A Novel Therapy for Huntington's Disease

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Editor's Note: In 1979, while at the National Institutes of Health, now Columbia University professor Nancy Wexler and colleagues traveled to Venezuela to study the world's largest family with Huntington's disease. That led to identifying the disease gene at the tip of human chromosome 4 and the race to find a drug that can treat people who carry the fatal gene prior to the onset of symptoms. Our author believes that a new strategy tied to turning off targeted genes could have profound implications for therapy development for Huntington's and other neurodegenerative diseases.

Altering the DNA sequence of a single gene can be enough to cause a fatal illness, and a medical specialty is devoted to the diagnosis and care of patients who have what doctors have labeled "genetic diseases." While most of these conditions are very rare (except in certain small human populations that exist in reproductive isolation[1]), there are thousands of genetic diseases, and at least half of pediatric patients admitted to major children's medical centers at any given time are afflicted with one of them.[2] Single gene mutations can also cause breast, ovarian, and colon cancer in adult patients.

Over the last century, our understanding of genetic disease has greatly advanced through the tireless work of clinicians and researchers, as the concept of one gene giving rise to one particular disorder evolved, and the modes of inheritance for different genetic diseases were defined. In the course of this work, certain such diseases (e.g. sickle cell anemia, cystic fibrosis, Duchenne muscular dystrophy, neurofibromatosis) became well known, almost always because they were the most common and the most tragic. Included in this group is a disorder that has been the focus of intense research efforts to define its cause and now to develop an effective treatment: Huntington's disease (HD).

In this primarily neuropsychiatric disorder, most affected individuals first suffer from an inability to control their movements, and develop signs of disease in their 30s or 40s. Because these uncontrolled movements can appear rhythmic, the disease was also named Huntington's chorea, from the Greek word for dance. The initial phase typically advances for a number of years before HD patients develop cognitive decline, which progresses until they can no longer perform the activities of daily living. At this point, patients are typically admitted to a skilled nursing facility, where they linger in decline for more years before passing away.

As a genetic disease, HD is recognized as a dominantly inherited disorder, meaning that only one of the two genes for the protein huntingtin needs to contain the disease-causing mutation for HD to develop. This also means that *each* child of a person with HD has a 50 percent chance of inheriting the defective gene and developing the disease. Men and women are at equal risk. Because individuals carrying the HD gene may not realize the disease runs in their family, they sometimes do

not learn that they will get HD until after they have had children, unknowingly placing their children at 50-50 risk of getting HD.

Finding the Huntington Gene

The fact that HD strikes individuals in the prime of life, robs them of their ability to control their movements and is ultimately passed on from parent to child (sometimes unknowingly), makes HD one of the most compelling and tragic of genetic diseases. For this reason, considerable attention was focused on identifying the gene that causes HD in hopes of finding a cure. In fact, when the tools of advanced genetic research developed a new type of genetic marker using molecular technology, HD was the very first genetic disorder to be "mapped" to a region of a specific chromosome (chromosome 4) in 1983 by a technique known as "linkage analysis."[3]

Because it was the first genetic disease to be mapped, physicians and researchers assumed that HD would be among the first whose cause would be found. But this turned out not to be the case. Indeed, while the gene defects responsible for *all* the other well-known genetic diseases were identified by using genetic mapping combined with the latest molecular cloning techniques, the HD gene remained elusive.

It would take the 1991 discovery of an entirely new type of genetic disease mutation in a very rare and unrelated neuromuscular disorder (X-linked spinal & bulbar muscular atrophy) to pave the way for the HD gene discovery.[4] This new type of mutation, known as a repeat expansion, occurs when a sequence of three nucleotides, or "triplet," is repeated too many times. In HD, the C-A-G sequence is repeated 37 or more times in one of a patient's two huntingtin genes, while individuals who do not develop HD have 4 to 35 repeats.[5]

The reason why the expanded C-A-G repeat causes a disease is that this three-nucleotide set within the huntingtin gene encodes the amino acid glutamine, and the expanded stretch of glutamine makes the protein "misfold." The misfolded protein can then no longer be broken down into its constituent amino acids, the process through which proteins are normally recycled when they become damaged from use and over time. Consequently, the abnormal huntingtin protein builds up

and interferes with the function of other proteins in neurons and other cells in the central nervous system.

