

Huntington's Research Points to Possible New Therapies

By studying the mutant protein made by the Huntington's gene, researchers have identified several candidate drugs that are starting clinical trials

The discovery of the gene that causes a life-threatening disease always sparks a wave of hope that it will lead to a cure. That certainly occurred in 1993 when researchers bagged the gene at fault in Huntington's disease, an inevitably fatal neurodegenerative condition that afflicts about 30,000 people in the United States alone. The gene hasn't so far provided a cure for Huntington's disease, let alone a therapy. But in the past few years, researchers have learned a great deal about how its product, a protein known as huntingtin (Htt), malfunctions and kills neurons when mutated—information that is suggesting therapeutic strategies.

Cell and animal studies have revealed that mutant Htt disrupts nerve cell function in several ways, all of which can contribute to neuronal death. Its actions range from disrupting normal gene activity patterns to interfering with the transport of proteins needed for nerve cell maintenance to overwhelming the cell with toxic amounts of calcium ions. "Talk to a dozen people, and you'll get a dozen different ideas" about what happens in Huntington's disease, says Christopher Ross of Johns Hopkins University School of Medicine in Baltimore, Maryland.

At present, no one knows whether any particular neuronal insult is primary, with the rest following as consequences, or whether they operate independently. "Some people believe there is a priming event you must find and address [for therapy], others that mutant Htt causes multiple problems," says Marian DiFiglia of Harvard's Massachusetts General Hospital in Boston.

Despite that uncertainty, researchers have already identified potential drugs that counteract some of the neuronal problems caused by mutant Htt and are beginning to move them into clinical trials. "It's an exciting time in this field. The pace is picking up, and things look brighter

than they did," says Steven Finkbeiner of the University of California, San Francisco.

Getting started

One of the first clues to how mutant Htt causes the brain degeneration of Huntington's disease came when researchers created a mouse model of the disease a few years after the responsible gene was discovered. The mutations causing the disease occur near the beginning of the gene, in a segment that codes for a repeating string of the amino acid glutamine. In the normal version of Htt, that string contains anywhere from six to 35 glutamines, but in Huntington's patients, the repeat contains 36 or more, with higher numbers producing earlier disease onset.

Over the years, researchers have identified

such polyglutamine expansions in the proteins encoded by about nine genes. In all cases, the gene mutations cause neurodegenerative diseases, none of which are completely understood.

Back in 1997, while trying to find out how glutamine expansions in Htt lead to the nerve cell death of Huntington's disease, Gillian Bates of King's College London and her colleagues introduced into mice the first (or N-terminal) coding segment of the human Htt gene bearing glutamine stretches of various lengths. Rodents with the longer expansions developed a fatal neurological disease with symptoms, such as abnormal movement coordination, similar to those of human Huntington's disease, the researchers reported.

They also saw abnormal deposits of the mutant Htt fragments, along with other proteins, in the animals' neurons, and these so-called inclusion bodies were present before symptoms developed. Subsequently, the Bates

team and others studied brain tissue taken during autopsies of Huntington's patients and found similar clumps in both the cell bodies of neurons and the long axonal projections through which they contact their target cells.

These findings suggested that Htt fragments containing extra glutamines cause at least some of the nerve damage of Huntington's disease and that their abnormal clumping contributes to this toxicity. Bolstering the idea that the fragments are toxic, researchers found that introducing them into cultured nerve cells leads both to clump formation and to the cells' death and that putting them in fruit flies produces a Huntington-like neurodegeneration in the small creatures.

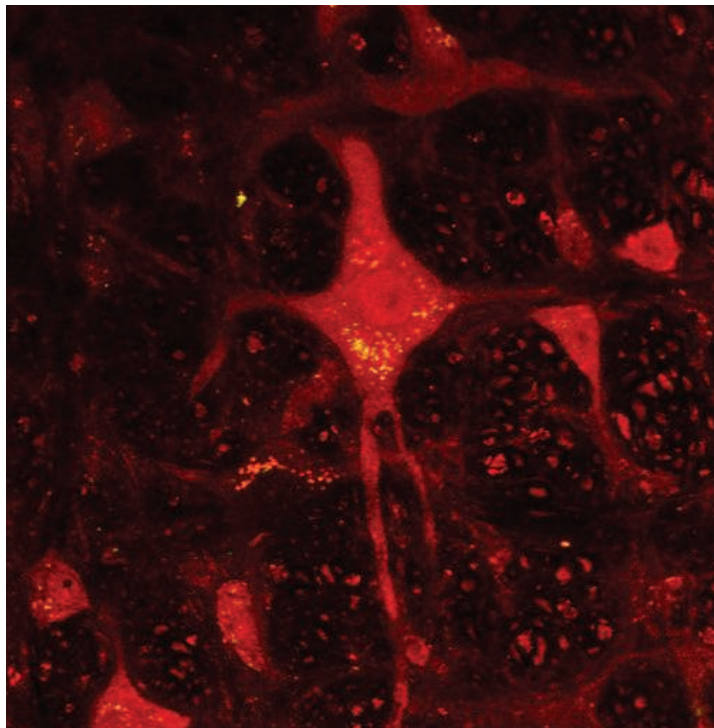
Researchers have also found that numerous protein-splitting enzymes, including certain caspases, can cleave Htt and release N-terminal fragments. As shown in 1996 by Michael Hayden's team at the University of British Columbia in Vancouver, Canada, this cleavage occurs in the brains of both normal humans and Huntington's patients, although only the mutant fragment is toxic.

