this was not the case for serum ferritin levels in any of the four studies.[9,11,21] Where assessed, in 53.5% to 97% of women, symptoms disappeared within the first 5 days after delivery.[9–11] Although the link between low functional iron and RLS symptoms during pregnancy has not yet been demonstrated conclusively, one possible explanation could be that increased blood and iron demand placed on the mother's body, especially during the later phases of pregnancy, could lead to desaturation of iron stores in anatomic regions pertinent to RLS and the development of RLS symptoms in women with a (genetic) predisposition for the disease. Alternatively, altered hormonal states or local compression could also play roles in precipitating RLS during pregnancy.

Compromised iron absorption from the duodenum has also been postulated to favor RLS. The prevalence of RLS in individuals with gastrointestinal disorders, such as celiac disease[22–24] and Crohn's disease,[25] is higher than in the general population: 31% versus 4% of 100 Italian patients with celiac disease and 100 age- and sex-matched controls[23] and 35% versus 10% of 85 U.S. celiac disease subjects and their spouses[24] reported RLS symptoms. Decreased hemoglobin levels ($P = 0.003$)[23] and serum iron deficiency (40% vs. 6%; $P < 0.001$)[24] were more prevalent in individuals suffering from celiac disease with RLS than in those without RLS. RLS occurred with a similarly high prevalence of 30.2% in 272 individuals suffering from Crohn's disease.[25] Some researchers also suggest that the increase in RLS prevalence in these populations could be related to alterations in iron metabolism brought about by the chronic inflammatory state observed in both Crohn's disease and celiac disease.[25] Here, the production for proinflammatory cytokines, such as interleukin-6, could result in increased production of hepcidin, a small protein, which slows down intestinal iron absorption.[25] Also, in this context, a new hypothesis suggests that certain drugs, such as proton pump inhibitors, may lead to the development of RLS symptoms by decreasing the amount of iron absorbed gastrointestinally specifically by changing duodenal pH,[26] although this has yet to be addressed in clinical studies.

Last, conditions that coincide with a large amount of blood loss—such as, for example, (repeated) blood donations[14–20] or (in single cases) heart transplantation,[27] therapeutic venesection,[28] or recurrent uterine myomas (personal observation)—also seem to possess the ability to both trigger and aggravate RLS.

However, though a correlation between low serum ferritin levels and severity of RLS symptoms has been reported in a sample of 1,012 elderly Turkish individuals admitted to hospital[29] as well as several smaller hospital-based studies,[30,31] three larger prospective general population-based studies totaling 1,780 individuals of European and Asian descent found no correlation between serum ferritin levels and the presence or absence of RLS.[32–34] Ferritin levels were also not significantly changed in lymphocytes from 24 individuals with RLS, when compared to 25 controls.[35] The reason for this remains unclear.

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It could be possible that changes in the iron metabolism in RLS are not reflected at the blood level because they primarily affect the central nervous system (CNS).[33] Alternatively, they could play a role only in a subset of RLS patients[32] (such as those recruited in the hospital-based studies) or be primarily reflected in the severity of the disease, rather than its existence or non-existence.

Model Systems

The clinical observation of a role of iron in RLS prompted an assessment of the different players in iron metabolism in a number of model systems ranging from cell culture and worm (discussed in the next section) to iron-deprivation studies in mice and rats. A total of 12 studies have examined the relationship between RLS and iron in mammalian model systems. Reminiscent of the human RLS phenotype, in the mouse, dietary iron deficiency (DID) was shown to increase awake time by decreasing rapid eye movement (REM) and non-REM sleep in the 4 hours preceding the lights-on (i.e., "night") phase, but not during the 12-hour lights-off phase, as assayed by polysomnography (PSG).[36] Further reminiscent of an RLS-like phenotype, DID mice also showed increased running wheel usage before the resting period.[37] In D3 receptor knockout (KO) mice, this phenotype was even more pronounced, suggesting an underlying DA-mediated mechanism.[37]

On a molecular level, iron chelation in rat primary dopaminergic neurons from the SN by desferoxamine induced apoptotic cell death.[38] In the murine CNS, DID resulted in differential changes in monoamine metabolism: DA transporter density decreased, whereas there was an increased metabolism of DA.[39] DID decreases iron concentration, transferrin saturation, hemoglobin, and hematocrit in peripheral blood and also iron concentration in the liver.[40] Moreover, iron deficits can also be found in the CNS. Whereas DID decreases iron concentrations in the ventral midbrain and nucleus accumbens, but not the prefrontal cortex, striatum, pons, or cerebellum during both light and dark phases, whole-brain iron is selectively decreased during the light phase only.[40] Overall, the investigators of this study argued that diurnal fluctuations in brain iron may have implications for neuronal functioning.[40] An important component of the RLS phenotype are sensory symptoms affecting primarily the legs, which can take on a number of different qualities. Interestingly, in the DID mouse model, iron-deficient mice were more sensitive to both acute pain in the hot plate test (Aδ-fibers) and persistent formalin-induced pain (C-fibers).[37,41] Here, too, D3-receptor knockdown further increased sensitivity,[37] implicating both DA and iron in the mechanism.

