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REVIEW

Iron, dopamine, genetics, and hormones in the pathophysiology of restless legs syndrome

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Abstract Restless legs syndrome (RLS) is a common, chronic neurologic condition, which causes a persistent urge to move the legs in the evening that interferes with sleep. Human and animal studies have been used to study the pathophysiologic state of RLS and much has been learned about the iron and dopamine systems in relation to RLS. Human neuropathologic and imaging studies have consistently shown decreased iron in different brain regions including substantia nigra and thalamus. These same areas also demonstrate a state of relative dopamine excess. While it is not known how these changes in dopamine or iron produce the symptoms of RLS, genetic and hormone studies of RLS have identified other biologic systems or genes, such as the endogenous opioid and melanocortin systems and *BTBD9* and *MEIS1*, that may explain some of the iron or dopamine changes in relation to RLS. This manuscript will review what is known about the pathophysiology of RLS, especially as it relates to changes in iron, dopamine, genetics, and hormonal systems.

Keywords Restless legs syndrome · Pathophysiology · Dopamine · Iron · Genetics

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Introduction

Restless legs syndrome (RLS), also known as Willis-Ekbom disease, is a chronic sensorimotor disorder characterized by an unrelenting urge to move the legs at night, preventing rest and sleep when it is most desired. These symptoms can cause significant discomfort and distress in patients with moderate-to-severe disease [1]. RLS is among the most common sleep disorders, having a prevalence of 5–10% in adults in North American and Western Europe [1, 2]. Women are affected twice more than men and incidence increases with age [3]. The diagnosis of RLS can be established by International RLS Study Group criteria using the mnemonic URGED: (1) urge to move the legs usually but not always accompanied by unpleasant or uncomfortable sensations; (2) rest worsens symptoms; (3) gyration or movement partially or totally relieves symptoms; (4) evening or nighttime worsening of symptoms; and (5) denial of another primary causation of the symptoms [4]. Two types of RLS phenotypes have been found: (1) early onset primary or idiopathic RLS, with a peak incidence at 20–40 years of age, familial, and slow disease evolution [5, 6] and (2) late-onset RLS with rapid progression, late peak onset after 40 years of age, and associated with other comorbidities, including neuropathy, iron deficiency anemia, and Parkinson's disease [5–7].

The pathophysiology of RLS is not fully understood. Iron and dopamine are the systems most extensively studied in relation to physiologic mechanisms underlying RLS. The increased prevalence of disease in the iron-deficient population and dramatic therapeutic response of RLS symptoms to dopamine-based medicines [8] have provided impetus to study these two systems in relation to RLS pathophysiology. Outside of the iron and dopamine systems, there are likely other pertinent biologic systems that play their role in producing RLS. These include the hypoxia sensing system, genes involved in neuronal functioning, and hormones that have various physiologic effects. In this review of the pathophysiology of RLS, we will discuss (1) iron and iron deficiency in relation to RLS, (2) the dopaminergic system and how it relates to iron deficiency in RLS, (3) the insight into RLS pathophysiology provided by genetic studies, and (4) the potential role of hormones in RLS.

Iron deficiency

In the 1950s, Norlander first described an association between iron deficiency and RLS [9]. Since then, the iron system has been extensively studied to clarify its role in RLS pathophysiology. Iron, an element essential to most cells in the body, is involved in many functions in the brain, including electron transfer reaction and neurotransmitter synthesis and degradation [10]. Iron imbalance has been linked to a variety of brain disorders, including Alzheimer's and Parkinson's diseases [11]. There is also evidence that links RLS to iron deficiency states, with a higher prevalence of RLS in persons having conditions that decrease iron availability, such as pregnancy and end stage renal disease [12, 13]. Furthermore, among persons with iron deficiency anemia, the prevalence of RLS has been found to be as high as 31% [14]. Despite this association, many patients with RLS have normal serum iron, yet serum ferritin, an iron storage and binding protein, often exists at low levels that inversely correlate with RLS symptom severity [15].

Whereas, iron in the serum is often normal, there more consistently seems to be a state of low iron in the brain of patients with RLS. This may relate to decreased permeability of iron across the blood brain barrier [10]. Low brain iron has been demonstrated in neuropathologic specimens, brain imaging, and cerebrospinal fluid (CSF) evaluation. In a magnetic resonance imaging (MRI) study of primary RLS patients, regional brain iron concentration was found to be significantly decreased in substantia nigra and these decreases were correlated to RLS symptom severity [16]. Other iron imaging studies of RLS patients have confirmed these findings of decreased brain iron in the substantia nigra of RLS patients, but also there is suggestion that this decreased brain iron may be more widespread [17, 18]. Also using MRI, Godau et al. [18] found that iron was decreased in all studied brain regions, including thalamus, caudate, substantia nigra, putamen, and white matter, but only significantly so in the former two areas.

