

Gene Editing Techniques Show Promise in Silencing or Inhibiting the Mutant Huntington's Disease Gene

BY JAMIE TALAN

ARTICLE IN BRIEF

Three different gene editing techniques have successfully inhibited or silenced the mutant huntingtin gene implicated in Huntington's disease.

CHICAGO—Gene silencing and editing techniques aimed at modifying the mutant huntingtin gene (HTT) to reduce levels of the protein promise to change the landscape for patients with Huntington's disease (HD), according to several reports presented at the Society for Neuroscience annual meeting here in October.

ANTISENSE OLIGONUCLEOTIDES

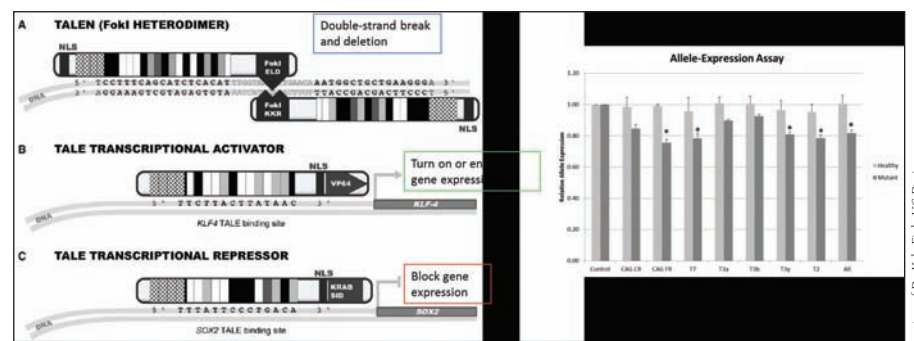
Scientists at the University of British Columbia's Centre for Molecular Medicine and Therapeutics (CMMT) identified eight of the most common genetic variations on the HTT gene associated with HD and tested specialized antisense oligonucleotides (ASOs) in blood cells from HD patients to assess whether the drugs can silence, or turn off, the mutated gene and reduce levels of the toxic huntingtin protein. The drugs work by binding to the messenger RNA and inducing degradation of the

transcript, preventing synthesis of the mutant protein.

Michael Hayden, MD, PhD, and his colleagues at the CMMT performed genetic analyses of thousands of HD patients and healthy controls to identify variations on the HTT gene that are common among HD patients. With these specific variations in hand, they developed ASOs that target each one of these genetic sequences and delivered a combination of ASO molecules to HD animals to see whether they work to reduce the toxic huntingtin protein. The normal huntingtin protein, critical for brain cells throughout life, is not targeted in this approach.

"We identified eight single nucleotide polymorphisms (SNPs) that are enriched on the mutant allele," said Nicholas Caron, PhD, a postdoctoral fellow in the Hayden laboratory who presented the latest data on this work. "We wanted maximum coverage for HD patients. The panel of ASOs we are now identifying and testing could ultimately help treat up to 85 percent of the HD population. We hope we can identify ASOs for each haplotype so we can tailor the medicine for an individual patient.

"We design ASOs to bind to that specific sequence on the allele and only silence the mutant copy," Dr. Caron explained. "The difference in our approach is that



THE MECHANISM BEHIND TALEs: Panels A, B, and C describe the different effector domains that can be attached to DNA-targeting proteins. Panel A represents obligate heterodimeric variants of the nuclease FokI (TALEN); the rationale for using a heterodimeric nuclease was to reduce potential off-target nuclease activity by necessitating complementary binding of the two TALE arrays to elicit a double-stranded break. The breaks were expected to be repaired by the highly efficient single-strand annealing pathway, resulting in the deletion of CAG repeats. The B panel represents a transcriptional activator that can turn on or enhance gene expression, though this was not used for Huntington's. Panel C represents a transcriptional repressor that would silence or block gene expression. To achieve the allele-specific transcriptional repression of mutant huntingtin, the researchers designed TALEs to target unique DNA sequences in the mutant allele.

other ASOs target the wild-type and mutant alleles. But the wild-type protein is important for neuronal health."

Dr. Hayden's team has evaluated ASOs that target one of the HD SNPs they identified, and they are now using a potent and well tolerated ASO in animals to see whether it is effective at reducing the mutant huntingtin protein. They are developing a panel of ASOs targeting HD SNPs specific to the three most common HD HTT haplotypes.

Lowering mutant huntingtin in animal models of HD has proven effective at reducing the motor and behavioral symptoms, as well as the neuropathology observed in the brain. RNA interference methods are also being developed by other groups to reduce the huntingtin protein.