Because of the positive effect of dopaminergic treatment, it is assumed that changes in DA neurotransmitter systems play a significant role in RLS pathophysiology. The DA neurons of the diencephalic A11 region have been proposed by some to be critical in this because they represent an important dopaminergic cerebrospinal connection. Accordingly, the effect of DID has also been examined in a murine model of bilateral 6-OHDA-induced lesions of the A11 dopaminergic neurons. 6-OHDA lesions of the A11 lead to attenuated expression of DA and homovanillic acid (HVA) as well as D2 and D3 receptors in the lumbar spinal cord. When combined with DID, D2 receptor expression further decreases and increased locomotor activity is observed.[42–44] Moreover, 6-OHDA lesions exaggerated DID-induced iron deprivation in the brain and spinal cord.[43] Taken together, these results illustrate the close relationship between the dopaminergic system and iron metabolism in the CNS. Next to the known involvement of iron as a cofactor in DA biosynthesis, Thy-1, which has a role in neurotransmitter release, has been highlighted as a possible connector because its expression is decreased by iron deficiency in cell and rat models as well as in the substantia nigra (SN) of RLS patients.[45] Overall, it is interesting to note that the combination of iron deprivation and dopaminergic alteration can model both motor and sensory aspects of the RLS phenotype, although still in a rather crude way. Yet, one caveat has to be that it is uncertain whether the A11 region targeted in some models is truly pertinent to RLS because the evidence available to date leaves room for doubt.

Genetics

Approaches to directly identify genetic causes of brain and systemic iron status imbalances in RLS are, to date, quite limited. Only two genes encoding key components of iron metabolism have been screened for genetic variants and mutations in RLS patients in candidate gene approaches: *DMT1* and mitochondrial ferritin (*FtMt*). Mutation screening of exons and selected intronic regions in *DMT1* in 179 cases, 180 controls, and 110 families yielded no RLS-specific variant or association to RLS.[46] *FtMt* was sequenced in 23 RLS cases and 342 controls, and only one putative risk variant specific to the RLS population (p.G185D) was found.[47] However, data from large-scale sequencing show this variant to be a low-frequency variant (allele frequency of 0.18% in the National Heart, Lung and Blood Institute (NHLBI) Gene Ontology (GO) Exome Sequencing Project) also found in controls. Limitations of both studies are the small sample size and their low power to detect rare variants and variants with small effect size. However, even when common variants in 111 genes of known relevance to iron metabolism were analyzed for a possible association with the RLS phenotype in a larger cases/control sample (n = 954/1,814 in the discovery step and n = 735/736 and 736/735 in the first and second replication step, respectively), no significant associations were identified.[48] Whether this equates to a lack of a role of (common) genetic variation in iron metabolism in RLS or is simply the result of the fact that the relevant iron factor was not on the list of candidate genes or a dilution of the effect by an intermediate trait (i.e., serum iron and ferritin levels),[48] remains to be established.

Genome-wide association studies (GWAS) for RLS have identified RLS-associated risk variants in six genomic regions containing the genes *MEIS1*, *BTBD9*, *MAP2K5/SKOR1*, *PTPRD*, and *TOX3/BC034767*.[49–52] None of these factors

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play a known role in iron metabolism. To identify previously unknown iron genes, GWAS have also been conducted for iron metabolism parameters in human serum and plasma and for iron deficiency.[53–58] Interestingly, none of the genomic regions and genes identified in these studies overlap with any of the RLS-associated regions. Therefore, the RLS-associated genes could either play no role in iron metabolism at all, have an effect on iron metabolism that is limited to the CNS and cannot be detected by serum measurements, or be too rare or of too small effect size to be detected by the current GWAS.

Functional studies in animal models and human tissue already provide starting points to answer these questions for two of the RLS-associated genes, *MEIS1* and *BTBD9*. In *Caenorhabditis elegans*, it was shown that the worm *MEIS1* ortholog, *unc-62*, is involved in iron metabolism. It requires iron for its influence on the worm's lifespan and a knockdown of unc-62 increases the expression of ferritins in the worm.[59] However, no other components of iron metabolism were studied, thus limiting the informative value of the study. In humans, the investigators examined the expression of various key components of iron metabolism in specific brain regions (pons and thalamus) and lymphoblastoid cell lines (LCLs) of RLS cases.[59] These were selected based on a previously described strong risk haplotype for RLS in *MEIS1* (odds ratio: 2.7), which is defined by two specific risk variants (rs12469063 and rs6710341).[52] By comparing both mRNA and protein expression levels between homozygous carriers of the risk and homozygous carriers of the nonrisk haplotype, a significant difference in expression levels of heavy- and light-chain ferritin and DMT1 between these two classes was detected in the thalamus ($P < 0.05$), but not in pons or LCLs.[59] The additional iron genes screened (*ACO1*, *IREB2*, ceruloplasmin, hepcidin, ferroportin, transferrin, and transferrin receptor 1 and 2) did not show differential expression. Although these observations point to a link between *MEIS1* and brain iron metabolism, the exact interactions and dependencies of *MEIS1* and iron are still unclear. The observation of increased ferritin levels in carriers of the RLS risk haplotype, however, is in stark conflict with the consistent observation of reduced serum ferritin levels in RLS patients and the observed association of RLS and iron deficiency. Unfortunately, serum iron and ferritin concentrations of the brain donors are not known. Also, the lack of association of common variation in genes involved in iron metabolism and RLS argues against a direct genotypic link.[48] Moreover, it still remains to be established whether the thalamus is an RLS-relevant brain region at all and thus whether the observed differences in iron-related gene expression play a role in RLS pathophysiology.