Postmortem neuropathologic studies have also found low iron in persons with primary RLS. Immunohistochemistry of RLS brain samples has shown markedly decreased staining for both iron and H-ferritin, a ubiquitous iron storage protein [19], in the substantia nigra of RLS patients [20]. Another study by the same group identified a defect in iron regulatory protein-1 in neuromelanin cells, which destabilizes transferrin receptor mRNA and leads to cellular iron deficiency in RLS patients [21]. Several studies have demonstrated reduced ferritin in cerebrospinal fluid (CSF) of RLS patients, consistent with an iron-deficient state [22–24]. In addition, increased CSF transferrin levels have been demonstrated in RLS patients compared to controls without RLS [22–24]. This research has also extended into the rodent. Studies demonstrated that mice fed a diet deficient in iron had disturbed sleep [25] and a reduction in ventral midbrain and nucleus accumbens iron levels [26]. Interestingly, the extent to which iron was deficient in brain was dependent on the diurnal cycle, which may relate to the diurnal nature of RLS symptoms in humans.

The iron system has intricate ties to the dopaminergic system as will be outlined in the following section. However, there may also be another pathway that has ties to iron and may have involvement in RLS pathophysiology. A study in six RLS patients showed increased HIF-1α immunoreactivity in the substantia nigra as compared to healthy controls [27]. HIF-1, a heterodimer of HIF-1α and HIF-1β, is a transcription factor which activates a variety of genes in response to hypoxia [28]. This pathway has the potential to affect iron levels in the brain by affecting intestinal iron absorption and through interaction with the transferrin receptor, whose gene contains an HIF binding sequence [29]. Binding by HIF-1α to this site leads to an increase in mRNA expression for the transferrin receptor, with this imbalance potentially affecting the flow of iron at the blood–brain barrier. An increase in transferrin receptor would favor increased brain iron, but only if normal or increased transferrin were present. In RLS patients compared to controls without RLS, transferrin has been demonstrated to be low in myelinated white matter [30], but high in choroid plexus [31], CSF [24], and neuromelanin cells in substantia nigra [21], and normal in peripheral lymphocytes [32]. So still, it is uncertain what role, if any, hypoxia plays in the pathophysiology of RLS.

Iron deficiency and its relation to the dopamine system

The link between iron and RLS symptoms may further be explained by its relationship to dopamine. Iron exists throughout the brain and is an essential cofactor in monoamine neurotransmitter synthesis [10, 33]. Neuromelanin-containing neurons in substantia nigra have weak acid cation exchange properties which leads to the binding of metals such as iron, manganese, copper, and still others [34]. Of course, these same neurons are responsible for the production of dopamine, and not coincidentally, iron is an essential cofactor of tyrosine hydroxylase, the rate-limiting enzyme of L-DOPA synthesis. In other words, in the brain, iron and dopamine co-localize and are intimately related to one another. The prototypical disease where abnormalities in one of these systems may affect the other is Parkinson's disease (PD). In PD, iron levels are increased in substantia nigra and many have hypothesized that this iron overload leads to oxidative stress-induced neurodegeneration and thus PD [35]. The situation is quite different in RLS, where iron levels are low in the brain. Our knowledge of the effect of this low iron on dopamine dynamics comes from animal and human studies of iron deficiency and RLS.

Rodents fed a diet low in iron and thus developing iron deficiency display poor sleep, increased tactile pain response, and increased locomotor activity [25, 36]. These iron-deficient rats demonstrate high levels of tyrosine hydroxylase in caudate, putamen, and ventral midbrain [37]. Congruent with this finding, extracellular dopamine levels sampled by microdialysis are increased in iron-deficient rats [38]. There is suggestion that this increased extracellular dopamine results from decreased extraction by dopamine transporter, whose levels are also low [39]. Dopamine receptor density is also affected by iron deficiency. Animal studies have shown that iron-deficient rats have decreased dopamine D1 and D2 receptors in the caudate and putamen, but similar levels in ventral midbrain, compared to normally fed animals [40–42]. Although, both D1 and D2 receptor subtype levels are decreased in iron-deficient animals, only D2 receptor levels correlate ($r = 0.91$) with iron concentration in caudate-putamen.

