HDBuzz

Huntington's disease research news. In plain language. Written by scientists. For the global HD community.

Cut-and-paste DNA: fixing mutations with 'genome editing'

Scientists make precise changes to the DNA of a live animal. Could it work for human genetic diseases?

By Dr Jeff Carroll on July 18, 2011
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What if we could edit the DNA of patients to remove the Huntington's disease mutation altogether? Sounds like science fiction, but new research in an animal model of hemophilia suggests that it can work - and now HD researchers are on the case.

DNA, RNA and protein

Every case of Huntington's disease is caused by a DNA mutation. The DNA code is written in four 'letters' that scientists refer to as bases. The four bases are adenine, cytosine, guanine and thymine - abbreviated as A, C, G and T.

Normally, near one end of the huntingtin gene, there's a stretch of 17 or so repeated C-A-G bases. In people with HD, the normal C-A-G stretch is longer, due to a kind of genetic stutter. That's the 'triplet repeat expansion' that causes HD.

Genome editing uses special molecular scissors to cut DNA in cells at exact places. Then new DNA is spliced in at the cut site.

Genes are the blueprint for everything a cell does. They're spelled out in DNA. When a gene is switched on, first the cell makes a 'working copy' of the gene, by copying the DNA into a message molecule made from a chemical cousin, RNA.

These RNA message molecules are used to direct the construction of proteins from amino acid building blocks. In Huntington's disease, the damage is done by the mutant huntingtin protein - not the huntingtin gene (made from DNA), or the RNA message molecule.

Gene silencing - shooting the messenger

There is a lot of excitement about **gene silencing**. In this approach to therapy, specially designed molecules find the HD gene's RNA message and tell the cell to get rid of it. The gene itself still exists in the DNA in every cell of the person, but because the message is destroyed, less mutant huntingtin protein is made.

Several different strategies are being tried to silence huntingin RNA, including antisense oligonucleotides and RNA interference. You can read more in our Gene Silencing Primer.

The next frontier - genome editing?

Gene silencing is definitely one of the most important therapeutic approaches to Huntington's disease. But what if we could actually go one further and remove the mutation that causes HD from the DNA of patients?

The idea seemed completely impossible until recently. Cells have mechanisms that repair DNA if it's altered, and every cell in the body has the same DNA. So the idea is much more radical than gene silencing.

Recently, though, a technology called **genome editing** has been developed. This approach uses a custom-made molecular machine called a **zinc-finger nuclease** to actually edit the DNA of a cell.

Zinc-finger nucleases are molecules with two special components.

The zinc finger bit can recognize and stick to specific sequences of DNA. That allows the machine to find a very specific point in the DNA code. And that exact point can be specified by the scientist designing the molecule.

Once the zinc fingers have brought the machine to a specific place in the DNA, the second part of the machine - the 'nuclease' - is brought into action. This little machine makes precise cuts in both strands of the DNA.

> "The mice were cured of hemophilia by editing the genome of their liver cells to correct the defective gene."

Cells are evolved to hate breaks in DNA, because breaks can lead to harmful mutations. So whenever a break occurs, the cell's repair machinery kicks in to try to fix it.

Here's the really clever part. If a little bit of custom-made DNA is supplied along with the zinc finger nuclease, the cell's repair machinery can be 'hijacked' to replace the normal DNA at the site of the cut.

Genome editing allows scientists to consider something that's never been possible - actually altering the DNA of a cell, to end up with any sequence we can design. In essence, the zinc finger nucleases make a cut in the DNA, and a different DNA sequence can then be pasted in.

Correcting hemophilia with genome editing

Genome editing sounds all very well in a test tube, but could we actually use it to treat diseases?

Recent work from the group of Prof Katherine High at the University of Pennsylvania suggests that it *is* possible. She studies a disease called **Hemophilia**, which reduces the ability of the blood to clot. That's bad news because it can lead to dangerous, uncontrolled bleeding.

Hemophilia B in people is caused by mutations in the F9 gene. F9 is a critical component of the blood clotting mechanism. Mutations in the F9 gene are scattered across the gene - different people have mutations at different spots. That's very different from Huntington's disease, where every patient has a mutation in the same place.