For *BTBD9*, a GWAS in Icelandic and U.S. subjects with RLS and/or periodic limb movements in sleep (PLMS) showed an association of the RLS risk variant, rs3923809, with serum ferritin levels. In a sample of 965 Icelandic individuals (362 men and 603 women; RLS cases and their relatives), serum ferritin levels were found to be decreased by 13% per copy of the risk allele ($P = 0.002$).[50] However, there were large differences in the observed effect in heterozygous carriers of the risk allele when analyzing men or women only. In men, the difference between homozygous nonrisk allele carriers and heterozygous risk allele carriers was only ~2% to 3%, whereas in women the difference was ~20%. These gender-specific discrepancies were not discussed further in the study. Interestingly, another study also found an association between another RLS-associated common variant in *BTBD9* (rs9296249) and serum ferritin levels ≤12 ng/mL in 1,302 female Danish blood donors ($P = 0.03$).[60] At the same time, none of eight single-nucleotide polymorphisms of known association with the RLS phenotype (including rs9357271 and rs3923809 in *BTBD9*) were associated with serum concentrations of iron or ferritin in a total of 3,447 individuals belonging to the KORA general population cohort,[48] suggesting that this association, if it truly exists, could be gender specific.

Additional results regarding BTBD9's involvement in iron metabolism and RLS stem from a murine *Btbd9* KO model, where an increase in serum iron levels was observed in homozygous KO animals when compared to wild-type (WT) animals.[61] Ferritin or other measures of iron were not assayed and only one specific brain structure, the striatum was analyzed, but no difference in iron content was found between homozygous KO and WT mice.[61] Furthermore, overexpression of BTBD9 in HEK cells yields increased ferritin expression and may arise through a BTBD9-dependent attenuation of the level of IRP2, which, in turn, drives ferritin expression.[62]

Although several studies insinuate a role for BTBD9 in iron metabolism and RLS pathophysiology, the diverse and partly conflicting nature of the results makes it difficult to come to a final conclusion at present. Different measures of iron metabolism in varying tissues were used, thus rendering it impossible to directly compare and relate the results of the different studies. Some results are in contrast with observations in RLS patients, for example, increased ferritin levels in knock-down or KO models of *MEIS1* and *BTBD9* homologs, whereas RLS patients usually show low ferritin levels even in spite of decreased blood and CNS expression of at least *MEIS1* dependent on the risk haplotype.

Still, first interesting observations of a possible link between RLS-associated genes and iron metabolism have been made, but confirmation in independent studies and further exploration of a possible role in RLS pathophysiology is warranted.

Imaging Studies

To date, eight MRI studies assessing cerebral iron content have been performed with regard to RLS (Table 1). Though one study in 12 RLS cases and 12 controls did not see any differences in iron deposits in 12 brain regions by T2*,[63] another study demonstrated significantly decreased iron in the SN and marginally decreased iron in the putamen of 5 individuals with idiopathic RLS (iRLS) without iron deficiency, compared to 5 controls, using R_2' (the reversible portion of the transverse relaxation time) as a measure of regional brain iron.[64] This finding was corroborated in a cohort of 22 individuals with early-onset iRLS (<45 years) with attenuated "iron index" or iron concentration (i.e., decreased R_2' signal), compared to 39 con-

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TABLE 1 Iron imaging studies in RLS