Human studies in RLS also support the notion of there being a hyperdopaminergic state in RLS. As iron is a cofactor of tyrosine hydroxylase, the rate-limiting enzyme of L-DOPA synthesis, one might expect that iron deficiency would result in decreased tyrosine hydroxylase. Human postmortem studies have shown just the opposite, increased tyrosine hydroxylase in substantia nigra of RLS patients [37]. In this study, there were no significant changes in these areas for levels of dopamine D1 receptor, dopamine transporters, or vesicular monoamine transporter, but there were significantly decreased levels of dopamine D2 receptors in putamen. These results suggest that there may be an increase in dopamine levels in RLS as tyrosine hydroxylase activity increases to produce more L-DOPA and dopamine receptors are downregulated. Furthermore, iron deficiency may cause these changes in the dopamine system, such as in rat models of iron deficiency [43].

The earliest report of human CSF dopamine metabolite sampling in RLS was in one RLS patient done in 1985, where free dopamine and homovanillic acid levels were increased [44]. In subsequent larger studies on this topic, levels of the dopamine metabolite, 3-ortho-methyldopa (3-OMD), were demonstrated to be high [45, 46]. 3-OMD is a product of a minor alternative pathway for the metabolism of L-DOPA [45]. The increased 3-OMD could be the result of decreased amino-acid decarboxylase activity, an excessive COMT and MAT activity, an excessive production of levodopa by tyrosine hydroxylase (TH), or some combination of all these. In a more detailed study of dopamine metabolites in human CSF, Earley et al. [46] again showed that 3-OMD levels were increased in CSF of persons with RLS compared to controls. CSF samples were taken both day and night. Unlike in controls where 3-OMD levels increased slightly at night, in RLS patients, 3-OMD levels decreased at night by about one-third, suggesting that in RLS at night, there may be a relative dopamine deficiency, yet on the background of a total hyperdopaminergic state.

Therefore, the sum of animal and human studies of the dopamine system in RLS suggests a hyperdopaminergic state, at least an increase in extracellular dopamine.

Treatment of RLS, a hyperdopaminergic state, with more dopamine?

If RLS is a hyperdopaminergic state, the obvious question becomes, "Why does treatment with dopamine agonist or L-DOPA decrease symptoms of RLS?" In RLS, total dopaminergic activity is increased, and as a result, dopamine receptors are downregulated. In RLS, as stated above, while total dopamine activity is likely heightened, there is circadian profile of dopamine activity that reflects hyperfunctioning in the morning and throughout the day, then relative hypofunctioning in the evening and nighttime. The dopamine receptor downregulation coupled with low dopamine activity at night may create a state of dopamine deficiency in the nighttime when RLS symptoms occur. Thus, providing additional dopamine activity, through dopamine agonism or increased L-DOPA, helps to relieve RLS symptoms. Unfortunately, this control of RLS symptoms by dopamine-based medicines may not persist. As dopaminergic activity is consistently supplemented

with medicine, dopamine receptors may become further downregulated, and dopamine requirements may increase, while dopamine deficiency at night may become more severe and occur for longer periods. This dopamine dysregulation theory may be the pathophysiologic basis for the clinical phenomenon of augmentation, in which there is a worsening of RLS symptoms occurring earlier in the day with greater severity in the setting of taking dopaminergic medication. As dopaminergic requirements increase, dopamine receptors may become further downregulated or desensitized. At the same time, the endogenous dopamine output may increase. The sum of these changes in the dopamine system is hypothesized to result in a worsening of RLS symptoms, including generalization of symptoms to the daytime and an increase in the severity of the symptoms. Augmentation was described by Christopher Earley and Richard Allen, who both advocate for the dysregulation of the dopamine system [47, 48].

Genetics of restless legs syndrome

Genetics seems to play an important role in the pathophysiology of RLS. RLS is often familial [49–51] and displays an autosomal dominant pattern of inheritance with anticipation [52, 53]. It is likely the early onset phenotype of RLS, rather than the secondary, late-onset RLS, that follows a genetic pattern [5, 6, 46]. Positive genetic findings in RLS have come through familial linkage and genome-wide association studies (GWAS). Genetic linkage studies have found gene regions that are associated with RLS, loci RLS1 through RLS5, but have not implicated specific genes [54, 55]. Alternatively, the majority of recent genetic evidence related to RLS has come via GWAS. Up to this point, significant genome-wide association has been found for single nucleotide polymorphisms (SNPs) in the following genes: *BTBD9, MEIS1, PTPRD, MAP2K5, SKOR1*, and *TOX3* [56–60]. It is also important to mention that PLMS, which occur in the majority of RLS patients and track with a familial diagnosis of RLS, is associated with SNPs in each of the above genes related to RLS. [56, 61] PLMS have been described as an endophenotype of RLS [51]. In the following sections, we will discuss the role of different genes and their possible contribution to the pathophysiology of RLS.