High's team had a very clever idea for replacing defective F9 genes using genome editing. Working with a company called Sangamo BioSciences, they designed a zinc-finger nuclease to put a cut very early in the F9 gene. They then added a DNA template that included a normal copy of the F9 gene. When the template and the zinc-finger nuclease were put into cells, some of the cells ended up with normal F9 genes in their DNA. In effect, the scientists had precisely spliced a new F9 gene where the old mutant copy was.

But could this ever work in the complex setting of a live animal? Most clotting proteins are made in the liver, so for Hemophilia B patients the important thing to do is to repair the F9 gene in the liver.

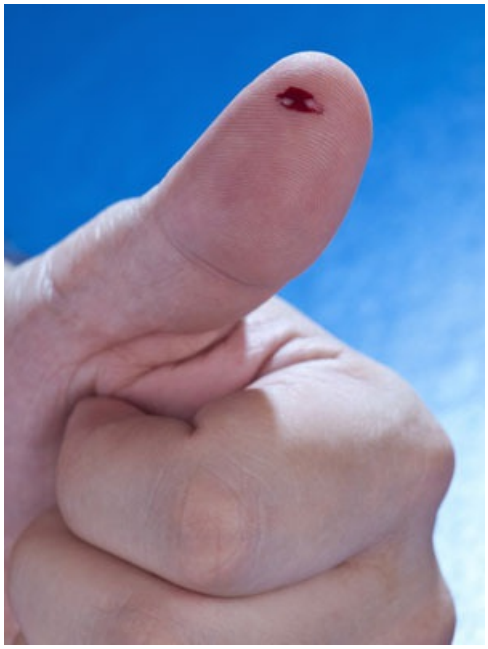
High's team used genetically altered mice with a mutated human F9 gene in the liver. They then injected these mice with a virus carrying a cocktail of zinc-finger nuclease and a DNA template including a new, healthy copy of the F9 gene.

Amazingly, after being injected with these viruses, the protein corresponding to the healthy F9 gene was found in the blood of the mice.

That means the viruses worked: they inserted a new copy of the F9 gene to the liver of mice and the cells actually started using it.

Of course, the most important test for a therapy is whether it corrects symptoms of the disease it's meant to treat. Mice and humans with hemophilia B have very slow clotting times - which can lead to serious bleeding problems.

But in the mice treated with viruses carrying the zinc-finger nucleases and the healthy F9 gene, the problems in clotting time were almost completely corrected. Essentially, the mice were cured of hemophilia by editing the genome of their liver cells to correct the defective gene.



People with hemophilia have blood that doesn't clot properly. Genome editing restored normal blood clotting in mice with the hemophilia mutation.

Could this help with HD?

It hasn't escaped anyone's attention that genome editing technology could be very powerful for Huntington's disease.

Because HD is always caused by an expansion of the same C-A-G tract in the same genetic location, it's possible to imagine using genome editing to remove some of those "extra" CAGs. In effect, this would cut the mutation right out of a cell's DNA.

There are a couple of problems that will need to be overcome before it becomes a reality. In the hemophilia mice, a healthy gene was added without removing the defective gene. That wouldn't work in HD because it's a toxic protein, not a missing protein, that causes the problems. So the technique will need to be modified to snip out the harmful CAGs, or deactivate the mutant gene, instead.

In addition, it will be more difficult to get the zinc finger nuclease treatment into brain cells than into liver cells.

Excitingly, though, genome editing research has already begun in Huntington's disease. CHDI, the leading organization funding HD research world-wide, has set up an HD genome editing program.

In a blog post, CHDI vice-president Ignacio Munoz-Sanjuan said, "after more than 2 years of trying, Sangamo and CHDI are now partners. Let's never give up having 'science fiction' dreams - one never knows how far science and technology will take humanity."

It will take several years before genome editing can be retooled to work in the brain of Huntington's disease patients - but this positive result represents a new avenue of research with great potential.

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

Glossary

ASOs A type of gene silencing treatment in which specially designed DNA molecules are used to switch off a gene

Zinc-finger Nucleases Molecular machines that attach to a specific DNA sequence and then cut the DNA strand

huntingtin protein The protein produced by the HD gene.

RNA interference A type of gene silencing treatment in which specially designed RNA molecules are used to switch off a gene

gene silencing An approach to treating HD that uses targeted molecules to tell cells not to produce the harmful huntingtin protein

Genome Editing The use of zinc-finger nucleases to make changes in DNA. 'Genome' is a word for all the DNA we each have.

amino acid the building blocks that proteins are made from

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.