	Imaging Method	Cases/Controls	Outcome	Serum Ft
Allen et al.[64]	MRI (R2′)	5 vs. 5	↓ iron in SN and putamen in RLS patients	N/A
Earley et al.[65]	MRI (R2′)	22 early, 19 late onset, 39 controls	↓ iron in SN and putamen in RLS patients	Individuals with serum Ft <18 μg/L were excluded, between group differences not evaluated
Haba-Rubio et al.[66]	MRI (R2′)	2 vs. 9	↓ iron in SN, red nucleus and putamen in hemochromatosis patients with RLS	N/A
Astrakas et al.[67]	MRI (T2)	25 vs. 12	↓ iron in SNpc and trend toward ↓ iron in caudate and dentate nucleus in patients with RLS	No difference between groups
Godau et al.[69]	MRI (T2) TCS	6 vs. 19	Multiregional brain iron deficiency in RLS patients	For RLS 174.8 ± 50.7 ng/mL; N/A for controls
Knake et al.[63]	MRI (T2*)	12 vs. 12	No differences in iron deposition between cases and controls in 12 brain regions	111.9 ± 63.4 vs. 124.7 ± 171.8 ng/mL
Margariti et al.[68]	MRI (T2)	11 versus 11	↑ iron in GP and STN in RLS patients	N/A
Rizzo et al.[70]	MRI (phase imaging)	15 versus 15	↓ iron in SN, thalamus, putamen, and pallidum of RLS patients	71 ± 45 versus 82 ± 48 ng/mL
Schmidauer et al.[71]	TCS	20 RLS versus 20 PD versus 20 controls	↓ iron in SN in RLS patients	For RLS 78.4 ± 16.9 ng/mL; N/A for PD and controls
Godau et al.[72]	TCS	49 versus 49	↓ iron in SN in RLS patients	N/A
Pedroso et al.[73]	TCS	30 individuals with RLS and either PD or Machado-Joseph disease	↓ iron in SN correlates with RLS severity	N/A

N/A, not available.

trols and 19 late-onset iRLS (>45 years) subjects. The comparison between the early- and late-onset cohorts was not significant.[65] The iron index reflects only ferritin-bound iron and not total tissue iron, without differentiating between H- and L-ferritin. R2′ MRI also revealed low iron levels in the SN, red nucleus, and pallidum of 2 patients with hemochromatosis and severe RLS, compared to 9 healthy volunteers.[66] This indicates that RLS symptoms are correlated with low brain-iron levels and not necessarily low serum-iron levels. Yet, patients with hemochromatosis without RLS would constitute a better control sample and could exclude the possibility that hemochromatosis *per se* is a confounding factor. Astrakas et al. were able to both confirm and extend the above results by demonstrating that a nigral iron decrease in RLS is not an effect secondary to treatment. By T2 relaxometry, they found significantly lower iron content of SNc alongside a tendency toward lower iron content in the caudate and dentate nucleus in 25 patients with late-onset untreated iRLS and 12 age- and sex-matched controls.[67] No significant correlation between T2 values and serum ferritin levels, disease duration, or severity scores on the International Restless Leg Syndrome and Johns Hopkins restless legs severity scale was noted. Contrary to this, 11 unmedicated patients with early-onset iRLS did not show differential nigral (SNc and SNr) iron content, compared to 11 matched control subjects. Conversely, iron content of the globus pallidus (GP) and the STN was increased in RLS subjects, whereas voxel-based morphometry did not indicate volume changes in any of the brain regions during nighttime hours.[68] Another group demonstrated increased mean T2 values in 11 assessed brain regions in 6 therapy-naïve individuals with iRLS, in comparison to 19 controls, although this difference was statistically significant only for the caudate head as well as three thalamic regions (medial/dorsal/ventral), leading the investigators to suggest a multiregional (global) brain-iron deficiency in RLS.[69] Most recently, phase imaging was used to show decreased iron content in the SN, thalamus, putamen, and pallidum of 15 RLS patients, compared to 15 controls.[70]

Another imaging technique, transcranial sonography (TCS) of the SN, supported the above finding of altered brain-iron content in RLS (Table 1). Twenty patients with iRLS without iron deficiency showed significantly reduced hyperechogenicity (indicative of decreased iron content), compared to 20 age-matched controls and 20 patients with idiopathic Parkinson's disease (PD) without RLS. In 10 individuals with RLS, but only in 1 control, no hyperechogenic signal was found. Treatment and age at onset did not have a significant influence and there was no correlation between serum ferritin levels and area of hyperechogenicity in the RLS group.[71] This finding was replicated in an independent set of 49 RLS patients and 49 age- and sex-matched controls. The investigators of the latter study further describe sum values of both sides below 0.20 cm^2 to indicate SN hypoechogenicity and decreased iron content.[72] Moreover, mean MRI T2 values of 11 different brain regions showed moderate inverse correlation with SN echogenicity,[69] and SN echogenicity was also inversely correlated with RLS symptom severity in 30 individuals with RLS and either PD or Machado-Joseph disease.[73]

Overall, these studies provide evidence for a dysregulated brain-iron content in individuals with RLS, with most showing a lack of iron in most regions examined, which most frequently included both the SN and the putamen (Table 1). Yet, though several studies see the strongest evidence for this in the SN, it is

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not known whether this is really the brain region responsible for the clinical phenotype observed in RLS. Also, at this point, it is unclear whether the changes in iron content observed in the different brain regions are indeed a functional correlate of the disease or merely a by-product of global changes in iron metabolism in RLS. Also, though the imaging techniques used can suggest altered iron content to underlie the changes noted, they are not specific enough to prove that changed iron concentrations are indeed responsible because, for example, changed tissue concentrations of other elements (i.e., calcium) would yield similar MRI results.[74]

