New technique for revealing genetic repeats yields surprising insights into Huntington's disease

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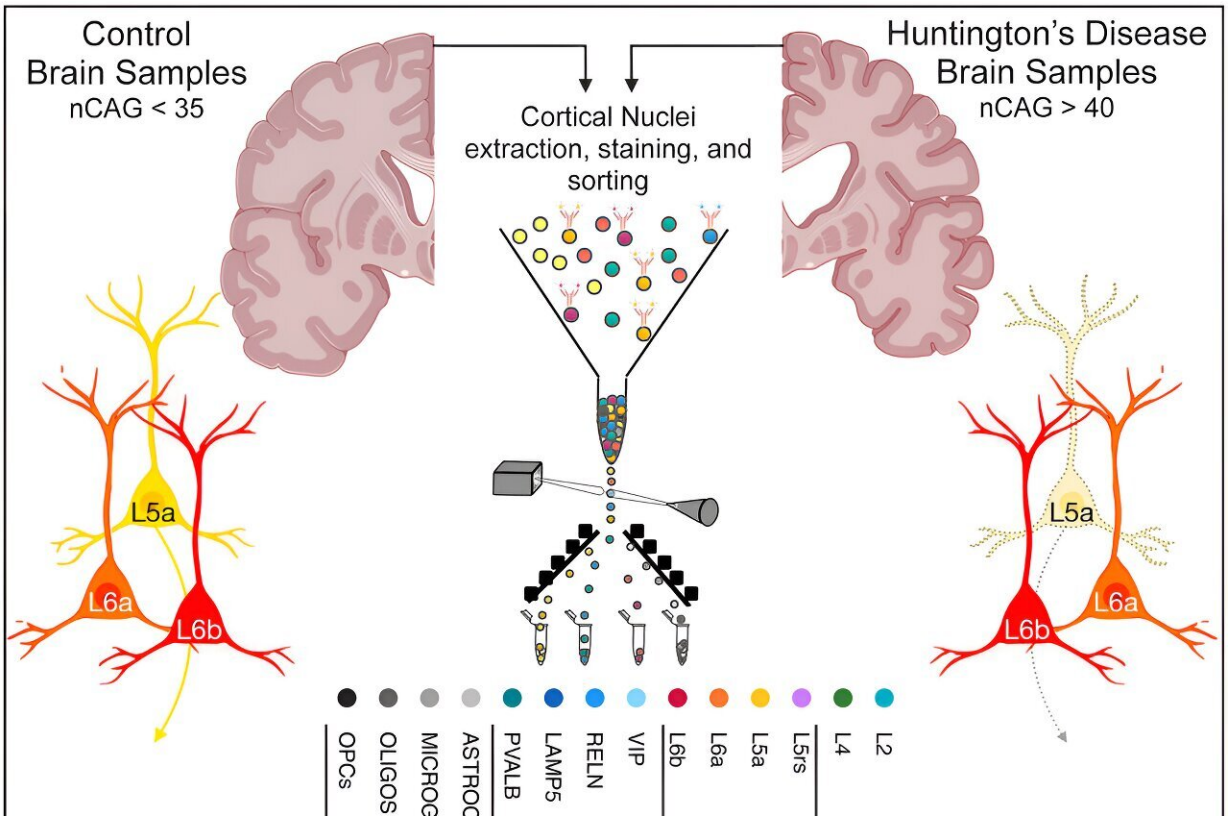

Credit: *Neuron* (2024). DOI: 10.1016/j.neuron.2023.12.009

Neurodegenerative diseases are among the most complex human ailments, and their exact causes and mechanisms are the subject of

ongoing research and debate. When it comes to Huntington's disease, steadily accumulating evidence over the past 30 years has led to a model of molecular events that explains several key features of the disease, including why it has an earlier onset in some people and why it causes symptoms such as involuntary movements and mood swings.

But two new complementary papers from The Rockefeller University suggest that this may not be the whole story.

Huntington's is caused by somatic CAG expansions in which a triplet repeat of DNA bases in a mutated Huntingtin (mHTT) gene increase in number throughout life, leading to cell death. As described in *Nature Genetics* and in *Neuron*, the Rockefeller scientists used a custom technique to reveal that these genetic repeats are unstable, and likely producing more toxic proteins, only in select brain cell types. Moreover, some cells they studied proved surprisingly resilient to CAG repeat expansion.

The findings both illuminate cellular nuances of a still-mysterious condition and provide potential targets for therapeutic interventions in the future.

"What we didn't expect was to see that cells carrying long CAG repeats can survive," says Nathaniel Heintz, head of Rockefeller's Laboratory of Molecular Biology. "That's very surprising. If expansion is not sufficient for death, what else is required?"

A troublesome trio

In mHTT, the repeated DNA bases—cytosine, adenine, and guanine—form lengthy and unstable stretches, resulting in mutant proteins that cause damage to some neural cells.

