Fruit flies with Restless Legs Syndrome point to a genetic cause

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When flies are made to lose a gene with links to Restless Legs Syndrome (RLS), they suffer the same sleep disturbances and restlessness that human patients do. The findings reported online on May 31 in *Current Biology* strongly suggest a genetic basis for RLS, a condition in which patients complain of an irresistible urge to move that gets worse as they try to rest.

Scientists have discovered that mutations in the gene BTBD9, which is linked with restless legs syndrome (RLS) in humans, disturb sleep in fruit flies. The mutant flies wake up more often during sleep periods, which resembles a key feature of human RLS.

The same mutations in BTBD9 also reduce levels of the neurotransmitter dopamine in the flies. Some kind of deficiency in dopamine signaling is thought to lie behind RLS in humans.

The results are published in the journal Current Biology.

"Flies and humans are distant from each other on the evolutionary tree, yet the same gene seems to be regulating a fundamental process in both organisms and affecting how soundly they sleep," says senior author Subhabrata Sanyal, PhD, assistant professor of cell biology at Emory University School of Medicine.

People with RLS experience unpleasant sensations in their legs and urges to move them, interfering with the ability to sleep. Genetics plays a

major role in RLS, and most people with RLS have a close family member with the disorder. A variant in the BTBD9 gene accounts for about half of the risk for RLS in the population, according to multiple genetic studies.

While medications exist to treat RLS, in some patients they are ineffective or have side effects. Researchers don't have a good understanding of what is going wrong in the nervous system in people affected by RLS, or what the BTBD9 gene does. Studying the fly version of BTBD9 could shed light on the basic biology and eventually lead to improved treatments for humans.

Postdoctoral fellow Amanda Freeman, the first author of the paper, examined flies' sleep behavior by putting individual flies into tubes with infrared sensors, which can detect when a fly moves across the middle of the tube. If a fly doesn't cross the beam for five minutes, it's considered asleep. She found that the BTBD9 mutant flies woke up more often during the night.

Disabling BTBD9 also makes flies more mobile while awake. Mutant flies confined in a tube move back and forth more often, leading Freeman and Sanyal to dub the mutant flies "wanderlust."

"People with RLS usually find it very difficult to sit and keep their legs as still as possible," she says. "We can't ask the flies to sit still, so we put them in a confined space."

In addition, the mutant flies have half the normal level of the neurotransmitter dopamine. Pramipexole, a drug that modulates dopamine activity and is FDA-approved to treat RLS in humans, can help the mutant flies sleep more soundly.

The mutant flies have a lifespan that is about 25 percent shorter: half of

them are dead at 30 days compared to 40 days.

"What we see strongly suggests that BTBD9 is regulating dopamine, either its production or its packaging," Sanyal says. "The flies may have alterations in several basic functions, such as sleep and locomotion, because of the changes in dopamine."

Another connection to RLS is that BTBD9 may be having its effects on dopamine indirectly via iron, which is required for dopamine synthesis. Iron deficiency exacerbates RLS and iron supplements can help alleviate its symptoms. Freeman and Sanyal found that in human cells, BTBD9 appears to regulate production of ferritin, an important iron storage protein.

The mutations the Emory team studied completely disable the fly BTBD9 gene. In contrast, variants within the BTBD9 gene are linked strongly with RLS but don't have an obvious impact on the gene's function, according to co-author David Rye, MD, PhD. Rye is professor of neurology at Emory University School of Medicine and director of research for Emory Healthcare's sleep program.

"That's what's so remarkable about this result," Rye says. "The genetic context may be different, but the effects of the mutation are consistent with RLS and the same modifying factors, such as dopamine and iron, are involved."

Freeman and Sanyal say their next task is to define BTBD9's function in the fly nervous system. BTBD9 shares some similarity with a group of genes involved in breaking down and removing waste proteins. Researchers elsewhere recently found that a related gene is also involved in sleep regulation; the mutant flies in that study were named "insomniac."

More information: A. Freeman, E. Pranski, D. Miller, S. Radmard, D. Bernhard, H. Jinnah, R. Betarbet, D. Rye, and S. Sanyal. Sleep fragmentation and motor restlessness in a Drosophila model of Restless Legs Syndrome. *Curr Biol* (2012).

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