Iron Pathology (Postmortem)

Neuropathologic studies, however, lend further support to the assumption that changed tissue-iron content is truly responsible for the observed imaging differences. Decreased staining for iron and H-ferritin in the SN was observed in 7 brains of individuals with iRLS as diagnosed according to the International RLS Study Group (IRLSSG) criteria, compared to 5 age-matched controls without history of neurologic disease. Furthermore, neuromelanin-containing cells of the SN showed immunostaining increased transferrin (Tf) and increased MTP1 and DMT1 immunostaining in RLS patients. The finding of diminished staining for the transferrin receptors (TfR) in these cells contradicts the notion of iron deficiency, but could, nonetheless, be explained by a defect in TfR expression regulation.[75] Upon isolation of nigral neuromelanin cells from 4 RLS and 4 control brains by laser capture microdissection, immunoblotting also showed decreased expression of TfR and IRP1. IRP1 post-transcriptionally regulates the expression of the TfR, implicating a possible mechanism for the iron deficiency.[76] Further along this line, the iron-regulatory hormone, hepcidin, interacts with ferroportin to halt iron release from the cells. By immunoblotting, prohepcidin was significantly up-regulated in neuromelanin cells, the SN, and the putamen of primary RLS samples (n = 8), compared to controls (n = 5). Speculatively, this increase of hepcidin in the RLS parenchyma could serve as a mechanism to maintain iron inside the neurons. It is possible that hormonal iron signaling in the CNS could be involved in the pathology of RLS or in the maintenance of iron homeostasis.[77] Levels of FtMt were also increased in the SN (specifically, neuromelanin-containing neurons), but not the putamen of RLS patients (n = 8), compared to controls (n = 8). Cytochrome c oxidase staining indicated an increase in the number of mitochondria in the SN of RLS brains. According to the investigators, this augmentation in mitochondrial number may reflect an attempt to correct a metabolic insufficiency in these neurons that may lead to cytosolic iron deficiency.[78]

Most recently, neuropathological studies have been expanded to include brain regions other than the striatum and putamen. Tf and ferritin H (H-Ft) were reported to also be decreased specifically in central myelin in 11 RLS brains, when compared to 11 controls.[79] At the cerebrospinal fluid/blood–brain interface (CSF/BBI; choroid plexus and brain microvasculature), differences in the pattern of expression of iron-management proteins existed between 18 (choroids plexus) or 11 (microvasculature) neurologically healthy controls and 14 individuals with iRLS. In the choroid plexus, iron and H-Ft were reduced, whereas DMT1, ferroportin, Tf, and TfR staining were increased. The microvasculature showed attenuated H-Ft, Tf, and TfR expression in RLS samples with concomitantly decreased iron regulatory activity.[80] These results implicate changes in iron uptake and storage at the CSF/BBI in RLS pathophysiology for the first time. Though this hypothesis is intriguing, additional follow-up and replication is needed to substantiate this concept.

A major drawback of all the studies mentioned above is the fact that, to date, the number of RLS brains biobanked worldwide is quite limited, and that, to our knowledge, there is a significant overlap between the sets of brains used in the studies. Though this will hopefully change in the future, with efforts in Europe to establish a large brain bank for RLS currently underway, a second caveat, that of possible treatment-induced neuropathological changes, is more difficult to address.

Iron in the CSF

If CNS and blood–brain-barrier iron status and regulation are indeed important to RLS pathophysiology, this could be reflected in the CSF. A decrease in CSF Ft levels alongside an increase in CSF Tf levels was observed in patients with RLS, compared to healthy controls. There was no difference in serum Ft and Tf levels between the two groups.[81] This finding could echo lower total brain-iron concentration in RLS patients. A second study, this time using psychophysiological insomnia patients as controls in order to exclude sleep loss as a confounding factor produced the same results. Furthermore, CSF iron was also significantly lower in the RLS group of patients.[82]

In order to reveal possible diurnal variation in CSF iron regulation, Earley et al. examined nighttime (10 PM) CSF Ft, Tf, and iron levels of 30 patients with iRLS (15 early- and 15 late-onset) and 22 age- and sex-matched controls.[83] This study showed diurnal variation in CSF iron regulation only in RLS patients, with the daytime samples (10 AM) presenting the most pronounced differences in CSF ferritin and Tf levels between the two groups. The early-onset, but not the late-onset, RLS group had significantly lower CSF ferritin levels than controls, whereas no difference in CSF Tf and iron levels was observed. To further delineate the changes in CSF Ft levels between patients with iRLS and control individuals, Clardy et al. quantified H- and L-Ft subunit levels in nighttime CSF.[77] They found a significant decrease of both subunits only in early-onset RLS with normal total protein amounts in CSF, possibly reflecting a chronic, active iron insufficiency in the brains of individuals with early-onset RLS.

