Scientists shed light on cause of spastic paraplegia

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Scientists at The Scripps Research Institute (TSRI) have discovered that a gene mutation linked to hereditary spastic paraplegia, a disabling neurological disorder, interferes with the normal breakdown of triglyceride fat molecules in the brain. The TSRI researchers found large droplets of triglycerides within the neurons of mice modeling the disease.

The findings, reported this week online ahead of print by the journal *Proceedings of the National Academy of Sciences*, point the way to potential therapies and showcase an investigative strategy that should be useful in determining the biochemical causes of other genetic illnesses. Scientists in recent decades have linked thousands of gene mutations to human diseases, yet many of the genes in question code for proteins of unknown function.

"We often need to understand the protein function that is disrupted by a gene mutation, if we're going to understand the mechanistic basis for the disease and move towards developing a therapy, and that is what we've tried to do here," said Benjamin F. Cravatt, professor and chair of TSRI's Department of Chemical Physiology.

There is currently no treatment for hereditary spastic paraplegia (HSP), a set of genetic illnesses whose symptoms include muscle weakness and stiffness, and in some cases cognitive impairments. About 100,000 people worldwide live with HSP.

Uncovering Clues

In the new study, Cravatt and members of his laboratory, including graduate student Jordon Inloes and postdoctoral fellow Ku-Lung Hsu, focused on DDHD2, an enzyme of unclear function whose gene is mutated in a subset of HSP cases. "These cases involving DDHD2 disruption feature cognitive defects as well as spasticity and muscle wasting, so they're among the more devastating forms of this illness," said Cravatt.

To start, the researchers created a mouse model of DDHD2-related HSP, in which a targeted deletion from the DDHD2 gene eliminated the expression of the DDHD2 protein. "These mice showed symptoms similar to those of HSP patients, including abnormal gait and lower performance on tests of movement and cognition," said Inloes.

Prior research had suggested that the DDHD2 enzyme is expressed in the brain and is involved somehow in lipid metabolism. One study reported elevated levels of an unknown fat molecule in the brains of DDHD2-mutant HSP patients. Cravatt's team compared the tissues of the no-DDHD2 mice to the tissues of mice with normal versions of the gene, and also found that the mutant mice had much higher levels of a type of fat molecule, principally in the brain.

Using a set of sophisticated "lipidomics" tests to analyze the accumulating fat molecules, they identified them as triglycerides—a major component of stored fat in the body, and a risk factor for obesity, atherosclerosis and type 2 diabetes.

"We were able to show as well, using both light microscopy and electron microscopy, that droplets of triglyceride-rich fat are present in the neurons of DDHD2-knockout mice, in several brain regions, but are not present in normal mice," said Inloes.

For the next phase of the study, Cravatt's team developed a more precise tool for studying DDHD2's function: a specific inhibitor of the DDHD2 enzyme, one of a set of powerful enzyme-blocking compounds they had identified in a study reported last year. "After four days of treatment with this inhibitor, normal mice showed an increase in brain triglycerides," said Inloes. "This suggests that DDHD2 normally breaks down triglycerides, and its inactivity allows triglycerides to build up."

Finally the team confirmed DDHD2's role in triglyceride metabolism by showing that triglycerides are rapidly broken down into smaller fatty acids in its presence.

"These findings give us some insight, at least, into the biochemical basis of the HSP syndrome," said Cravatt.

Looking Ahead

Future projects in this line of inquiry, he adds, include a study of how triglyceride droplets in neurons lead to impairments of movement and cognition, and research on potential therapies to counter these effects, including the possible use of diacylglycerol transferase (DGAT) inhibitors, which reduce the natural production of triglycerides.

Cravatt also notes that the same approach used in this study can be applied to other enzymes in DDHD2's class (serine hydrolases), whose dysfunctions cause human neurological disorders.

More information: The hereditary spastic paraplegia-related enzyme DDHD2 is a principal brain triglyceride lipase, *PNAS*, www.pnas.org/cgi/doi/10.1073/pnas.1413706111

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