A CLINICAL TRIAL OF AN ASO

The first clinical trial using a non-selective HTT silencing approach (targeting the wild-type and mutant genes) is now underway. The trial, which is sponsored by Isis Pharmaceuticals and Roche, will enroll a few dozen early-stage HD patients at six sites across Canada and Europe. It is a dose-escalating safety

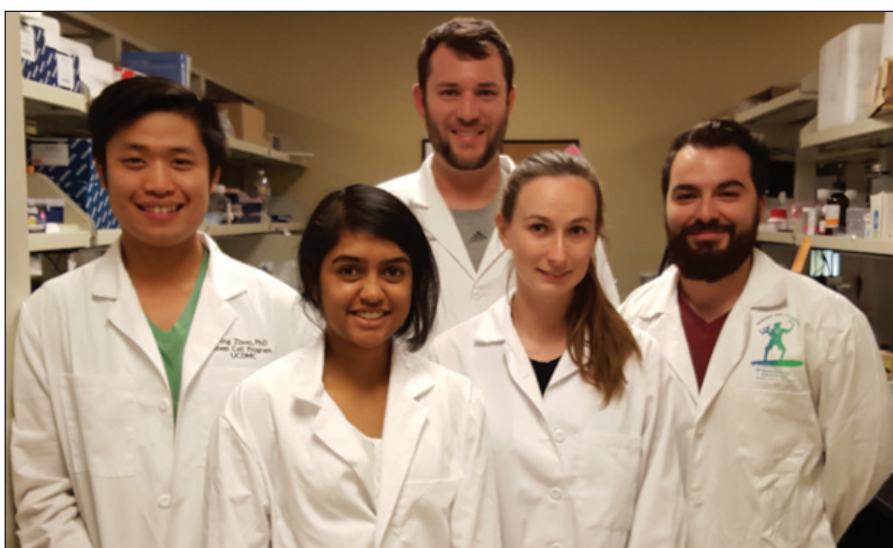
trial. The drug, ISIS-HTTRx, is injected into the spinal fluid. The researchers will measure the level of the mutant protein in the CSF. Sarah Tabrizi, MD, PhD, director of the Huntington's Disease Centre at University College London's Institute of Neurology, is the chief clinical investigator of the trial.

"Huntington's is ideally suited to this innovative therapeutic technology because it comes with genetic certainty: everyone with the mutant gene will get the disease at some point. We designed ISIS-HTTRx to target the huntingtin gene and reduce the production of huntingtin protein," said C. Frank Bennett, PhD, senior vice president of research at Isis Pharmaceuticals.

WILL DNA-BINDING MOLECULES WORK?

Scientists at the Institute for Regenerative Cures and the Genome Center at the University of California, Davis, are using transcription-like effectors (TALEs) in primary human HD fibroblasts and neurons to see if they can reduce mutant huntingtin. TALEs are DNA-binding molecules that can be designed to target

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THE UNIVERSITY OF CALIFORNIA, DAVIS RESEARCH TEAM is shown here: Dr. Kyle Fink (center) with (left to right) Peter Deng, Anvita Komarla, Dr. Audrey Torrest, and Joseph Aprile.

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single nucleotide polymorphisms in the mutant allele. The TALE molecules either cause a CAG collapse in the expanded mutant allele or provide transcriptional repression of the mutant gene.

Kyle Fink, PhD, a postdoctoral fellow at the university, explained that his group tested the TALEs in human HD fibroblasts and induced neurons made from these skin cells. They have done previous work to show overproduction of reactive oxygen species (ROS) and altered mitochondrial function that results in oxidative damage, neuronal dysfunction, and cell death in the HD cell lines.

In the study reported at the Society for Neuroscience meeting, the researchers treated human HD fibroblasts with each TALE-specific SNP or TALE-FokI, which was developed by fusing TALEs to a snippet of the FokI endonuclease or KRAB domain.

The TALE recognizes sequences in DNA and makes a double-stranded cut, which would collapse the CAG length down to a non-disease stage, or, when using KRAB, cause transcriptional repression the mutant allele.

Dr. Fink reported that there was a significant reduction in the aggregated mutant huntingtin protein. The allele-specific expression was measured using an SNP genotyping assay, and the protein aggregates were quantified with Western blots for anti-ubiquitin and anti-huntingtin antibodies. They also measured cell growth, ROS levels, and ROS-induced DNA damage in the mitochondrial genome as biomarkers of the downstream dysfunction in these cells.

Both TALE strategies targeted specific sites within only the mutant huntingtin gene. TALEs are one of three engineered

nuclease approaches that have been tested for the treatment of human diseases.

