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The molecular basis of restless legs syndrome

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Restless legs syndrome (RLS) disrupts sleep in a substantial proportion of the population and is associated with higher cross-sectional rates of affective illness and cardiovascular disease. While dopamine and iron availability in the brain modulate emergence of symptoms, and dopamine agonists and iron alleviate the sensory symptoms and motor signs of RLS, the biology of the disorder is incompletely understood. Genetic factors, as opposed to environmental ones, account for most of the disease variance. The at-risk allelic variants exist in non-coding regions of at least six genes rendering it a complex genetic disease. Nonetheless, these provide the first hypothesis independent clues that advance a better understanding of RLS pathophysiology.

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Introduction

Restless legs syndrome (RLS) is a common, complex, trait sensory experience characterized by an insatiable urge to move one's legs, that is, often painful, and that interferes with sleep onset and maintenance in a clinically meaningful way in 0.5–1.0% of children and teenagers [1] and 2–3% of adults [2]. The negative impact of RLS upon quality of life can be substantial, and potentially accounts for a near doubling of cross-sectional rates of anxiety/depression and cardiovascular disease in the disorder [3,4••]. Motor signs of RLS include non-volitional myoclonus (*viz.*, periodic leg movements; PLMs) while awake and asleep that are an informative endophenotype [5]. Expressivity is influenced by aging, female sex, pregnancy, deficient stores of mobilizable iron, genes, and many common medical conditions and diseases of the nervous system [3]. Circadian and homeostatic influences appear to independently impact the emergence of RLS and PLMs in the evening and around bedtime, and early in the sleep period [6–10]. Pharmacological agonists of D_2 and D_3 dopamine receptors, opioids, and derivatives of gabapentin are effective treatments for both the sensory symptoms (RLS) and its motor signs (PLMs) [3]. There remains no clear consensus as to whether a hypo-dopaminergic or hyper-dopaminergic state lies at the core of RLS/PLMs and which brain circuits are principally responsible [4••].

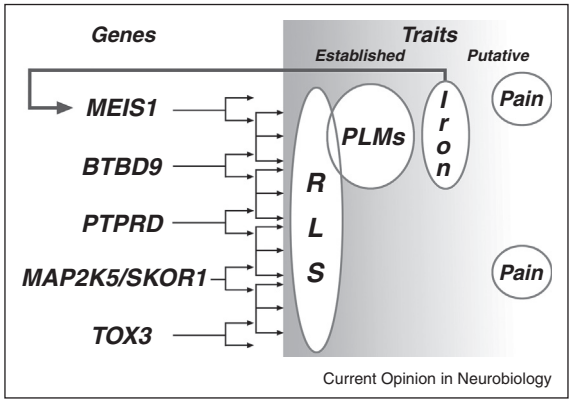
Human genetics

The picture of how variants in the sequence of the human genome confer risk of RLS/PLMs is beginning to emerge. Independent genome-wide association studies (GWAS) in diverse populations of Northern European origin suggest involvement of six different genes which are widely expressed in the central nervous system and other organs: *BTBD9*, *MEIS1*, *PTPRD*, *MAP2K5*, *SKOR1*, and *TOX3* [11••,12••,13,14•,15•,16]. Together, the allelic variants implicating these six genes account for nearly 80% of the population attributable risk for RLS. The at-risk SNPs are common and are located within non-coding, intronic, or intergenic regions. Association of the SNP *rs3923809* in *BTBD9* increases risk of RLS and PLMs (even in the absence of RLS sensory symptoms) by 70–100%. Nearly one-half of those of northern European ancestry are homozygous for this risk allele, so by itself, it appears to play a role in at least 50% of RLS cases. A minor allele of *rs113851554* in *MEIS1* nearly doubles one's risk and is in a known regulatory element associated with reductions in *MEIS1* mRNA and protein expression [17]. RLS susceptibility variants associated with *PTPRD*, *MAP2K5*, *SKOR1*, and *TOX3* confer more modest risks. Almost nothing is known about how factors generally acknowledged to influence RLS expressivity (e.g. female sex, advanced age, and iron decrements) [4••] interact with the at-risk variants. Copy number and structural variants (e.g. insertions or deletions) that might disrupt the function of the implicated genes and thereby shed light upon the pathophysiology of RLS/PLMs have not been reported. Similarly, informative *cis* (local) or *trans* (distant) regulatory effects upon gene and protein expression and splice variants associated with the at-risk alleles have not been reported [11••,12••,13,17]. The coding regions and exon–intron boundaries of *BTBD9* [18] and *MEIS1* [17–19] also lack any common functional polymorphisms. Several rare exonic variants in *MEIS1* have been reported; however, they do not segregate faithfully with the RLS phenotype [18,19]. Nonetheless, these two genetic susceptibility factors are plausible contributors to disease pathophysiology because: first, there is a dose effect of the *BTBD9* at-risk allele upon decrements in iron stores in a population enriched for RLS [11••]; second, a *MEIS1* at-risk haplotype associates with aberrant iron homeostasis [20•]; third, the dosage of the *BTBD9*

[11••] and *MEIS1* (H. Stefansson and D. Rye, unpublished observations) risk alleles predict PLMs number; fourth, ethnic frequencies of the at-risk alleles generally mirror the different prevalences of RLS reported across the world [16]; fifth, RLS can be modeled in animals by genetic disruptions of *BTBD9* [21••,22••]; and sixth, manipulations of *BTBD9* in animals and cultured cells disrupt iron and dopamine homeostasis (see below) [21••,22••].