Iron in the Therapy of RLS

Low-normal ferritin levels <50 ng/mL are known to coincide with severe symptoms of RLS.[30] Accordingly, a total of three studies were conducted assessing whether oral iron treatment

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could improve RLS symptoms. The number of patients included in each study was small (Table 2). In an open-label trial, O'Keeffe et al. administered 600 mg of oral ferrous sulfate per day to an elderly patient population (n = 15) with a mean serum Ft level of 32.5 ng/mL. This led to an average increase in Ft of 34 ng/mL alongside an improvement of RLS symptoms in all, with the strongest effects observed in individuals with Ft levels <18 ng/mL.[31] More recently, Wang et al. showed a significant improvement in IRLSSG severity scores (10.3 vs. 1.1 points; $P = 0.01$) in 11 individuals with RLS who had received 650 mg of oral ferrous sulfate daily during a 12-week period, compared to 7 placebo-treated individuals.[45] It is interesting to note that this study was limited to RLS patients with low-normal ferritin levels (15–75 ng/mL; mean, 40.6 ± 15.3).[45] Conversely, in a placebo-controlled, randomized, double-blind investigation of 8 RLS subjects and 13 controls with mean Ft levels of 134.8 and 100.6 ng/mL, respectively, this effect was not observed.[84] Overall, oral iron therapy was primarily effective in individuals with low-normal baseline serum Ft, and the possibility of an RLS endophenotype with low peripheral iron stores in which therapeutic iron supplementation is especially effective has been postulated.[45]

As far as clinical studies are concerned, more emphasis has been placed on the investigation of IV iron substitution in RLS (Table 3). After Nordlander's initial report,[2] a total of nine trials were conducted to assess the efficacy and safety of IV iron therapy in RLS. Similar to the situation found with regard to oral iron supplementation, differing results were reported: In 11 individuals with secondary RLS resulting from to end-stage renal disease (ESRD) who received 1,000 mg of IV iron dextran, RLS symptoms improved after 1 and 2 weeks, in comparison to 14 RLS patients who had received IV saline ($P = 0.03$ and 0.01; −2 and −3 points on an RLS severity scale from 0 to 10).[85] In a retrospective evaluation of 25 RLS patients who had been treated with 1,000 mg of iron dextran, 73.9% reported some degree of improvement of RLS symptoms. Duration of treatment effect, however, was highly variable and ranged from 1 to 60 weeks.[86] The same IV iron formulation, when used in a prospective study, also showed moderate-to-complete improvement of all RLS symptoms in 68% of 25 RLS patients. Improvement, as measured by the Korean version of the IRLSSG severity scale, however, did not correlate with either serum or CSF Ft levels at baseline or 3 weeks after treatment.[87] IV iron dextran was also used by Earley et al. in 10 individuals with iRLS in an open-label fashion.[88] Two weeks after infusion of 1,000 mg of iron dextran, RLS symptoms and periodic leg movements in sleep (PLMS; the motor component of RLS in sleep) were markedly improved, as measured on the global rating scale (0–6 points; 54 ± 41%; $P < 0.002$) and by actigraphy (28 ± 32% PLMS/h; $P = 0.01$).[88] The investigators of this study also noted that, despite an overall positive effect on RLS symptoms, 40% of the subjects did not respond. Although mean serum Ft levels did not differ significantly between responders and nonresponders, they were, overall, higher in the later group (72.2 ± 72.0 vs. 104.36 ± 53.2 ng/mL; $P = 0.76$). In responders, treatment effect lasted from 3 to 36 months (mean, 11.3).

TABLE 2 Clinical Studies on oral iron substitution in RLS

	Type of Study	Number of Cases/Controls	Treatment Regime	Baseline Ft	Outcome	Other
O'Keeffe et al.[31]	Oral ferrous sulfate, open label	15	200 mg ferrous sulfate 3×/day for 2 months	<100 ng/mL (32.5 [6–124] ng/mL)	At 8–20 weeks, all improved, those with baseline Ft <18 ng/mL the most	Elderly patients only
Davis et al.[84]	Oral ferrous sulfate versus placebo, randomized, double-blind	8 versus 13	325 mg ferrous sulfate 2×/day for 12 weeks	134.8 (9–680) versus 100.6 (8–335) ng/mL	At 14 weeks, no difference in quality of sleep/life or frequency of RLS	Including secondary RLS, on medication
Wang et al.[45]	Oral ferrous sulfate versus placebo, randomized, double-blind	7 versus 11	325 mg of ferrous sulfate plus 100 mg of vitamin C 2×/d for 12 weeks	15 to 75 ng/mL (40.6 vs.36.7)	At 12 weeks, IRLSSG score −10 versus −1, trend toward improved quality of life	

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TABLE 3 Clinical studies on IV iron substitution in RLS