Protein misfolding is the core problem in HD. Researchers studying HD at the molecular level have shown that abnormal huntingtin can interfere with as many as a dozen basic cellular processes.[6] Their findings illustrate the challenge of discovering a treatment for HD: it is difficult to pinpoint which pathological event (or events) is most responsible for the degeneration and demise of neurons in the brains of HD patients.

Strategies for Treatment

Given the complexity of fixing multiple processes all at once in the HD brain, researchers sought other treatment approaches. If the disease is caused by the production of an abnormal protein, researchers reasoned, why not destroy the protein itself? This concept gave rise to attempts to target the abnormal huntingtin protein with antibodies. But this would require that the antibody enter the brain cells wherever the toxic protein is present, and to do this efficiently without causing side effects is very challenging. So instead of targeting the mutant protein itself for destruction, researchers chose to focus on its Ribonucleic acid (RNA), because various technologies were being developed to permit the efficient and specific targeting of RNA molecules, even in the brain. (Recall that the DNA sequence, or deoxyribonucleic acid sequence, of each gene is transcribed into a molecule of messenger RNA [mRNA], whose triplets code for amino acids that are translated into the gene's protein.)

Two different techniques were developed to target the huntingtin RNA. Both achieved specificity by using a defined sequence of the RNA long enough to be bound only by a manufactured complementary nucleotide sequence. The first approach used a strategy known as RNA interference, which co-opts a natural system of RNA destruction to target the huntingtin RNA. This work initially showed great promise in a mouse model.[7]

This approach, however, requires that the RNA sequence of interest be carried on a virus, injected into the brain regions affected by HD. Thus, to extend this therapy to human HD patients would necessitate an invasive neurosurgical procedure, which is feasible but less than ideal. For the

second strategy, researchers prepared a sequence of nucleotides—an "oligonucleotide"—complementary to the huntingtin RNA. The idea is for the synthetic "antisense" oligonucleotide to block the ability of the "sense" huntingtin sequence to produce its protein.

Antisense oligonucleotides, or ASOs, have been employed to turn off genes for more than three decades. As shown more than two decades ago, they work, but only for a limited time, because they are degraded quickly—which is fine for certain experimental systems (e.g., frog oocytes where you want to affect embryonic development at a specific short-lived stage), but not for therapeutic applications. For ASOs to be practical as potential therapies, their stability and safety would need to be markedly improved.

Drug Development Enters the Picture

A number of biotechnology and pharmaceutical companies have realized the potential of ASOs, and one, Ionis Pharmaceuticals, has been a pioneer in modifying the chemistry of nucleotides to achieve stability and safety. Their two-decade effort has yielded an ASO formulation that we know can be safely and effectively delivered to the human central nervous system, because its application in treatment of another devastating genetic disorder, infantile-onset spinal muscular atrophy (SMA), was approved for use in December 2016. This ASO drug, known by the trade name Spinraza, has emerged as a mainstay of therapy for SMA and represents the first truly compelling disease-modifying therapy for any neurodegenerative disease.

In the case of HD, the ASO strategy is to introduce an antisense oligonucleotide that will bind to the huntingtin RNA and form a heteroduplex (DNA ASO + huntingtin RNA). This hybrid molecule is degraded by RNase H, an enzyme in the nucleus of all cells that acts as surveillance against the heteroduplexes that naturally form with invasion by certain viruses.

The beauty of the ASO-based RNase activation system is that when RNase H degrades the bound huntingtin RNA it leaves the DNA ASO intact to seek out another huntingtin RNA, permitting the process of degradation to be repeated. Working with Ionis Pharmaceuticals, researchers vetted the huntingtin ASO strategy in HD transgenic mice and achieved considerable success.[8] Based upon this work and on safety studies in rodents and monkeys, Ionis Pharmaceuticals received approval to

move forward with a Phase 1 safety trial, which began in 2016. This study recruited human HD patients who received injections of the ASO drug, Ionis-HTT-Rx (or RG6042), into the lumbar spine (similar to epidural anesthesia delivery). This delivery scheme has been very well tolerated and there have been no untoward side effects from the treatment itself.

Because HD progresses slowly, demonstrating that a drug therapy slows the disease process requires careful observations of patients over at least two to three years. As clinical trials are very expensive, sometimes costing upwards of $25 million—even for a rare disease like HD where study cohorts are typically smaller—it would be helpful to know whether the therapy has a beneficial effect before proceeding with a large-scale study. To assess how well the ASO therapy works for HD, researchers developed an assay to detect huntingtin protein in the cerebrospinal fluid (CSF) that surrounds the brain, obtained with a lumbar puncture (i.e. through a needle inserted into the spinal canal).