If the pathology of Huntington's disease indeed depends on Htt being cleaved, then inhibitors

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Points of contention. Although Htt aggregates (bright dots) in brain neurons are a common pathological feature of Huntington's disease, it's currently unclear whether they kill the neurons or are protective.

Some Potential Huntington's Drugs

DRUG	ACTIONS	STATUS IN HUNTINGTON'S
Coenzyme Q10	Antioxidant; protects mitochondria	Late clinical trials
Creatine	Stabilizes mitochondria	Early clinical trials
Cystamine	Decreases Htt aggregation?	Early clinical trials
Geldanamycin	Decreases Htt aggregation	Preclinical
HDAC inhibitors (butyrates, SAHA)	Counters Htt's transcription effects	Early clinical trials
Memantine	Neurotransmission	Early clinical trials
Minocycline	Decreases cell death, Htt aggregation	Early clinical trials
Paroxetine	Protects neurons by increasing BDNF	Preclinical
Rapamycin	Decreases Htt aggregation by stimulating autophagy	Preclinical

of the enzymes responsible offer a potential therapy. In fact, researchers have found that treatments that inhibit or inactivate caspase activity decrease the toxicity of mutant Htt in both cultured cells and Huntington's mice.

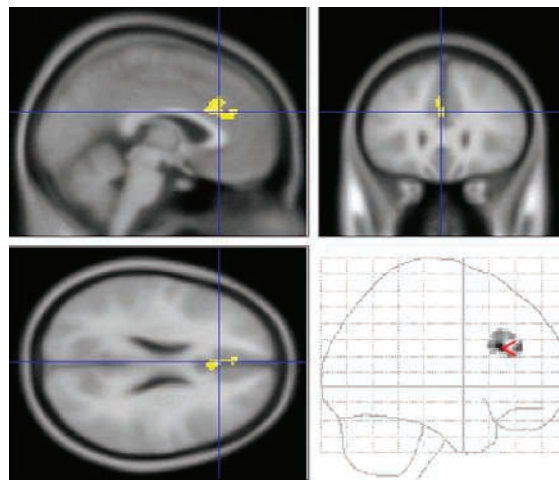
Although the idea that N-terminal fragments of mutant Htt are toxic has been gaining acceptance, the role of the aggregates remains controversial. Some evidence indicates that they contribute to Huntington's pathology, whereas other findings suggest that, far from causing harm, they serve to protect cells from mutant Htt's toxic effects. This debate mirrors a similar one in Alzheimer's disease in which researchers are wrestling to determine whether deposits of a protein fragment called β amyloid are toxic to brain neurons or are protective because they take the real culprit—soluble β amyloid—out of action.

The evidence that Htt aggregates are harmful includes demonstrations that inhibiting their formation reduces the toxicity of mutant Htt. For example, in the 18 January issue of the *Proceedings of the National Academy of Sciences*, a multi-institutional team led by Anne Young and Aleksey Kazantsev of Massachusetts General Hospital reported that feeding a compound, called C2-8, to fruit flies engineered to express mutant Htt prevents aggregate formation and reduces neurodegeneration in the insects' photoreceptor neurons.

David Rubinsztein of the Cambridge Institute for Medical Research in the United Kingdom and his colleagues have also found that the drug rapamycin suppresses Htt aggregate formation and decreases related toxicity in cultured neurons and in fruit fly and mouse models. The drug seems to work by enhancing a recycling process

called autophagy that cells use to destroy excess or damaged proteins. The process involves moving the proteins into small membranous sacs called lysosomes, which are essentially the cell's garbage-disposal units. The lysosomes are packed with enzymes that break down proteins and other cellular constituents. In the past few years, researchers, including DiFiglia and Rubinsztein, have found that autophagy helps clear Htt from nerve cells, thereby decreasing aggregate formation in the cells.