	Type of Study	Number of Patients	Treatment Regime	Baseline Ft	Outcome	Other
Nordlander et al.[2]	IV iron, open label	22		N/A	21/22 "responders"	
Sloland et al.[85]	IV iron dextran versus placebo, randomized, double-blind	11 versus 14	1,000 mg of iron dextran once	87 (47–371) versus 175 (60–336) ng/mL	At 1 and 2 weeks, improved RLS score (7–4) in those treated, at 4 weeks, effect only marginal	2-degree RLS (all ESRD)
Earley et al.[83,88]	IV iron dextran, open label; IV iron gluconate for follow-up	10 (5 in follow-up)	1,000 mg of iron dextran once; 3× 150 mg of iron gluconate if symptoms and ferritin <300 ng/mL	85.0 (±64.0) ng/mL	At 2 weeks, 6/10 "responders," mean "response" time 11 months, 44% drop in PLMS/h, rapid decrease in Ft levels; 2 of 5 still on only IV iron after 130 weeks	MRI, actigraphy
Grote et al.[90]	IV iron sucrose versus placebo, randomized, double-blind	29 versus 31	5× 200 mg of iron sucrose once	<45 ng/mL, 20.1 versus 20.4 ng/mL	At 7 weeks, IRLSSG score 12 versus 20 from 24, ESS did not improve; at 52 weeks, 80% IV iron versus 40% placebo without drugs	
Earley et al.[89]	IV iron sucrose versus placebo, randomized, double-blind	11 versus 7	500 mg of iron sucrose once	70 (±21) versus 78 (±47) ng/mL	At 2 weeks, placebo improved more (57% "responders")	MRI, CSF, PSG
Ondo[86]	IV iron dextran, retrospective analysis	25	1,000 mg of iron dextran	43.5 (±58.0) ng/mL	73.9% reported some degree of improvement; duration of response ranged from 1 to 60 weeks	
Allen et al.[91]	IV FCM versus placebo, randomized, double-blind	24 versus 22	2–3× 500 mg/1× 1,000 mg of FCM	28.1/70.0 (F/M) versus 24.8/58.7 (F/M)	At 4 weeks,11 of 24 versus 1 of 22 "responders," −9 versus −4 points on IRLSSG score, improved RLS-QoL, PLMS/h and FSS not significantly different; treatment regimes all the same	Actigraphy
Hornyak et al.[92]	IV FCM, prospective open label	20	500 mg of FCM	<45 ng/mL; mean, 30.1 ng/mL	60% reported some improvement of RLS symptoms ("responders"), IRLSSG score decreased by 10 points in "responders"	
Cho et al.[87]	IV iron dextran, low molecular weight prospective, open label	25	4× 250 mg of iron dextran	41.0 (±37.0) ng/mL	68% reported moderate to complete improvement of RLS symptoms, no correlation between "responder" status and serum and CSF Ft levels	CSF

F, female, M, male, RLS-QoL, RLS quality-of-life scale, FSS, fatigue severity scale.

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In the SN, increase in iron deposits resulting from the iron infusion was marginally significant ($P = 0.059$). Furthermore, it was noted that after the infusion, Ft levels decreased more rapidly than expected from physiological considerations (6.2 ± 3.1 vs. less than 1 mg/L per wk).[88] This phenomenon was also noted in a follow-up study in which 5 responders received additional infusions of iron gluconate. Here, the duration of the therapeutic effect was inversely proportional to the rate of Ft decline after infusion.[83]

Two independent studies assessed IV iron sucrose in the therapy of RLS in a randomized, placebo-controlled, double-blind design.[89,90] Grote et al. saw a significant difference in IRLSSG scores at 7 weeks after the infusion of five times 200 mg of iron sucrose (week 0: 24 vs. 26; week 7: 12 vs. 20; $P = 0.017$), but not at 11 weeks (week 0: 24 vs. 26; week 11: 7 vs. 17; $P = 0.123$). A similar study conducted by Earley et al was discontinued prematurely after 2 weeks because of a lack of effect.[89] Next to the total amount of iron sucrose given (1,000 vs. 500 mg), the main difference between the studies lies in the fact that serum Ft levels were much lower (20.1 ± 12 ng/mL) in the first than in the second (70.3 ± 21.5 ng/mL) study.

In November 2007, the most recent iron formulation, ferric carboxymaltose (FCM), was first released in Europe. In 24 individuals who had received 1,000 mg of IV FCM, a positive "response" to treatment was reported in 11 individuals, compared to 1 of 22 placebo-treated individuals. The IRLSSG score decreased by 9 points in the verum, compared to 4 points in the placebo, group.[91] Similarly, in 20 RLS patients with either iron deficiency or low-normal serum ferritin (<45 ng/mL) who received 500 mg of FCM as a single dose, 60% reported improved symptoms with IRLSSG scores decreasing from 28.3 ± 6.1 points at baseline to 18.3 ± 8.0 points after 3 weeks.[92] Responders showed a trend toward being younger, having lower baseline serum Ft levels and IRLSSG scores, and suffering from fewer comorbid conditions than nonresponders.[92]