Encouraging Indicators

At the end of last year, Ionis reported that analysis of CSF from HD patients receiving the ASO drug revealed a reduction of huntingtin protein of up to 60 percent after three months of treatment—an encouraging indication of effective engagement with the huntingtin RNA target. Based upon this result, Ionis Pharmaceuticals, together with Roche Pharmaceuticals (which has licensed the drug for further development), announced plans to advance the HD clinical trial to Phase 3, the final step in seeking approval for use in human patients. In the Phase 3 trial, patients will be followed for more than two years, in a double-blinded protocol where neither the patients nor the examining physicians will know who has received drug and who has received placebo. If all goes as planned, we will know if the Ionis-HTT-Rx ASO is an effective treatment by 2021.

The incredible progress made with HD ASO knock-down therapy could have profound implications for many other neurodegenerative diseases. Indeed, a clinical trial for amyotrophic lateral sclerosis (ALS, Lou Gehrig's disease) is about to begin; here, an ASO that targets mutant RNA in the most common familial form of ALS, known as C9orf72 ALS, will be evaluated in patients carrying this gene mutation. ASO drug development is also underway to target the RNA that encodes the protein tau, a protein believed to be involved in the pathogenesis of frontotemporal dementia and Alzheimer's

disease. As most neurodegenerative disorders involve a protein that misfolds and accumulates, the ASO strategy for reducing levels of RNAs that encode the toxic protein clearly holds great promise for a wide range of neurodegenerative diseases, from the very common to the exceedingly rare.

But the development of drugs to treat neurodegenerative disease is far from straightforward, with formidable challenges that could complicate the process of clinical testing and FDA approval. Indeed, experience with drug development for Alzheimer's disease (AD) may serve as a cautionary tale, as, with rare exception,[10] therapies that appeared very promising in experimental cell models and even in rodents have failed to show efficacy in human patients.[9] While some experts attributed such failures to an incomplete understanding of the disease process, others have expressed concern that treatments are being initiated in patients whose disease is too far advanced. This latter issue could be relevant to the ongoing clinical trial of ASO therapy for HD, in that by the time patients with HD (like those with AD or ALS) present with symptoms of their disease, they have already suffered the degeneration and death of numerous nerve cells.

So the question is: at what point has a patient lost too many neurons for meaningful therapeutic intervention to still be possible? Right now, we neither know the answer nor have sufficiently reliable animal models upon which to base a valid prediction. In their absence, it is entirely possible that we may develop a therapy that would be fully effective if offered to patients at the very start of their disease, but would fail when given to those with longstanding disease.

This is an important issue, as it is currently possible to test individuals at risk for HD based upon family history, and accurately determine whether they will get the disease in coming decades. Coupling presymptomatic diagnosis with ASO therapy might conceivably delay or even fully prevent the onset of HD in such individuals, but we do not yet have a clinical testing paradigm to support a prevention trial that needs to last 20 or more years. Hence, we are currently unable to establish clinical efficacy for what might be a highly effective therapy.

A Connection to Alzheimer's Diagnosis

While the situation for HD is straightforward because it is a genetic disease where presymptomatic testing is 99 percent accurate, recent research advances indicate that we are close to achieving

accurate presymptomatic diagnosis for AD as well. Two recent studies have reported that elevated levels of amyloid-beta protein in blood plasma can predict with high likelihood whether someone will develop AD 20 or 30 years later.[11,12] Here again we could be faced with the challenge of validating a potentially effective therapy, for purposes of FDA approval, that may not work in the symptomatic population on whom clinical testing is currently focused.

This is a vexing conundrum, because we do not wish to write off symptomatic patients as being beyond any hope of meaningful therapeutic intervention, yet the possibility of a continuum of therapeutic response in neurodegenerative disease is a reality we may need to face. The question becomes unavoidable as we enter an era in which powerful therapies will almost assuredly be developed, but the timing of their initiation and the selection of the appropriate patient population may require us to reevaluate the entire process of clinical testing and drug approval. If we do not grapple with these issues now, we might well dismiss a preventative "cure" for presymptomatic patients because the treatment was ineffective in patients with significant existing disease. For these reasons, the outcome of the Phase 3 clinical trial of ASO therapy in HD may not only tell us whether this particular drug is effective for affected HD patients, but more broadly illuminate the difficulties inherent in neurotherapeutic drug development.

