Teaching An Old Protein New Tricks: Developing A Novel Gene Therapy For Huntington's Disease

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Huntington’s disease is a neurodegenerative disorder caused by a genetic defect in the Huntington (HTT) gene. Huntington’s belongs to an elite class of diseases which are ‘monogenic’ - directly attributable to one gene- and ‘dominant’, meaning that the presence of even one mutant HTT gene can have catastrophic consequences for those carrying the defective gene. A child born to a parent with Huntington’s disease has a 50% chance of getting the disease, depending on which copy of the Huntington gene is inherited from the affected parent. The disease is most prevalent among Europeans and North Americans, but still relatively rare, affecting approximately 40-100 individuals per million.

The root of Huntington’s disease lies in the DNA of affected individuals. When a gene is expressed, the instructions for making a protein are encoded in RNA- DNA’s close but highly unstable cousin- and sent to a ribosome, the cell’s protein production machinery. The message is written using only four letters: A, C, T and G. These letters are assembled and ‘read’ as various three-letter combinations, with each combination specifying a particular amino acid. As the triplets are read the appropriate amino acids are then strung together to form a protein, which can then go on to carry out any number of functions within the cell.

When CAG appears, glutamine, an amino acid, is added to the growing protein chain. In the Huntington gene, there is a stretch of DNA where CAG is repeated anywhere from 10-29 times in people without Huntington’s disease. Patients with Huntington’s have an increased number of CAG repeats, ranging anywhere from 36-121 repeats. Correspondingly, the defective HTT gene with extra CAG repeats directs the synthesis of a mutant huntingtin protein which has an excess of glutamine added to it. The mutant Huntington protein (mHTT) is cleaved into small fragments in the cell which form tangled clumps known as protein aggregates. In the case of Huntington’s disease, these aggregates accumulate in nerve cells and eventually become toxic to the cell. As neurons die off a number of neurological symptoms begin to appear, including behavioral changes such as depression, irritability, or paranoia; dementia; impaired motor skills and balance; and speech impairment. Huntington’s chorea- derived from the Greek word for dance- refers to the jerky, uncontrollable movements exhibited by many Huntington’s patients.

The number of CAG repeats in a patient often correlates to the onset of symptoms: the more repeats there are in the gene, the earlier the symptoms of Huntington’s disease appear. Sometimes, when a long stretch of repeated DNA sequence is encountered in the process of copying DNA, the DNA replication machinery may lose track of its place in the tedium of copying the same thing ad nauseum and inadvertently add an extra CAG triplet. With each successive generation, therefore, the number of CAG repeats tends to increase slightly.

Most people with Huntington’s disease have inherited a single copy of a defective HTT gene from an affected parent, and that one mutant copy alone is enough to produce Huntington’s disease, even if they have a second ‘normal’ copy of the gene. Theoretically, if one were to interfere with HTT gene activity, the expression of the mutant huntingtin protein could be prevented as a treatment for Huntington’s disease. There would need to be a way to discriminate between the normal gene and the mutant gene, however- the protein encoded by the HTT gene is essential for embryonic development and the function and survival of nerve cells in the brain, and so therapeutic interference with HTT activity needs to be restricted to the mutant copy as much as possible.

In a recent study published in the Proceedings of the National Academy of Sciences, researchers used zinc finger proteins to preferentially restrict the expression of the mutant HTT gene while leaving the normal HTT gene relatively untouched. Zinc finger proteins are named for their finger-like protrusions which can ‘grasp’ DNA, RNA, or protein, and the zinc ions which are commonly found tucked into the protein to stabilize its shape. In this instance, researchers strung together multiple zinc finger proteins (ZFPs) which specifically stick to CAG repeats to form CAG-binding ZFP chains. After experimenting with different ZFP chain lengths, they settled on a chain made up of 11 ZFPs.

They then compared the ZFP chain’s ability to repress varying lengths of CAG repeats, because the primary difference between mutant HTT and normal HTT is the length of the CAG repeat region within the gene. To do this, they added to cells a long stretch of CAG repeats which, when read to build a protein, directed the formation of a small protein made of multiple glutamine molecules and a red or green fluorescent ‘tag’ on the end. A shorter stretch of CAG repeats tagged with the opposite color was simultaneously expressed. To compare the repression of the short vs. the long repeats, they only had to compare the dominant color in the cell: if the shorter stretch was tagged with green and the cell was mostly green with little red, that would indicate that the shorter glutamine repeat fragment was being expressed while the longer (red-tagged) fragment was repressed. If there was no effect, it would be expected that both the short and long fragments would be expressed in equal amounts, causing an even mixture of red and green within the cell. From this experiment researchers found that the longer stretches of glutamine repeats were significantly repressed by the ZFP chains while the shorter stretches were barely affected, and so this was their first ‘proof-of-concept’ before they attempted to prove that the effects of their ZFP chains are physiologically relevant.

Next, researchers expressed the 11-ZFP chain in cells derived from mice which are used as a ‘model’ for Huntington’s disease (in these
particular cells, part of the mouse HTT gene has been replaced with 111 CAG repeats). A similar preferential decrease in mutant Huntington gene expression was observed. To ensure that the ZFP chains were not inadvertently clamping onto other genes with CAG repeats, they tested the expression of other CAG repeat genes and found that they remained unaffected.

Finally, to demonstrate that the ZFP chain had a therapeutic effect on the symptoms of Huntington’s disease, researchers injected the brains of a particular strain of mice (another model of Huntington’s disease, as these mice have 115-160 CAG repeats and have measurable symptoms which correlate with those found in humans) with the DNA sequence coding for their ZFP chains. When the ZFP chains were formed in the brain after injection they repressed the expression of the mutant HTT gene, as observed in earlier experiments described above. By examining the brains of the treated mice researchers found that the number of protein aggregates formed from mutant Htt had decreased, nor was there evidence of dead or dying neurons. Live mice were then tested to see if the ‘Huntington’ symptoms had improved: normally, mutant mice clasp their paws together when lifted by the tail. In mutant mice where the ZFP chain was expressed, however, no such behavior appeared. Similarly, ZFP chain expression in mutant mice improved motor coordination to the extent that the treated mice were nearly indistinguishable from normal, healthy mice.

There are still major obstacles to developing this method into an effective form of gene therapy for Huntington’s patients: first, one can never predict the behavior of a protein in a novel environment, and so it would need to be demonstrated that in the human brain ZFP chains behave more or less the same as they did in the experiments described in the paper. The authors also point out that it is unknown whether ZFP chains would be produced at levels sufficient to achieve significant repression of the mutant HTT gene after injection into the brains of Huntington’s patients.

At present, only the symptoms of Huntington’s are treatable, although the disease is ultimately always fatal. Other studies of potential therapeutic interventions for the treatment of Huntington’s disease have utilized a number of different techniques; however, this study was the first to show that a gene could be repressed by a synthetic regulator of gene activity. Additionally, the authors of this study demonstrated that the ZFP chain they developed acted on the target gene- mutant HTT- but had little effect on the normal HTT gene, a quality that is particularly attractive when attempting to maintain the delicate physiological balance within the cell. While there are still issues which need to be addressed, researchers have laid the foundation for an entirely novel method of therapeutic intervention (“establish[ed] a proof-of-principle”, according to the authors of the paper). A treatment based on the method described in the paper may one day prove invaluable to Huntington’s patients, or it could potentially be modified and retooled to target other diseases of a similar nature.


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