Understanding expansions at the single cell level

Scientists have looked at CAG expansions in brains from people with HD to see which cells are affected

By Dr Rachel Harding | March 12, 2024 | Edited by Dr Sarah Hernandez and Dr Leora Fox

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In two recent studies, researchers looked at how different parts of the brain are affected by CAG expansions in Huntington's disease (HD) at the level of individual brain cells. The scientists looked at post-mortem brains from people with and without HD to track molecular changes in different brain regions called the cortex and striatum. These studies have provided new insights into what contributes to HD. Let's get into it!

Specific brain areas are prone to damage in HD

For a long time now, we have known that some areas of the brain are affected more than others in people with HD. Specific types of brain cells in these vulnerable parts of the brain tend to die off more quickly than others, in a process known as degeneration.

Post-mortem brain samples are extremely precious materials which help research to illuminate exactly what is going on in people with HD

However, the underlying reasons for why some cells are affected more than others are not very clear. Lots of researchers from around the world have been trying to figure this out, as it might shine a light on exactly how HD progresses and give us clues as to how we might treat it.

In two recent papers coming from the same laboratory at Rockefeller University in New York, scientists have looked very closely at molecular changes which happen in different types of brain cells in HD. Using generously donated post-mortem brain samples from people with or without HD, the team carefully separated the brain tissue into individual cells.

The two studies focused on different parts of the brain; the first concentrating on a region called the cortex, and the second looking into cells which make up the striatum and cerebellum. Each brain region is made up of lots of different types of cells so they used special markers to sort all the cells and work out which cells were which type. They were then able to measure all sorts of molecular changes from the different types of cells using cutting-edge genetics technologies.

Linking somatic expansion to when symptom start

> **“Thanks to tremendous advances in DNA sequencing technology, we can now look at how long the CAG number is in each individual cell ”**

One of the changes the scientists looked at in each cell was the CAG number in the huntingtin gene. HD is defined at the genetics level as people who have more than 36 repeating C-A-G DNA letters in their huntingtin gene, with most folks with HD having 40-50 CAGs compared to people without HD who have somewhere around 18 CAGs.

For some time now we have known that in certain types of cells this CAG number is not stable and will change over the course of someone’s lifetime, often getting much longer. This process of CAG increases in some cells is known as somatic expansion. It’s important to note that blood cells happen to be a cell type with stable CAG repeat numbers compared to other cell types. So if you received a genetic test when you were 18, that number will almost surely be the same if you were to get tested again at age 50.

Somatic expansion became a hot topic in HD research when studies of genetic modifiers, traits which change the age of symptom onset, pointed to the exact genes which we think control somatic expansion. Together, this suggests there is a link between how big a CAG number gets during the lifetime of someone with HD and how early they will experience symptoms of the disease.

Thanks to tremendous advances in DNA sequencing technology, we can now look at how long the CAG number is in each individual cell. In fact, this is exactly what the team from Rockefeller did. So what did they find?

Lots of scientists are studying somatic expansion, comparing their data and findings to try and work out how this process is involved in HD

Image credit: Joseph Mucira

Cool conclusions from the cortex

In the first study published at the start of this year, the scientists zoomed into a part of the brain called the cortex - the outer part of the brain with all the wrinkles. Studies which have done detailed brain scans of people with HD have shown a thinning of this part of the brain. They've also found that connections between brain cells are lost in this part of the brain over the course of the disease and that the cells tend to die early on. Changes in the cortex cause cognitive decline and psychiatric symptoms that many people with HD experience.

The scientists found that a specific type of brain cell, called Layer 5a corticostriatal projection neurons (phew, what a mouthful!), is lost in people with HD. These cells die early during the disease, in both humans and monkeys. While these cells are found in the wrinkly cortex, they connect all the way to the centre of the brain, to the striatum, the region that's most vulnerable in HD.

Interestingly, the team found that increases in the CAG number happen in many different types of nerve cells in the cortex, including those that remain relatively healthy. CAG increases were seen in the vulnerable Layer 5a brain cells but also in other cells called Betz cells, which aren't so badly affected by HD. This pointed the researchers to the conclusion that having an increase in CAG number is not enough on its own to cause cells to get sick.