Bio

Albert La Spada, M.D., Ph.D., is Professor of Neurology, Neurobiology, and Cell Biology at the Duke University School of Medicine, where he directs the Duke Center for Neurodegeneration & Neurotherapeutics. While a graduate student at the University of Pennsylvania School of Medicine, La Spada identified the cause of spinal and bulbar muscular atrophy as an expansion of a trinucleotide repeat in the androgen receptor gene. As the first disorder shown to be caused by an expanded repeat tract, this discovery of a novel type of genetic mutation led to the emergence of new field of study. La Spada's research remains focused on neurodegenerative disease, and he is seeking novel therapies to treat spinocerebellar ataxia type 7, spinal and bulbar muscular atrophy, Huntington 's disease, amyotrophic lateral sclerosis, and Parkinson's disease. He graduated *Summa Cum Laude* from the University of Pennsylvania with a Biology degree in 1986.

References

1. Peltonen, L., Jalanko, A., and Varilo, T. (1999) Molecular genetics of the Finnish disease heritage. *Hum Mol Genet* 8 (10), 1913-1923

2. McCandless, S. E., Brunger, J. W., and Cassidy, S. B. (2004) The burden of genetic disease on inpatient care in a children's hospital. *Am J Hum Genet* 74 (1), 121-127

3. Gusella, J. F., Wexler, N. S., Conneally, P. M., Naylor, S. L., Anderson, M. A., Tanzi, R. E., Watkins, P. C., Ottina, K., Wallace, M. R., Sakaguchi, A. Y., and et al. (1983) A polymorphic DNA marker genetically linked to Huntington's disease. *Nature* 306 (5940), 234-238

4. La Spada, A. R., Wilson, E. M., Lubahn, D. B., Harding, A. E., and Fischbeck, K. H. (1991) Androgen receptor gene mutations in X-linked spinal and bulbar muscular atrophy. *Nature* 352 (6330), 77-79

5. HD, and Group, C. R. (1993) A novel gene containing a trinucleotide repeat that is expanded and unstable on Huntington's disease chromosomes. *Cell* 72, 971-983

6. Gil, J. M., and Rego, A. C. (2008) Mechanisms of neurodegeneration in Huntington's disease. *Eur J Neurosci* 27 (11), 2803-2820

7. Harper, S. Q., Staber, P. D., He, X., Eliason, S. L., Martins, I. H., Mao, Q., Yang, L., Kotin, R. M., Paulson, H. L., and Davidson, B. L. (2005) RNA interference improves motor and neuropathological abnormalities in a Huntington's disease mouse model. *Proc Natl Acad Sci U S A* 102 (16), 5820-5825

8. Kordasiewicz, H. B., Stanek, L. M., Wancewicz, E. V., Mazur, C., McAlonis, M. M., Pytel, K. A., Artates, J. W., Weiss, A., Cheng, S. H., Shihabuddin, L. S., Hung, G., Bennett, C. F., and Cleveland, D. W. (2012) Sustained therapeutic reversal of Huntington's disease by transient repression of huntingtin synthesis. *Neuron* 74 (6), 1031-1044

9. Cummings, J. L., Morstorf, T., and Zhong, K. (2014) Alzheimer's disease drug-development pipeline: few candidates, frequent failures. *Alzheimers Res Ther* 6 (4), 37

10. Hung, S. Y., and Fu, W. M. (2017) Drug candidates in clinical trials for Alzheimer's disease. *J Biomed Sci* 24 (1), 47

11. Nakamura, A., Kaneko, N., Villemagne, V. L., Kato, T., Doecke, J., Dore, V., Fowler, C., Li, Q. X., Martins, R., Rowe, C., Tomita, T., Matsuzaki, K., Ishii, K., Ishii, K., Arahata, Y., Iwamoto, S., Ito, K., Tanaka, K., Masters, C. L., and Yanagisawa, K. (2018) High performance plasma amyloid-beta biomarkers for Alzheimer's disease. *Nature* 554 (7691), 249-254

12. Ovod, V., Ramsey, K. N., Mawuenyega, K. G., Bollinger, J. G., Hicks, T., Schneider, T., Sullivan, M., Paumier, K., Holtzman, D. M., Morris, J. C., Benzinger, T., Fagan, A. M., Patterson, B. W., and Bateman, R. J. (2017) Amyloid beta concentrations and stable isotope labeling kinetics of human plasma specific to central nervous system amyloidosis. *Alzheimers Dement* 13 (8), 841-849