Published in final edited form as:
Curr Biol. 2012 June 19; 22(12): R485–R486. doi:10.1016/j.cub.2012.05.004.

Neurological Disorders: Towards a Mechanistic Understanding of Restless Legs Syndrome

Paul J. Shaw[1] and **Stephen P. Duntley**[2]

Paul J. Shaw: shawp@pcg.wustl.edu

[1]Anatomy and Neurobiology, Washington University School of Medicine, 660 S. Euclid Ave Campus Box 8108, St. Louis, MO 63110, USA

[2]Department of Neurology, Washington University Sleep Medicine Center, 212 North Kingshighway, Suite 237, St. Louis, MO 63108, USA

Abstract

Restless legs syndrome is a curious neurological disorder of unknown aetiology. A new study has found that *Drosophila* mutants in the fly homologue of a human gene, *BTBD9*, that has been implicated as a risk factor for restless legs display important features of the syndrome.

Restless legs syndrome is a common neurological disorder characterized by an urge to move the legs associated with uncomfortable sensations that occur or worsen with rest, are relieved by movements, and are worse in the evening [1]. Motor restlessness is evident when awake and periodic limb movements frequently occur during sleep, resulting in sleep fragmentation and non-restorative sleep. When the syndrome is mild it may be a mere nuisance, but when severe it can be life-altering, resulting in profound sleep disruption, impaired daytime functioning, a global decrease in quality of life and possibly increased cardiovascular disease risk [2]. The diagnosis is made by clinical symptoms and there is no definitive confirmatory testing available. Although clinical research has demonstrated that restless legs syndrome is associated with iron deficiency [3] and can be treated pharmacologically with dopamine agonists [4], the underlying mechanisms are largely unknown and even localization of the primary pathology within the nervous system is uncertain. Population and family studies indicate that restless legs syndrome has a strong genetic component, so several groups have carried out genome-wide association studies to identify genes that confer susceptibility to restless legs syndrome [5]. These studies have successfully identified a number of genetic variants suspected to be involved in restless legs syndrome, including one named *BTBD9* [6,7]. While additional loci continue to be identified [8], it has been difficult to demonstrate that these susceptibility genes are causative for restless legs syndrome. In this issue of *Current Biology*, Freeman *et al.* [9] report how they used the molecular genetic tools available in the model genetic organism *Drosophila melanogaster* to functionally characterize the fly homologue of *BTBD9* (*dBTBD9*) as a causal factor in restless legs syndrome.

BTBD9 is a poorly characterized gene that accounts for approximately 50% of the population attributable risk for restless legs syndrome and is therefore an ideal candidate for functional analysis using *Drosophila* genetics. To begin, Freeman *et al.* [9] identified the fly homologue of *BTBD9* and created two null mutations in the gene. When they examined sleep in these mutants, they found that both mutant alleles caused severely fragmented nighttime sleep and an increase in the amount of waking after sleep onset, as is seen in human patients with restless legs syndrome. Because sleep fragmentation is only one component of restless legs syndrome, the authors needed to evaluate aspects of movement that have face-validity with symptoms seen in human patients with restless legs syndrome.

In humans, the diagnosis of restless legs syndrome is facilitated by the 'suggested immobilization test', in which periodic limb movements and leg discomfort are worsened when subjects are asked to sit in bed with their legs outstretched for an hour [10]. Intriguingly, when mutants in *dBTBD9* were confined to a small space, to simulate the suggested immobilization test, they showed significantly more locomoter behavior than controls. Moreover, when locomotor behavior was precisely quantified in an independent assay, the mutants were found to spend more time in longer uninterrupted bouts of walking than controls. Together, these data indicate that *dBTB9* mutant flies recapitulate key behavioral aspects of restless legs syndrome, including sleep fragmentation and motor restlessness.

To determine whether *dBTB9* would also show physiological changes found in restless legs syndrome patients, Freeman *et al.* [9] examined dopamine signaling and iron homeostasis in the mutant flies. Interestingly, they found that flies mutant for *dBTBD9* show dramatically reduced brain dopamine levels and that the abnormal sleep phenotype was completely rescued by administering mutant flies the dopamine D2 receptor agonist Pramipexole, which is used clinically in humans to treat restless legs syndrome. Importantly, Freeman *et al.* [9] used RNA interference (RNAi) to show that they could recapitulate restless legs syndrome symptoms by knocking-down *dBTBD9* expression in a subset of dopaminergic neurons. Finally, the authors over-expressed *BTBD9* in HEK cells to confirm that the gene does indeed play a role in iron homeostasis, acting via regulation of iron regulatory protein-2 (IRP2). It is worth noting that a homologue of another restless legs syndrome associated variant, *MEIS1*, has also been shown to affect iron homeostasis in *Caenorhabditis elegans* [11].

How does *BTBD9* regulate dopamine signalling, iron homeostasis and sleep? Although much more work needs to be done, Freeman *et al.* [9] propose that the BTBD9 protein belongs to a family of BTB-domain containing substrate adaptors for the Cullin-3 (Cul3) class of E3 ubiquitin ligases. Interestingly, *Cul3* has recently been shown to regulate sleep in flies [12], further supporting the idea that Cul3 has a role in sleep regulation. In the case of iron homeostasis, BTBD9 may act together with Cullin-3 to regulate levels of iron regulatory protein-2 (IRP2), which controls ferritin expression and thus iron metabolism. BTBD9 also seems to play a role in dopamine biosynthesis by unknown mechanisms. Several questions remain but they are now experimentally tractable. Are iron regulation and dopamine regulation mechanistically related, or are they independent processes? Is the specificity of *dBTBD9* within dopaminergic neurons due to restricted expression of *dBTBD9* in a defined set of dopaminergic neurons? Or, is the specificity of *dBTBD9* due to differences in the presence of Cul3 substrates in particular neurons? Is the ability of *dBTBD9* to influence sleep regulation confined to dopaminergic neurons or does dBTBD9 play a more fundamental role in regulating sleep and waking throughout the nervous system? Finally, which are the substrates for BTBD9 and Cul3, and how might they influence restless legs syndrome?