"It has been thought that reducing mutant huntingtin through protein interference or conditional gene knockout could prove to be an effective therapy for patients and reduce the associated downstream effects," said Dr. Fink. "We showed that the TALE-SNP and the TALE-FokI that is delivered into the HD

fibroblasts or HD neurons led to a significant reduction in aggregated proteins and mutant allele repression."

The research team conducted real-time quantitative assays to measure how much transcription of the mutated gene occurred. The technique lowered the expression of the mutant gene to near-normal levels. The expression of the healthy gene was not affected by the treatments.

"We are looking for ways to deliver TALEs with a higher efficiency," Dr. Fink added. "The technique we are now using is not feasible for human trials." The scientists are now trying to figure out the best way to deliver TALEs in an *in vivo* delivery system for use in humans. The work has not yet been applied in animal models.

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DR. MICHAEL HAYDEN and colleagues developed antisense oligonucleotides (ASOs) that targeted genetic variations on the huntingtin gene and delivered a combination of ASO molecules to Huntington's disease animals.

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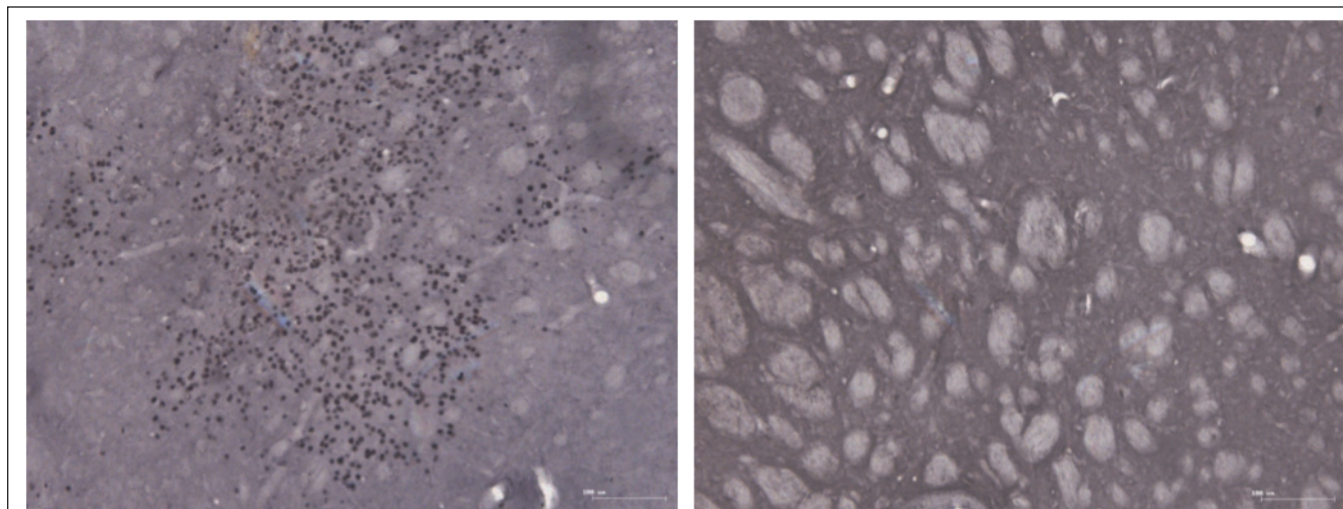
CRISPR: ADVANCES IN THE GENE EDITING TECHNOLOGY

Another gene editing technology has been used successfully to disrupt the mutant huntingtin gene in test tube studies and in animals. Nicolas Merienne, PhD, and his colleagues in the laboratory of cellular and molecular neurotherapies at Lausanne University Hospital in Switzerland, used the gene editing technology, referred to as clustered regularly interspaced short palindromic repeats (CRISPR), to test its power to edit out the mutant gene and alter the reading frame of the HTT gene that would lead to a loss of mutant HTT expression.

In the study, they incorporated genes coding for the CRISPR system into viral vectors and used a fluorescent reporter gene to measure the effect on brain cells. They introduced this system into neurons and astrocytes and showed that it was able to efficiently edit the targeted genes.

The researchers then used a viral vector to deliver it into the brains of adult HD mice, and found that the gene editing strategy successfully led to a strong reduction of mutant HTT aggregation, a key hallmark of HD pathology. The technique led to a 50 percent gene disruption in the test tube experiments.

"We've only done a local proof of principle in the striatum," Dr. Merienne said. "We have not looked for behavioral changes or functional recovery yet." He added that the researchers are now evaluating the impact of allele or non-allele-specific mutant HTT editing in human neurons cultured from HD patients.