One possible interpretation of the divergent results reported in these studies is the existence of different RLS endophenotypes that respond differently to iron substitution. For example, it would be imaginable that a subgroup of patients has persistently low Ft levels (possibly as a result of accelerated iron metabolism) and that it would be this group that would benefit more from iron substitution than those with normal or high basal ferritin. Moreover, the specific iron formulation also appears to affect the success of iron substitution in RLS. Although systematically only evaluated in regular blood donors with RLS,[93] IV iron substitution is likely to be more effective than oral substitution, but formal studies need yet to be conducted.[94] Also, iron sucrose did not seem to be as effective as the other substances administered in IV iron substitution. Yet, direct comparisons between the different formulations and (for the most part) different application schemes are lacking, and a Cochrane Review evaluating studies through early 2011 came to the conclusion that there is currently insufficient evidence to determine the true benefit of iron therapy in RLS.[94] Additionally, from the perspective of evidence-based medicine, each iron formulation should be treated as a separate drug, making transnational and meta-analyses difficult. Overall, FCM emerges as the formulation with the least side effects at present.

Iron and Augmentation

Furthermore, three publications have addressed the relationship between low Ft levels and augmentation, the paradoxical worsening of RLS symptoms under dopaminergic therapy.[95–97] In a first assessment, Trenkwalder et al. retrospectively compared patients who experienced symptoms of augmentation (n = 36; Ft, 85 ± 59 ng/mL) with those without augmentation (n = 302; Ft, 118 ± 108 ng/mL) in a therapeutic trial of cabergolin versus L-dopa and found significantly lower basal Ft levels in the augmented group ($P = 0.0062$).[95] In a second study, 19 of 162 RLS patients treated with dopaminergics developed augmentation, and of those, 31.1% had low-normal Ft levels <50 ng/mL and 10% had pathological Ft levels <20 ng/mL.[96] Age, gender, RLS etiology, previous augmentation, or other documented comorbidities did not differ between the two groups. RLS severity correlated inversely with Ft level.[96] Last, a trend toward lower Ft (82 ± 47 vs. 131 ± 88 ng/mL; $P = 0.06$) was also observed in 36 individuals who actually developed augmentation of 60 individuals with RLS who were given very high doses of L-dopa (up to 600 mg/day) in an attempt to trigger augmentation.[97] Although all three studies suggest a link between low Ft and risk for augmentation, it is unclear, however, how direct this link truly is. Here, too, it could be possible that an endophenotype of RLS exists that predisposes to augmentation and coincides with low Ft, but also with other features, such as, for example, a positive family history, which was not assessed in any of the studies thus far. Accordingly, it would be interesting to learn whether individuals with RLS secondary to other disorders, predisposing to low iron stores (e.g., pregnancy, compromised intestinal iron absorption, or continuous blood loss), are also more likely to develop augmentation.

Perspectives

Whereas an involvement of iron metabolism in RLS pathophysiology has been suspected for several decades and a number of pieces of evidence exist, the concrete role of iron in RLS remains ambiguous. However, understanding how iron is involved in RLS development will likely be very beneficial to both our scientific and clinical understanding of the disease. New data on a possible link between iron and the RLS susceptibility genes that seem to lie beyond already established "iron genes" is intriguing. Animal models provide an opportunity to further study this interesting potential link as has already been demonstrated by single studies. In this context, it is noteworthy that some characteristics reminiscent of the human RLS phenotype can be recapitulated in mammalian DID models and are exaggerated by functional DA deficits. In the future, they may also supply a possible model to study the environment-gene interaction likely to play a role in RLS.

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From a clinical standpoint, it appears important to determine which subsets of RLS patients benefit from iron substitution and which do not. It is imaginable that several iron endophenotypes exist in RLS and that, for example, individuals whose endophenotype coincides with low serum Ft benefit more from iron substitution than others. With regard to pathological and imaging studies, it is central to realize that, most recently, it has been shown that the genetic changes predisposing to RLS lead to alterations in the developing murine ganglionic eminences, the primordial basal ganglia, establishing RLS as a neurodevelopmental disorder.[98] However, all pathological and imaging studies performed to date assessed adults. And, in the worst case, all the changes observed are merely results of the truly underlying pathological change or the administered treatment. Nonetheless, these studies clearly substantiate the link between iron metabolism and RLS, especially in the CNS.

At the moment, the quality of the research in the field overall is compromised by the fact that, especially in the neuropathological studies, the same set of RLS patients has been investigated repeatedly. Accordingly, it would be desirable to expand the number of researchers and patients/samples in the field in order to be able to replicate the results already obtained and build upon already existing results. Also, from a non-RLS-specific perspective, it is interesting to further explore the role of iron in RLS pathophysiology because RLS represents one of few low-iron phenotypes in humans and could thus inform the physiologic role of iron in humans as well.

Author Roles

(1) Research Project: A. Conception, B. Organization, C. Execution; (2) Statistical Analysis: A. Design, B. Execution, C. Review and Critique; (3) Manuscript: A. Writing of the First Draft, B. Review and Critique.

E.C.S.: 1A, 1B, 1C, 2C, 3A, 3B

M.K.: 1A, 1B, 1C, 2C, 3A, 3B

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