BTBD9

The *BTBD*9 gene was first associated with RLS (combined with PLMS) in Icelandic and United States populations through genome-wide association [58]. This association was confirmed again through a genome-wide study by other groups in different populations from Europe and Canada [59, 62]. The presence of the intronic genetic variant increases the odds of having RLS by 50–80% [58]. The BTB domain is located on the short arm of chromosome 6 at position 21.2 (6p21.2) and was identified in three *Drosophila* lines for which it is named, broad complex, tramtrack, and brick-a-brack. The function of *BTBD9* is incompletely understood, but it is involved in numerous cellular functions, including ion channel gating, transcriptional regulation, and ubiquitination of proteins [63].

To better understand how *BTBD9* relates to RLS, animal models have been created to study the implications of abnormality in the *BTBD9* gene, and its relation to both the dopamine and iron systems. An RLS-like phenotype of restlessness and fragmented sleep was found in both Drosophila and mice with mutant *BTBD9* gene expression [64, 65]. RNA interference mediated knockdown of BTBD9 in the large subsets of dopaminergic neurons in Drosophila leads to sleep fragmentation and restlessness. Furthermore, restricted knockdown in dopaminergic neurons does not lead to these changes. Brain dopamine is reduced by 50% in *BTBD9* knockdown Drosophila compared to wild-type flies. A possible explanation for reduction in total dopamine levels is through decreased activity of Tyrosine hydroxylase, a rate-limiting enzyme in dopamine biosynthesis, but this finding was not evident in mutant flies. In addition to this, when mutant flies were given the dopamine agonist, pramipexole, to replenish dopamine, they showed marked improvement in sleep consolidation to control levels. [65]. The PC12 cells in rat pheochromocytoma showed that BTBD9 may play a role in synaptic vesicle transport [66]. On the other hand, increased expression of *BTBD9* leads to increased ferritin in embryonic kidney cells [65]. A proposed mechanism for RLS pathophysiology states that a deficiency of brain iron favors increased synaptic dopaminergic signaling and accounts for the clinical efficacy of dopamine agonists via inhibitory auto-receptors [50, 67]. Furthermore, *BTBD9* seems to serve as an adaptor for ubiquitin ligases, such as Cullin-3, which regulates circadian and sleep rhythms. In summary, these fly and mice *BTBD9* knockdown models suggest that a lack of *BTBD9* results in cellular iron storage mishandling, an inactivation of the biosynthetic enzyme, tyrosine hydroxylase, in dopaminergic neurons, and finally a fragmented phenotypic sleep pattern with increased movement.

MEIS1

The presence of the *MEIS1* risk allele is associated with a nearly 50% increased odds of having RLS [59]. *MEIS1* is located on the short arm of chromosome 2 at position 14

(2p14) and has a broad level of functions ranging from leukemogenesis, hematopoiesis [68] to endothelial cell development in mice [69] and distal limb formation in zebrafish [70]. *MEIS1* also regulates substance P expression in amygdala [71] and has association with iron and dopamine systems. It is strongly expressed in dopaminergic neurons of the substantia nigra and in the spinal cord. Furthermore, association with iron is suggested by findings of increased ferritin in a *Caenorhabditis elegans MEIS1* orthologue knockdown model, and reduced *MEIS1* expression in human cells cultured under iron deficiency conditions [72].

Other genes

Basic research to understand the association between RLS and genes other than *BTBD9* and *MEIS1* found through genome-wide scans has not been carried out, but there known function may relate to RLS. *MAP2K5* is important for muscle differentiation [73] and neuroprotection of dopaminergic neurons [74]. *SKOR1*, in contrast, is expressed in spinal dorsal horn interneurons during development and cerebellar Purkinje cells [75]. *PTPRD* encodes protein tyrosine phosphatases that regulate cell growth and differentiation [76]. *TOX3* encodes transcription factors which function to modify chromatin structure [77].

It is important to note that while these common variants have moderate effect sizes that they only account for a minority of the inheritance in RLS with estimates that they account for only 10% of the total genetic variability in RLS [78]. Copy number variants, deletions, and insertions of genes have been less reported on. In addition, as GWAS have gained in popularity over the past decade, less research has been carried out to determine to what extent rare variants explain RLS heritability.