> **"When the team looked at the CAG number in individual cell types, they found that the MSNs had the biggest increase in their CAG number "**

Study surprises from the striatum

In the second study, the researchers focused on the striatum, a brain region in the very center of our heads and the part of the brain most affected by HD. A type of brain cell, called medium spiny neurons or MSNs, are found in this part of the brain and are known by researchers to be the most vulnerable to death in folks with HD.

When the team looked at the CAG number in individual cell types from this part of the brain, they found that the MSNs had the biggest increase in their CAG number. However, other cells in the striatum which are not so affected in HD, such as a type of nerve cell called ChAT+, also had big changes in their CAG number.

The researchers looked at cells from the brain of someone with a similar brain disease to HD called SCA3 (spinocerebellar ataxia type 3), which is caused by an increase in CAGs in a gene called Ataxin3. Folks with SCA3 suffer loss of brain cells but this is not specific to the MSN cell type like it is in HD.

These studies were made possible by the selfless and generous donations of HD family members - thank you!

In this disease, they also found that the CAG number increased in the MSN cells, but not other types of brain cells, even though the MSN cells in these folks' brains weren't so affected. This means that MSNs may just be particularly prone to expanding CAG repeats, regardless of what gene has the long CAG repeats. However the ever expanding CAG repeat may not be the direct reason that those cells die.

So what does this all mean?

What both of these studies point to is the idea that increasing CAG number in HD could be just one of the necessary steps towards cells getting sick. On its own, somatic expansion might be insufficient to cause that cell to die, as the researchers report CAG expansions in cells not vulnerable to death in HD, like the Betz cells.

Both studies also looked at other features of these cells. They did a deep dive into the genes that are turned on or off in every cell in brains of people with and without HD. What they found is that HD causes global changes in the types of genes that are on or off. This has also been shown by many other researcher groups before. Researchers think that these changes may cause toxicity, affecting the health of the cells, eventually contributing to their death.

> **"What both of these studies point to is the idea that increasing CAG number in**

> **HD is only one-step towards cells getting sick ”**

The researchers think that connection issues could also be contributing to cell death. In the cortex, they found alterations in vulnerable cells that change the way they can connect and communicate with cells in other areas of the brain. This disconnection not only reduces the ability of one brain area to communicate with another, but it also weakens the cells themselves over time.

Other research groups are still testing the hypothesis that somatic expansion is the major driver of HD. Different scientists are using different technologies to measure the CAG numbers and early previews of these datasets at the recent therapeutics meeting suggest that this can lead to different results. We expect to see a lot more work in this space in the future.

Science only made possible by patient families

It is really important to note that nearly all of the findings of these two very important studies were made possible by the researchers having access to extremely precious post-mortem brain samples. In both studies, the scientists compared brain material from people either with or without HD, who had passed, who generously donated their brains to science to further research. This is an amazing selfless act that has tremendous impacts for research to better understand HD, with the ultimate aim of one day finding a drug to slow, stop, or reverse the disease.

Whilst brain donation is not something that everyone might be comfortable or able to do, if this is something you are interested in doing, the HDSA, HSC, the Brain Donor Project, and other patient organisations, have information and resources about what this decision involves and next steps.

Sarah Hernandez is an employee of the Hereditary Disease Foundation, which has provided or is providing funding to researchers who contribute to work mentioned in this article. Rachel Harding and Leora Fox have no conflicts to declare. For more information about our disclosure policy see our FAQ...

GLOSSARY

spinocerebellar ataxia A family of diseases which result in characteristic movement disorders. Many types of spinocerebellar ataxia are caused by the same type of mutation as HD – a CAG expansion.

therapeutics treatments

CAG repeat The stretch of DNA at the beginning of the HD gene, which contains the sequence CAG repeated many times, and is abnormally long in people who will

develop HD

neuron Brain cells that store and transmit information

somatic relating to the body