A comprehensive understanding of how the identified genetic susceptibility alleles lead to RLS remains elusive because: first, RLS-related risk alleles are in non-coding regions which are generally believed to subserve regulatory functions such as influencing when and where genes are turned 'on' and 'off'; second, there is evidence for pleiotropic and epigenetic effects; and third, phenocopies of RLS/PLMs exist. Single genes can affect multiple traits such as those comprising the RLS phenotypic spectrum (e.g. pleiotropy; see Figure 1). Decrements in mobilizable stores of iron as reflected in low serum ferritin levels, for example — often considered an intermediate trait essential for the clinical syndrome of RLS [4••] — may be modified by pleiotropic actions of the same genes that independently influence RLS (Figure 1). Conversely, iron may have epigenetic effects upon gene expression in individuals harboring RLS susceptibility variants. This hypothesis is supported by reports that *MEIS1* expression is reduced in human cells cultured under iron-deficient conditions [20•]. Epigenetic effects of iron upon *BTBD9* may also exist given that its RLS at-risk allele does not associate with low serum ferritin levels in the general population [23••], but does so in a population enriched for RLS [11••]. Asymptomatic manifestations of RLS that predate emergence of sensory complaints, yet are impacted by some of the same genetic factors, are also problematic; for example, in the instance of PLMs that often reflect a *forme fruste* of RLS. Thus, since RLS is a complex trait, a comprehensive determination of the genetic architecture will require — at a minimum — phenotyping of asymptomatic individuals for PLMs. An additional confound is the potential tissue specific regulation of genes implicated in RLS, as has been suggested for *MEIS1* [20•]. Increased co-morbidity of RLS-like symptoms with other medical conditions represents either an opportunity to identify biological factors that influence trait expressivity by way of the at-risk alleles or problematic phenocopies. For example, RLS/PLMs are exceedingly common in end-stage renal disease [4••], and are associated with *BTBD9* and *MEIS1* variants [24]; however, these associations are not discernible in multiple sclerosis patients in whom RLS-like symptoms and PLMs emerge at a frequency greater than that of controls [25].

Figure 1



Established and putative influences of RLS susceptibility genes upon traits comprising the RLS phenotypic spectrum. Genome wide association studies have identified multiple variants largely in introns that confer risk of RLS including: *MEIS1*, *BTBD9*, *PTPRD*, *MAP2K5/SKOR1*, and *TOX3*. Deciphering how these individual genes and molecular pathways contribute to the clinical syndrome of RLS is complicated by the potential differential effect(s) that a single allele might have on multiple traits (i.e. pleiotropy) such as: PLMs number, iron metabolism, and the perception of pain. Epigenetic effects of iron upon expression of *MEIS1*, age, sex, and tissue dependent effects upon gene expression, and the existence of RLS/PLMs phenocopies are also significant challenges to further delineation of this genetic architecture (see text for details). *Abbreviations*: PLMs, periodic leg movements of sleep; RLS, restless legs syndrome.

Experimental studies

Echoing a theme emerging from GWAS of many common diseases, the variants conferring susceptibility to RLS/PLMs do not provide a parsimonious explanation for how and why symptoms and signs emerge. The at-risk SNPs in each instance implicate genes that are widely expressed in the central nervous system and other organs.

BTBD9

Though much is known of the versatile BTB-domain-containing protein family, exceedingly little is known about the function of BTBD9 itself. The protein harbors three highly conserved peptide domains, in order from amino to carboxy terminus: a BTB domain, a BACK domain, and several Kelch repeats. The BTB domain is a phylogenetically conserved protein–protein interaction motif named for the three *Drosophila* lines in which it was originally identified: broad complex, tramtrack, and bric-à-brac. BTB containing proteins participate in a wide range of cellular functions, including transcriptional regulation, cytoskeleton dynamics, ion channel assembly and gating, and targeting proteins for ubiquitination [26,27]. The BACK (for BTB and C-terminal Kelch) domain is highly conserved across metazoan genomes, and may facilitate substrate orientation during ubiquitin ligation [28,29]. Kelch repeats interact with actin and play a role in cytoskeletal/microfilament orientation [30].

Animal models have proven powerful in unraveling the molecular mechanisms of BTBD9's causative role in RLS [21••,22••]. The function of BTBD9 was unknown when