Interruptions are encouraged

A CRISPR-based approach called “base editing” is being explored to develop a new potential treatment for Huntington's disease. Editing a single letter in the genetic code with base editing may be the key for delaying HD symptoms, maybe by a decade.

By Dr Chris Kay | October 14, 2024 | Edited by Dr Sarah Hernandez

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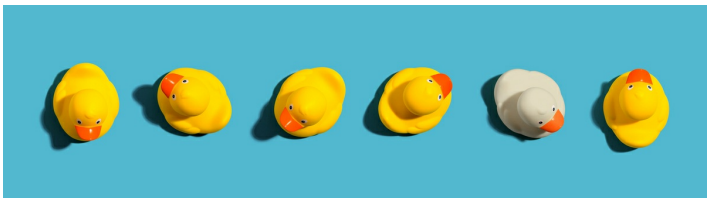
Scientists searching for new ways to stop Huntington’s disease (HD) have focused in on the repeating C-A-G letters of genetic code that cause the disease. That’s because the exact way these C-A-G letters repeat may have a big impact on when and whether someone develops HD. A group in Boston led by Dr. Jong-Min Lee recently applied a cutting-edge technology to try to change the letters of the C-A-G repeat in cells grown in a dish and mice that model HD. Were they successful? And what could this mean for future therapeutic approaches?

Three repeating letters – and an interruption to the repeat

The genetic code of every living organism is made up of 4 letters – C, A, G, and T. They’re combined in different ways to make every gene in our body, like letters on each page of a book. That’s a lot of diversity for just 4 letters! HD is caused by a stretch of repeating C-A-G letters in the huntingtin gene – like three letters repeated on one specific page of the book. People who develop HD are born with 36 or more CAG repeats, one after the other at least 36 times, like this on the page: ...CAG CAG CAG CAG CAG...



HD is caused by a long stretch in the genetic code repeating the letters C-A-G. But those CAGs are frequently interrupted by C-A-A near the end of the repeating sequence. These 3 letter “words” code for the same protein building block, so the protein made from the genetic code is the same. However, that change can make a big difference for when symptoms appear.

In most people, however, these repeating CAG letters actually have a slight imperfection near the end, which looks like this: ...CAG CAG CAG **CAA** CAG. Notice those three CAA letters? Scientists call this the “CAG repeat interruption”, because it “interrupts” the

repeating CAG letters. The CAG repeat interruption is found in the DNA of almost everyone, including people who have the gene that causes HD.

Two words, one meaning

Letters in the genetic code are grouped by threes to create “words” that code for building blocks to create proteins. C-A-G codes for a protein building block called “glutamine”. This is why you may have heard of the CAG repeats referred to as a “polyglutamine” stretch – there’s lots of glutamines in a row.

But C-A-G isn’t the only word that codes forglutamine. C-A-A does as well! This means when the CAG repeat is interrupted by CAA, it doesn’t change the protein word that’s spelled. It still codes for glutamine.

> **“What is especially interesting about people without the CAG repeat interruption is that they develop HD much earlier than expected – about 12 years earlier. And people with an *extra* interruption appear to develop HD later – perhaps 5 year later. ”**

It’s noteworthy that if you get a genetic test for HD and you’re told that you have a certain number of repeats, like 42 for example, that number is the pure CAG repeats. They’re not including any CAG repeat interruptions you may have in your genetic code. There may be more glutamines present, but the CAG repeat stretch, uninterrupted, is 42.

More interruptions please

Not long ago, HD researchers discovered that some people with HD have noCAG repeat interruption – and some even have an extra interruption in the CAG repeat!

What is especially interesting about people without theCAG repeat interruption is that they develop HD much earlier than expected – about 12 years earlier. And people with an *extra* interruption appear to develop HD later – perhaps 5 year later. So there may be something special about those CAA letters that interrupt the repeated CAG letters. Losing the interruption in the middle of CAG letters might make HD symptoms appear earlier – and an extra interruption in the middle of CAG letters might make HD symptoms appear later.

"Base editing" is a new cutting-edge CRISPR-based technology that allows researchers to edit a single letter in the genetic code with the precision of a homing missile. Using this technique, HD researchers are working to change CAG to CAA with the hopes of delaying symptom onset.

Image credit: Rags Fehrenbach

Could adding extra CAG repeat interruptions into the DNA of people with HD help delay or slow symptoms? Changing the DNA of a person is no easy task, but a group of scientists led by Dr. Lee decided to try a cutting-edge approach to introduce more CAG repeat interruptions into cells grown in a dish and mice, as a proof-of-concept to seeing if it would be possible in people.

Changing bases

Dr. Lee's group teamed up with Dr. Ben Kleinstiver, an expert in "base editing", to try out their idea of adding more CAG repeat interruptions. What's base editing? It's basically a new technology that allows you to change a specific letter on a specific page of the book. It's targeted to a specific letter like a homing missile. The technology is based on CRISPR discoveries that have been used to create medicines that recently received regulatory agency approval. Base editing is basically based on CRISPR. Ok, I'll stop with the bad puns.

Base editing is hot-off-the-press technology, so scientists are still working out the kinks. They don't really know which ingredients of base editing work best to change specific letters in DNA sequences. So Dr. Lee's group tried a bunch of different combinations of

ingredients to see what happened in cells in a dish that have a CAG repeat resembling the repeat in people who have the gene for HD.

> **“In a few combinations of base editing ingredients, up to 50% of cells in a dish had CAA interruptions added to the CAG repeat sequence. That’s pretty amazing! ”**

In a few combinations of base editing ingredients, up to 50% of cells in a dish had CAA interruptions added to the CAG repeat sequence. That’s pretty amazing! Scientists can’t yet control exactly where these interruptions are added in the CAG repeat, but a few of the letter changes even looked like the extra interruption we see in people with delayed onset of HD symptoms.

Not quite ready for prime time

Getting all the ingredients to make base editing work into people is a big task, similar to the hurdles facing CRISPR therapeutics for HD. This is really hard if you’re trying to get all those ingredients into the brain, where people who have the gene for HD need them.

But scientists are a tenacious bunch of people and Dr. Lee’s group was not about to let the challenge stop them from trying. His group tried the best combinations of base editing ingredients in mice that have the CAG repeat from people that have the gene for HD, with some early indicators that the approach may be working to add interruptions.

We will no doubt hear more about this work in the future, and learn more about whether adding interruptions to the CAG repeat would be a new promising approach to slow HD. Stay tuned!

The authors have no conflicts of interest to declare.For more information about our disclosure policy see our FAQ...

GLOSSARY

PolyQ A description of HD and other diseases that are caused by abnormal expansion of stretches of DNA containing the sequence CAG repeated many times. Too many CAGs in a gene results in proteins with too many ‘glutamine’ building blocks, and glutamine is represented by the symbol Q.

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

glutamine the amino acid building block that is repeated too many times at the beginning of the mutant huntingtin protein

CRISPR A system for editing DNA in precise ways