But autophagy apparently can't keep mutant Htt in check forever. The Rubinsztein team found that when rapamycin treatment began 33 hours after introducing DNA encoding the mutant N-terminal fragment of Htt into cultured neurons, it had no effect on aggregation or cell death, whereas it worked



Trouble sign. An area in the brain cortex (yellow) of people who are carrying a mutant Htt gene but have not yet developed Huntington's symptoms has lower activity during a cognitive task than it does in noncarriers.

just fine when introduced 15 hours after the gene transfer. "In the later stages [of Huntington's disease], autophagy might fail and allow the mutant protein to accumulate," DiFiglia says. These results suggest that if drugs such as rapamycin ever reach the clinic, they will have to be given before such a stage is reached.

Not all the researchers who have looked at Htt aggregation have found it harmful, however. In work described last fall in *Nature*, Finkbeiner and his colleagues found in lab cultures that many mouse neurons die even though they lack Htt inclusions. Indeed, the neurons with the inclusions survived better than those without, whereas neurons with lots of diffuse Htt had very poor survival. More recently, the Hayden group has confirmed this in mice, showing that animals with massive inclusions show no signs of neurodegeneration.

Work by Ross with Michelle Poirier and other Hopkins colleagues has shown that the inclusions develop in a stepwise process, with small, soluble aggregates forming first. The Finkbeiner and Hayden teams' results suggest that this diffuse Htt is the toxic form and that sequestering it in insoluble aggregates actually helps protect neurons. "At the moment, it looks like earlier forms of certain kinds of aggregates cause the problems," says Erich Wanker of the Max Delbrück Center for Molecular Medicine in Berlin, Germany, whose team has also looked at how the aggregates form.

Into the nucleus

No matter which form of mutant Htt is toxic, there is no doubt that the extra glutamines in the protein cause a raft of problems for nerve cells, including disruption of gene activity. Normal Htt binds to various transcription factors, proteins that control gene expression, and is thus part of the cell's gene regulatory machinery. Researchers, including Ross, Leslie Thompson of the University of California (UC), Irvine, and Dimitri Krainc of Massachusetts General Hospital, have found that the mutant N-terminal fragment of Htt disrupts this machinery. In some cases, it does this by binding to transcription factors and taking them out of action. This prevents the factors from turning on needed genes such as those encoding certain nerve cell growth factors.

Mutant Htt also indirectly alters gene expression by binding to and inhibiting a histone acetylase, an enzyme that marks genes for activity by tagging their associated histone proteins with acetyl

groups. This suggests another way of decreasing Htt's toxicity. "You can compensate for the decreased acetylation by using an inhibitor of the enzyme that removes the acetyl groups," says Thompson, who is one of the researchers who made the histone acetylase connection.

A few years ago, Thompson, working with Lawrence Marsh, also at UC Irvine, and colleagues, showed that this works in fruit fly models of Huntington's disease. Treating the animals with either sodium butyrate or a drug known as SAHA, both of which inhibit the enzyme that removes acetyl groups from histone, slowed down neurodegeneration in the flies. At about the same time, Bates and others obtained similar results with SAHA in Huntington's mice. Both of these deacetylation inhibitors are being explored for use in cancer therapy and are now also moving toward clinical trials for Huntington's disease.

Mutant Htt may also cause neuronal damage by increasing the activity of certain cell death-related genes, a new study suggests. Evidence has accumulated that the mitochondria, the small structures that produce most of the cell's energy, malfunction in animal Huntington's models and in human patients. In work reported in the 7 July issue of *Neuron*, Akira Sawa, working with Ross, Solomon Snyder, and colleagues at Johns Hopkins University School of Medicine, found that mutant Htt, but not the normal version, binds to a transcription factor called p53. Best known as one of the body's major tumor suppressors, p53 controls several genes, including some affecting mitochondria. Sawa's team found that the increase in p53 activity that occurs when it binds mutant Htt causes an abnormal flow of calcium ions across the mitochondrial membrane, thereby disrupting function of the organelles and leading to nerve cell death.

Sawa and his colleagues also showed that they could prevent the mitochondrial dysfunction by inactivating the p53 gene in cultured mouse brain neurons carrying a toxic Htt N-terminal fragment. Similar p53 inactivation suppresses neurodegeneration in Huntington's mice and flies, an indication that it might be another drug target. But Sawa cautions, "p53 is so important for our life that complete p53 suppression could be bad for us." Instead, he suggests,

scientists should look for molecules that either block the p53-mutant Htt interaction or inhibit the products of the mitochondrial genes activated as a result of that interaction.