CRISPR-Cas9 Editing: In a mouse model, the injection of lentiviral vectors led to the expression of a mutant fragment of the huntingtin gene; the dark spots, which show gene expression (left), are absent after CRISPR-Cas9 editing (right). The CRISPR-Cas9 editing system reduced mutant huntingtin aggregation in the mouse striatum.

"CRISPR is by far the most efficient system," said Nicole Déglon, PhD, head of the laboratory at Lausanne University Hospital. "We are still trying to identify the efficient and safe system to use in humans. One of the major concerns is treating enough cells in the brain to have a therapeutic effect. We have to be absolutely sure that we are not doing anything else off of our target gene."

EXPERTS COMMENT

"The challenge with Huntington's has been trying to figure out how to help the cell once the horse is out of the barn," said Walter J. Koroshetz, MD, FAAN, director of the National Institute for Neurological Disorders and Stroke. "The genetic approaches hold promise to intervene at the most proximal cause

of the neurodegeneration; it turns the problem into an engineering problem of delivery. It is an attractive strategy."

Christopher Ross, MD, PhD, a professor of psychiatry, neurology, pharmacology, and neuroscience at Johns Hopkins

cell models of HD and HD patients. She also studies possible therapeutic treatments, including small antibodies specific for the huntingtin protein and ASOs.

"These approaches make a lot of sense," she said, commenting on the

Dr. Michael Hayden's team is developing a panel of ASOs targeting HD SNPs specific to the three most common Huntington's disease haplotypes.

University School of Medicine and director of the Baltimore Huntington's Disease Center at Hopkins, said that the nature of HD — it is a triplet repeat expansion — is that it is hard to get an intervention to the mutated allele without affecting the wild-type allele.

"One strategy is to target other parts of the HD message whose sequence is specific to the mutant allele," Dr. Ross said. "This strategy is attempting to correct the gene defect, and another difficulty is that you need to get every cell, or at least a significant number of them. Delivery of these therapeutic agents into relevant areas of the brain is very challenging. You use these technologies to edit the mutant gene, and thereby disrupt its ability to transcribe mRNA to make mutant protein.

"These are still very early days, but the gene editing strategy is still tremendously exciting and novel," he added. "A few years ago no one would have imagined gene editing technology, so who knows what will come in the future. The difficulty is to imagine a viral delivery system that would target all the relevant cells in the HD brain."

Willeke van Roon-Mom, PhD, an assistant professor at Leiden University Medical Center in the Netherlands, also studies transcriptional changes in both

studies, noting that these approaches could reduce up to 50 percent of the mutant huntingtin protein. "Scientists can design a drug that only targets the sequence in which they are interested. I think this strategy will work. But you always have patients who don't have the common SNPs, so other strategies are needed too." •

THE MECHANISMS BEHIND SILENCING OR REPRESSING THE MUTANT HUNTINGTON GENE

- **Antisense Oligonucleotides (ASOs):** Antisense oligonucleotides, synthetic drugs that are complementary for a specific segment of DNA, bind to messenger RNA to prevent gene expression. Each ASO is designed for a specific gene target. In the case of Huntington's disease, the challenge is that each ASO has to be delivered to each cell containing the mutant huntingtin gene.
- **Transcription Activator-Like Effectors (TALEs):** TALEs are DNA-binding molecules that can be designed to target single nucleotide polymorphisms in the mutant allele. These "designer" DNA-binding proteins can be adapted with different effector domains to create a double-stranded break (nuclease) or single-stranded break (nickase); they can enhance gene expression or repress transcription. The nucleases used in this strategy activate cellular DNA repair pathways. The TALE molecules either cause a CAG collapse in the expanded mutant allele or provide transcriptional repression of the mutant gene.
- **Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)/Cas9:** The latest generation of gene editing techniques is the CRISPR-Cas9 technique, which uses RNA-guided engineered nucleases to target a specific gene. CRISPR allows multiple genes to be edited at the same time. Researchers can literally apply thousands of guide RNAs for the cas9 system at once and test them in a very high-throughput manner.

—Jamie Talan

LINK UP FOR MORE INFORMATION:

- Society for Neuroscience abstract: Caron NS, Southwell AL, Cay C, et al. Toward developing a personalized allele-specific gene silencing therapy for Huntington disease: <http://bit.ly/NT-SFN-genesilencing>
- Society for Neuroscience abstract: Fink K, Torrest A, Deng A, et al. Allele-specific silencing of the mutant huntingtin gene in primary fibroblast and neural cultures with transcription activator-like effectors: <http://bit.ly/HD-TALES>
- Society for Neuroscience abstract: Merienne N, Meunier C, Zimmer G, et al. Genetic editing with the CRISPR/Cas9 system for Huntington's disease: <http://bit.ly/NT-CRISPR-HE>