Hormones and restless legs syndrome

A hormonal basis for RLS is implicated by its diurnal nature, therapeutic responsiveness to dopaminergic agents, and association with hormonally dynamic states, including pregnancy. Wetter et al. [79] examined diurnal variation of prolactin, cortisol, and human growth hormone, but found no difference in hormone levels in RLS patients. A separate study found that RLS patients compared to control subjects exhibit greater inhibition of prolactin and greater increase in growth hormone, following challenge with L-dopa, and that this difference was only seen at night [80].

RLS is exceedingly common in pregnancy, affecting up to 23–26% of women by the third trimester [12, 81]. It is likely that low levels of iron during pregnancy may precipitate RLS but this is not certain. There is some evidence that female hormone changes during pregnancy may be associated with RLS. Pregnant women with RLS compared to those without RLS have increased levels of estradiol, but not progesterone, follicle stimulating hormone, luteinizing hormone, or testosterone [82]. Involvement of estrogen in RLS pathogenesis is suggested by data showing that estrogen replacement therapy usage is associated with a greater than twofold increased risk of developing RLS [83].

There has been some study of the glucocorticoid pathway in RLS. While cortisol levels are normal in RLS patients, adrenocorticotropin hormone levels are slightly lower in persons with RLS than those without RLS [84]. Evening infusion of hydrocortisone has also been shown to decrease sensory discomfort by as much as 50% compared to placebo [85]. Alternatively, in the rat, the administration of either adrenocorticotropin or alpha-melanocyte stimulating hormone (α-MSH) has been shown to create an RLS-like state, which consists of increased locomotion, insomnia, and periodic hindlimb movements during sleep [86] and in humans, intravenous administration of α-MSH.

It is well known that RLS symptoms are exquisitely responsive to opioid therapy, and because of this, there has been study of the endogenous opiate system in RLS. Neuropathologic investigation has revealed a reduction in β-endorphin and met-enkephalin positive cells in the thalamus but not substantia nigra of RLS patients compared to controls [87]. A nuclear imaging study demonstrated a negative correlation between RLS severity and opiate receptor binding in the medial thalamus, orbitofrontal cortex, and amygdala [88]. It is intriguing that β-endorphin, adrenocorticotropin hormone, and α-MSH share the same precursor hormone, pro-opiomelanocortin. The latter of these hormones have antagonistic roles. β-endorphin promotes analgesia, feeding, and inactivity, while α-MSH stimulates movement, sensitizes to pain, and promotes anorexia [89–91]. There is also interaction of dopamine with these hormones, as dopamine inhibits secretion of both β-endorphin and α-MSH [92, 93]. These opioid and melanocortin hormone systems deserve further study as they may relate to RLS pathophysiology (Table 1).

Conclusion

The pathologic state of RLS is characterized by central nervous system iron deficiency and net dopamine excess. While the dopamine and iron systems have been extensively studied in relation to RLS, it is currently not known how these states of iron deficiency and dopamine excess arise. Genetic studies and consideration of hormonal pathways have revealed abnormalities in biologic systems

Table 1 Changes in iron, dopamine, and hormones in restless legs syndrome

Serum	Cerebrospinal fluid	Brain
Iron ↔ [59, 61]	Ferritin ↓ [20, 30, 31]	Iron ↓ in S. nigra by MRI [34], pathology [74]
Ferritin ↓ [59, 61]	Transferrin ↑ [20, 30, 31]	Iron ↓ in Thalamus, caudate, S. nigra, putamen [28]
		Ferritin ↓ in S. nigra [74]
		Transferrin ↓ in S. nigra [22]
Cortisol ↔ [42, 53]	3-OMD ↑ [3, 80]	Tyrosine hydroxylase ↑ in S. nigra [46]
ACTH ↓ [42]		Dopamine-1 receptor in S. nigra [46]
		Dopamine-2 receptor ↓ in putamen [46]
		β-endorphin in thalamus ↓ [79]
		β-endorphin in S. nigra ↔ [79]

↔ no change, ↑ increased levels, ↓ decreased levels, *ACTH* adrenocorticotropin hormone, *3-OMD* 3-ortho-methyldopa, *S. nigra* substantia nigra

external to the confines of dopamine and iron. Nevertheless, these pathways, including BTBD9, MEIS1, endogenous opioid, and melanocortin, have important interactions with the dopamine and iron systems and thus may not be completely extrinsic to them. In the end, it is likely that the true pathophysiologic story of RLS is much more complicated than involvement of one or two biologic systems, and it will take a great deal of human and animal research to clarify the mechanisms underlying RLS.

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Compliance with ethical standards

Conflicts of interest The authors have no conflicts of interest to report.

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