A continuing mystery concerns the fact that the medium spiny neurons of the striatum of the brain are the first to degenerate in Huntington's disease. Hayden's team, working with that of Ilya Bezprozvanny at the University of Texas Southwestern Medical Center in Dallas and Lynn Raymond of the

Protective actions lost

Because the huntingtin mutation is dominant—causing disease if only one gene is inherited—researchers have focused mainly on the idea that the mutated protein has gained a toxic function. But recent work suggests that glutamine expansions can also contribute to neurodegeneration by reducing normal Htt's protective effects on neurons.

About a year ago, Frédéric Saudou of the Institut Curie in Paris and his colleagues found that normal Htt is part of the machinery that transports a nerve growth factor called brain-derived neurotrophic factor (BDNF) along their axons. Because of the structural changes caused by the additional glutamines, mutant Htt can't perform this transport function and in fact disrupts normal Htt interaction with the transport machinery.

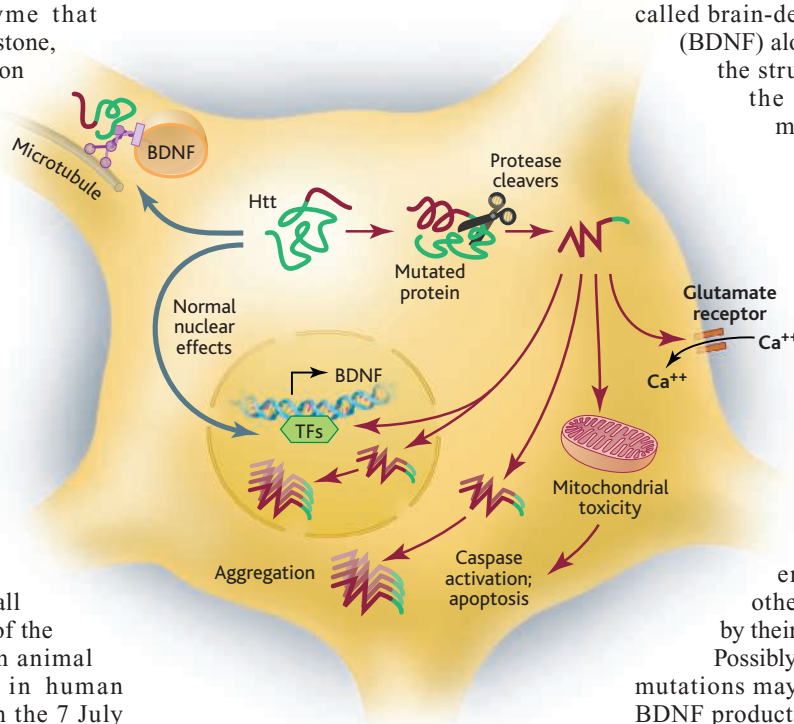
As a result, the neurons lose part of their supply of BDNF, which the cells need to survive. Saudou speculates that this may be one reason that medium spiny neurons are hit first in Huntington's disease. They're especially dependent on BDNF produced by other brain cells and taken up by their axons.

Possibly making matters worse, Htt mutations may also lead to a decline in BDNF production by medium spiny neurons. Elena Cattaneo and her colleagues at the University of Milan, Italy, have found that normal Htt helps turn up the activity of genes encoding BDNF and other proteins needed for neuronal maintenance. As a result, the BDNF supply will be further reduced when Htt is mutated, producing a double whammy for the cells.

If any of these revelations about the causes of Huntington's pathology ever generate effective treatments, they may have to be started early in life. Recent brain imaging studies from Ross's team, including Sarah Reading and Elizabeth Aylward, show that the degeneration in the striatum of the Huntington's brain is visible at least 11 years before a person develops symptoms, usually in their 40s.

Ross notes that this means there is a long time to try to halt the decline before a person gets sick. "What I've found most gratifying is that we've gone from a single gene to multiple possible mechanisms," he says. "Now we need to find which are most important and will lead to therapy."

—JEAN MARX



Many paths. The mutated Htt protein can disrupt neuronal functioning in several ways, some of which are shown in this diagram.

University of British Columbia, produced lab cultures of these neurons from mice carrying a full-length mutant Htt and compared the responses of those neurons to the excitatory neurotransmitter glutamate with those of neurons from normal mice. The modified medium spiny neurons experienced a much greater influx of calcium ions—and died—as a result, the researchers reported in the 15 February *Proceedings of the National Academy of Sciences*.

What's more, Hayden says, these changes occur very early. They were present in neurons studied just after the animals are born—well before disease symptoms develop. Hayden suggests that the receptors through which glutamate exerts its effects are possible targets for Huntington's therapies. In support of that idea, he and his colleagues found that drugs that block their activity suppressed the glutamate-induced death of cultured medium spiny neurons.