The number of CAG repeats closely corresponds to disease risk: People with more than 40 repeats will inevitably develop Huntington's, usually between ages 30 and 50. The longer the CAG segments, the earlier the onset of symptoms. As neurodegeneration snowballs, the disease turns fatal, because we lack treatments that slow or prevent the somatic expansion and accumulation of mutant huntingtin.

The molecular processes underlying CAG expansion and cell death are not understood. Over the decades, the Heintz lab has generated deep, high-resolution transcriptional and epigenetic data about the mammalian brain that has, among other things, resulted in the discovery of interesting new modifications to the neuronal genome that may play critical roles in brain aging and degeneration. More recently, the team decided to apply similar approaches to the study of Huntington's.

"Generating high-quality data from postmortem samples in early stages of the disease can give us insight into what predisposes certain cell types to succumb," Heintz says.

New methods

To do so, Kert Mätlik, a research associate in Heintz's lab and first author of the *Nature Genetics* paper, adapted a cellular analysis method called FANS (fluorescence-activated nuclear sorting) to molecularly profile specific cells in the striatum, a region of the deep brain connected to motor control and cognition that is greatly affected by Huntington's and Parkinson's disease.

By analyzing striatal cells from people who died of Huntington's—and donated their brains to science—Mätlik found that the repeat segments were particularly unstable in medium spiny neurons (MSNs), the most common striatal neurons. These are also the cells that are known to be lost from the striatum during the progression of Huntington's disease.

Mätlik noticed that the levels of two DNA repair proteins MSH2 and MSH3, forming the MutSβ complex, were especially high in MSNs. In dividing cells, the job of these proteins is to initiate the repair of mismatched DNA strands, thus stabilizing the genome by preventing mutations that could otherwise lead to cancer.

Yet when it comes to CAG repeats, these proteins appear to promote CAG expansion rather than prevent it. "Their elevated levels could really be doing these neurons a disservice," Mätlik says.

Though the resilience of most other striatal cell types could be explained by the stability of CAG repeats in them, Mätlik was surprised to find not all cells with CAG expansions are impacted in the same way. "That led us to believe that while CAG expansion is a key first step in the pathogenesis of Huntington's, it doesn't cause cell death in all cases. Some other process is playing a role," says Mätlik.

A new cell type on the map

In the second study, published in *Neuron*, first author Christina Pressl, an instructor in clinical investigations at Rockefeller, developed the sFANS (serial fluorescence-activated nuclear sorting) method to isolate different cell types in five regions of the cortex—the motor, cingulate, visual, insular, and prefrontal cortices—in 13 early-stage HD brains.

The cortex is composed of six layers, each numbered corresponding to its depth; higher numbers equal deeper layers. The majority of its cells are pyramidal neurons, which use dendrites to take in synaptic inputs and axons to send out action potentials, mostly to other parts of the brain. Some of these transmissions travel as far as the spinal cord.

Pressl used sFANS and deep molecular profiling to identify 16 cell types. "We immediately saw a specific set of cells that was majorly

disturbed," she says.

In the motor region of the cortex, pyramidal cells in layers five and six had dramatically long CAG repeats in comparison to all other cortical cell types. By employing sFANS followed by single-cell RNA sequencing, the researchers showed that only layer 5A cells were more likely to die.

With this discovery, "we've put another cell type on the map for increased vulnerability for Huntington's disease," Pressl notes.

Why 5A cells were the least resilient, however, "is really puzzling," she says. One potential reason is that they project to the striatum, and connectivity between those regions has been shown to falter in Huntington's disease. "Perhaps the vulnerable 5A cells found in the cortex are connected to the vulnerable MSN cells found in the striatum," Pressl says. "When it comes to Huntington's, the entire neural network breaks down at some point."

Exploring core mysteries

Heintz's team will pursue multiple lines of inquiry from these findings. Mätlik will seek a clearer understanding of how the MutSβ complex harms rather than heals in MSNs. "Our findings have contributed to the interest these proteins have raised as potential targets for therapeutic intervention for Huntington's," Mätlik notes.

Pressl will investigate the cortex, which is largely unexplored in neurodegenerative diseases: "I'm very excited to apply our strategies to multiple cortical regions of Alzheimer's brains," she says.

For Heintz, fundamental questions about the disease remain. "Is there a specific length of repeats at which the cells become dysfunctional?" he

asks. "If a cell has repeats but does not die, is it dysfunctional enough to cause symptoms? At what CAG repeat length do the cells die, and does it differ depending on the cell type? We need to understand these things in order to develop new treatments for this devastating disease."

More information: Kert Mätlik et al, Cell-type-specific CAG repeat expansions and toxicity of mutant Huntingtin in human striatum and cerebellum, *Nature Genetics* (2024). DOI: 10.1038/s41588-024-01653-6

Christina Pressl et al, Selective vulnerability of layer 5a corticostriatal neurons in Huntington's disease, *Neuron* (2024). DOI: 10.1016/j.neuron.2023.12.009

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