Genome-wide association studies have succeeded in identifying a large number of disease susceptibility genes. Nonetheless, it is frequently difficult to collect additional data that can provide causative or functional insight into the underlying molecular mechanisms and this is only complicated further when evaluating complex traits that are differentially influenced by environmental conditions. With this in mind, one of the most compelling aspects of the approach of Freeman *et al.* [9] is the successful use of a model system to functionally characterize a susceptibility gene that was originally identified in human genome-wide association studies. A similar strategy has recently been used to functionally examine genes identified in a genome-wide association study for loci influencing the burden of Alzheimer's disease [13] and to extend the results from a small genetic study on short sleeping humans

[14]. In this regard, it is important to note that tools have been developed so that orthologous genes can be quickly identified in *Drosophila* and other model organisms [15]. These tools will certainly expedite the ability of labs to identify and then test candidate genes from genome-wide association studies to provide critical insight into mechanism and causation of human disease states. Thus, the use of model systems to functionally characterize genes identified with genome-wide association studies is a move in the right direction.

References

1. Yeh P, Walters AS, Tsuang JW. Restless legs syndrome: a comprehensive overview on its epidemiology, risk factors, and treatment. Subst Use Misuse. 2011; 47:99–107. [PubMed: 22066751]
2. Walters AS, Rye DB. Evidence continues to mount on the relationship of restless legs syndromee/periodic limb movements in sleep to hypertension, cardiovascular disease, and stroke. Sleep. 2010; 33:287. [PubMed: 20337185]
3. Trenkwalder C, Paulus W. Restless legs syndrome: pathophysiology, clinical presentation and management. Nat Rev Neurol. 2010; 6:337–346. [PubMed: 20531433]
4. Clemens S, Rye D, Hochman S. Restless legs syndrome: revisiting the dopamine hypothesis from the spinal cord perspective. Neurology. 2006; 67:125–130. [PubMed: 16832090]
5. Sehgal A, Mignot E. Genetics of sleep and sleep disorders. Cell. 2011; 146:194–207. [PubMed: 21784243]
6. Yang Q, Li L, Chen Q, Foldvary-Schaefer N, Ondo WG, Wang QK. Association studies of variants in MEIS1, BTBD9, and MAP2K5/SKOR1 with restless legs syndromee in a US population. Sleep Med. 2011; 12:800–804. [PubMed: 21925394]
7. Stefansson H, Rye DB, Hicks A, Petursson H, Ingason A, Thorgeirsson TE, Palsson S, Sigmundsson T, Sigurdsson AP, Eiriksdottir I, et al. A genetic risk factor for periodic limb movements in sleep. N Engl J Med. 2007; 357:639–647. [PubMed: 17634447]
8. Skehan EB, Abdulrahim MM, Parfrey NA, Hand CK. A novel locus for restless legs syndromee maps to chromosome 19p in an Irish pedigree. Neurogenetics. 2012; 13:125–132. [PubMed: 22411506]
9. Freeman A, Pranski E, Miller RD, Radmard S, Bernhard D, Jinnah H, Betarbet R, Rye DB, Sanyal S. Sleep fragmentation and motor restlessness in a Drosophila model of restless legs syndrome. Curr Biol. 2012; 22:1142–1148. [PubMed: 22658601]
10. Michaud M, Lavigne G, Desautels A, Poirier G, Montplaisir J. Effects of immobility on sensory and motor symptoms of restless legs syndromee. Mov Dis Off J Mov Dis Soc. 2002; 17:112–115.
11. Catoire H, Dion PA, Xiong L, Amari M, Gaudet R, Girard SL, Noreau A, Gaspar C, Turecki G, Montplaisir JY, et al. Restless legs syndrome-associated MEIS1 risk variant influences iron homeostasis. Ann Neurol. 2011; 70:170–175. [PubMed: 21710629]
12. Stavropoulos N, Young MW. insomniac and Cullin-3 regulate sleep and wakefulness in Drosophila. Neuron. 2011; 72:964–976. [PubMed: 22196332]
13. Shulman JM, Chipendo P, Chibnik LB, Aubin C, Tran D, Keenan BT, Kramer PL, Schneider JA, Bennett DA, Feany MB, et al. Functional screening of Alzheimer pathology genome-wide association signals in *Drosophila*. Am J Hum Genet. 2011; 88:232–238. [PubMed: 21295279]
14. He Y, Jones CR, Fujiki N, Xu Y, Guo B, Holder JL Jr, Rossner MJ, Nishino S, Fu YH. The transcriptional repressor DEC2 regulates sleep length in mammals. Science. 2009; 325:866–870. [PubMed: 19679812]
15. Hu Y, Flockhart I, Vinayagam A, Bergwitz C, Berger B, Perrimon N, Mohr SE. An integrative approach to ortholog prediction for disease-focused and other functional studies. BMC Bioinf. 2011; 12:357.