BAC to basics: a more accurate mouse model for Huntington's disease

A genetically-tweaked Huntington's disease mouse model shows a tendency for the CAG repeat to grow, just like we see in humans with the mutation.

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A Californian research group has made a new mouse model of Huntington's disease that is much more like human HD than ever before. How could it help us work out exactly how the mutation causes HD in people?

CAGs and repeat instability

Huntington's disease (HD) is caused when three DNA 'letters' – C, A and G – are repeated over and over too many times in the huntingtin gene. And, on average, the more CAGs a person has in that gene, the earlier they will tend to develop symptoms of HD.

Mice and people are very different, so it's important to try to make HD animal models that are closer to how the HD gene causes the disease in people

What you may not know is that in people with HD, the CAG repeat is unstable and tends to get bigger throughout their life, especially in the cells of the brain. We have known about this for a while, but in recent years big genetic studies have really focused the spotlight of research on what makes repeats unstable.

That's because they found that people with a bigger tendency for the CAG repeat to grow were, on average, the people whose HD progressed more rapidly, even allowing for the number of CAG repeats their cells started off with.

Even more importantly, these genetic studies showed that almost all the non-huntingtin genes that affect the progression of HD are likely involved in causing or preventing repeat expansion.

However, while we know repeat expansion is important, and that ultimately it causes the neuronal damage we see in Huntington's disease, we still aren't sure about all the steps in between. There is lots of research going on right now working with cell and animal models of Huntington's disease to try and answer that question. This work is vital, because in order to design new treatments we need to understand each step in the disease, from repeat expansion to neuronal damage.

BAC to basics

This is where William Yang and his team at University of California Los Angeles come in, with a new HD mouse model called '**BAC-CAG**'. Its name comes from how it was made; BAC stands for 'bacterial artificial chromosome', which describes how they managed to put the whole human Huntingtin gene into the mouse's genome.

Usually, scientists want their disease model to be as consistent as possible over time, so previous HD mice, made before we appreciated just how important repeat expansion is, had DNA sequences that were engineered to be stable throughout the mouse's life. That was done by interrupting the CAG stretch in DNA with 'CAA' sequences. These CAAs don't alter the protein that gets made by the gene, but they do stop the repeat from getting bigger. However, Yang's new BAC-CAG mouse has an **uninterrupted** CAG repeat – making it closer to the DNA sequence we see in humans with HD.

What do the new mice look like?

> **"Importantly, just like in humans, the BAC-CAG mice showed CAG expansion that was most pronounced in the striatum. "**

The first step with a new mouse model of HD is to check whether it develops any HD-like symptoms. Indeed, the BAC-CAG mice developed problems with their movement and had disrupted sleep. In humans, the bit of the brain affected earliest and most prominently is a set of brain cells called 'medium spiny neurons', in a deep area called the striatum. Importantly, the BAC-CAG mice showed early loss of these very neurons, as well as some inflammation in this area. And just like in human HD, the mutant huntingtin protein built up into clumps called aggregates inside the neurons of the BAC-CAG mouse striatum.

As we discussed above, the CAG repeat grows throughout life, but the rate varies in different tissues throughout the body. In parts unaffected by HD it's relatively stable – which is why the CAG count on a blood test generally doesn't change – but in the striatum it can show huge expansion. Importantly, just like in humans, the BAC-CAG mice showed CAG expansion that was most pronounced in the striatum.

We know that in the striatum of people with HD, the carefully controlled switching on and off of lots of genes is disrupted. Previous similar mouse models only showed a fraction of this disruption, but in BAC-CAG Yang's team saw a much bigger disruption, and found it closely resembled what we see in humans.

Using the new mice to study CAG instability

Having shown that BAC-CAG mice closely mimic what we see in people with HD, Yang and colleagues could then go on to look for the link between repeat CAG instability and disease.

You will recall that previous mouse models have CAA interruptions to keep the repeat length stable. Both CAG and CAA tell the cell to insert a glutamine building block when it's making the huntingtin protein, so regardless of whether the repeat is made up just of CAGs, or if it has some interrupting CAAs, the protein will contain a long line of glutamines.

When Yang and colleagues compared lots of different mouse models they found that the amount of disruption to genetic switching was affected by the number of pure, uninterrupted CAGs in the huntingtin gene. However, this disruption did not relate to the number of glutamines in the protein. That means it is the number of uninterrupted CAGs that sets the course of HD. This echoes what we see in humans - but still doesn't tell us how repeat expansion causes disease.

In BAC-CAG mice, Yang's team found that the more repeat expansion there was in the brain, the more disrupted their movement and sleep were. While we've suspected this from genetic studies in people, this is the first time repeat expansion has been directly linked to symptoms in HD mice; it really strengthens the argument that expansion has a key role in causing the disease.

The tendency of CAG repeats to expand was previously one aspect of HD that was missing from mouse models – until now

What does this mean for humans?

So how could CAG repeat expansion be causing HD? We've long thought it was by making a protein that was toxic, for example by aggregating in cells and causing them to die. But there are several other ways the CAG repeat in DNA could be toxic to the striatum.

For example, there are a few steps between the gene and the protein it makes; first the instructions in the DNA are copied into a related molecule called RNA, and then that gets 'translated' into protein. Yang and colleagues found abnormal RNA molecules were being made from the huntingtin gene, and other groups have previously shown these RNA molecules can be toxic to cells. They also found that RNA from the huntingtin gene was building up in cells - the sort of thing that has been shown to cause other degenerative diseases like myotonic dystrophy.

Reading DNA is usually a very organised process that starts at the start of a gene and works its way along in the correct direction. However, when there is a long repetitive stretch of DNA, like in HD, sometimes the machinery gets lost, and the process kicks off within the repeat itself, and can go in either direction. This backwards reading can make lots of different kinds of strange little proteins, some of which can be toxic. Yang and colleagues showed that these little proteins were being produced in the brains of the BAC-CAG mice, but that it only started late in the disease course, long after they had started developing symptoms. They concluded this process could contribute to the late stages of HD, but is unlikely to a key early step.

What's next?

So where does this leave us? Have we joined the dots between CAG repeat expansion and neuronal damage? Not quite yet, but the BAC-CAG mouse has helped us work out which processes might be involved, and which are less likely to be responsible. This mouse has helped cement CAG expansion as a key early event in HD. Repeat expansion in turn leads to neuronal damage, and the processes along the way likely involve a combination of disrupting genetic switching, the building-up of proteins within neurons, inflammation and toxic RNA.

There is still much more work to do to figure out which of these processes are most important for causing the neuronal damage in HD. And good news - **the drugs are coming**! New treatments targeting repeat expansion, are expected to enter clinical trials this year, and Yang's work with the new mouse gives us reason to hope that they will be effective at stopping these harmful processes before neuronal damage happens.

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

GLOSSARY

huntingtin protein The protein produced by the HD gene.

inflammation Activation of the immune system, thought to be involved in the HD disease process

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

aggregate Lumps of protein that form inside cells in Huntington's disease and some other degenerative diseases

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

neuron Brain cells that store and transmit information

genome the name given to all the genes that contain the complete instructions for making a person or other organism

RNA the chemical, similar to DNA, that makes up the 'message' molecules that cells use as working copies of genes, when manufacturing proteins.

BAC an abbreviation for 'bacterial artificial chromosome'
