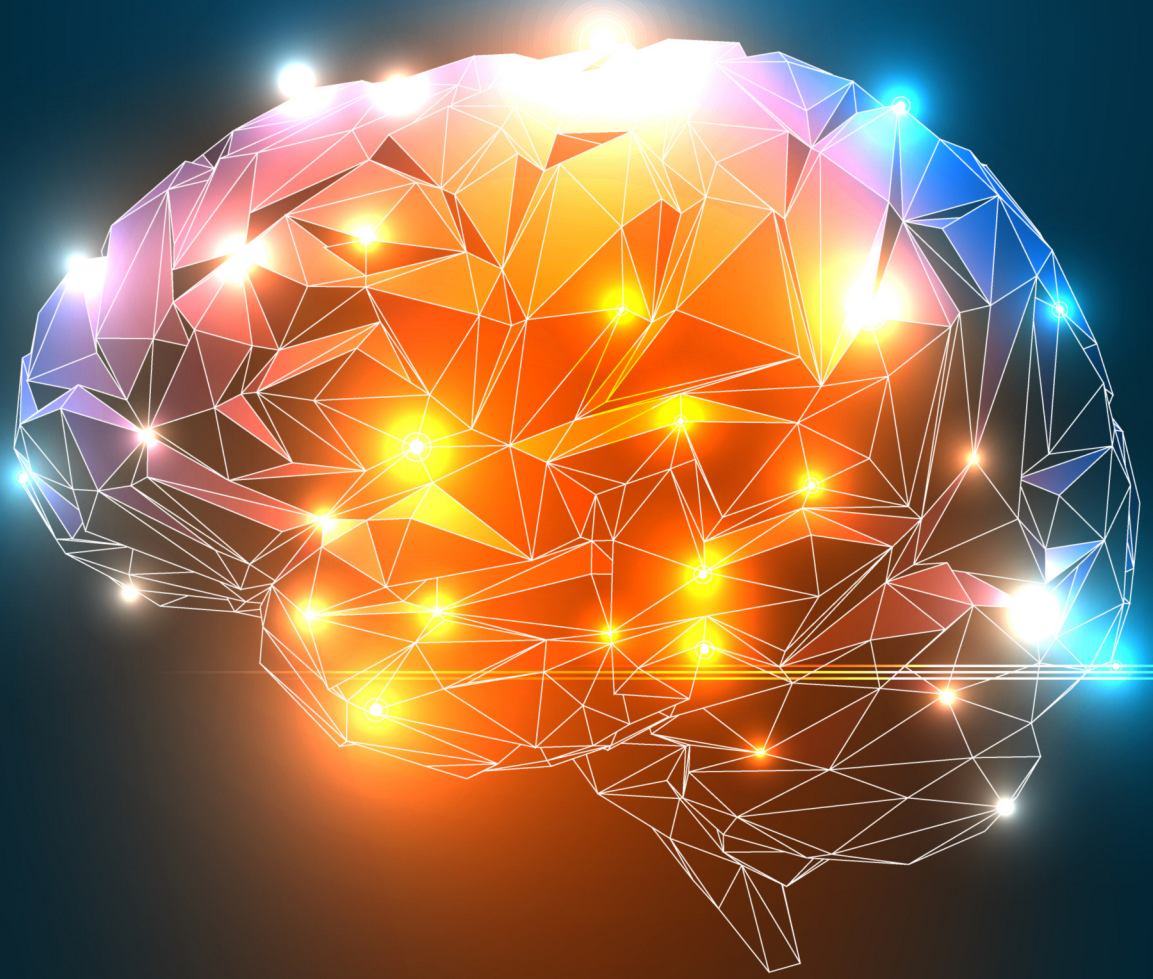


Gene expression and Huntington's disease

NYU School of Medicine

A hallmark of many neurodegenerative disorders is the appearance and persistence of aggregates in neuronal cells. Recent evidence suggests normal interactions that occur between proteins and nucleic acids (RNA) in these cells may become seeds for the formation of aggregates under prolonged stressful conditions. Such irreversible assemblies of proteins and RNA are considered toxic and impair neuronal functions. Mutations in proteins that bind RNA (RNA binding proteins) have been implicated in neurodegenerative disorders such as amyotrophic lateral sclerosis, spinal muscular atrophy, and fragile X syndrome.

Huntington's disease is a heritable neurodegenerative disease caused by a mutation in the Huntingtin gene, HTT. The mutation results in an increased number of repeats (greater than 40) of the amino acid glutamine in the Huntingtin protein (HTT). A normal HTT protein has between 7 and 35 glutamines. The mutant HTT protein is toxic to cells and causes cell death over time. The HTT protein is present in all human cells throughout life. Despite this, neurons are most vulnerable to death. The normal HTT protein has been implicated in many cellular functions. My lab discovered new functions of HTT in gene expression. Understanding these functions and how they are altered in the cells that harbour a mutant HTT gene shall reveal pathways and targets for therapeutic intervention to prevent the death of neurons.

Genes are blueprints for making proteins

The process by which the information encoded in a gene is converted to a gene product is termed gene expression. This occurs in two steps: transcription and translation. During transcription, an enzyme makes a copy of the gene. The copy is termed messenger RNA (mRNA) or transcript. During translation, a multi-protein complex called the ribosome decodes the mRNA and generates a string of amino acids. The string of amino acids folds itself to create stable structures known as proteins.

The program of gene expression is specific to each cell-type. The process is highly regulated both temporally and spatially to preserve the specific identity of each cell. Regulation of gene expression occurs during transcription and post-transcriptional events that involve processing of the synthesized mRNA. A category of proteins called RNA binding proteins (mentioned above) bind newly made mRNA and control its stability and location within the cell. Some RNA binding proteins do this by interacting with one end of the mRNA molecule, forming a stable RNA-protein complex. RNA binding proteins can influence gene expression through the assembly of multiple RNA-protein complexes thus sequestering mRNAs. When cells are stressed these complexes grow in size and number and become visible under a microscope. They are called granules, which are reversible aggregates of mRNA-binding and mRNA-degrading proteins that trap mRNAs in the cell. Through degradation or temporary storage of mRNAs, these granules reduce the amount of protein that can be made at any given time thus affecting gene expression.

Gene expression can also be regulated post-transcriptionally through the transport of mRNA transcript to specific locations in the cell. This is particularly important in neurons. Neurons are highly asymmetric cells with long

branches that send signals from the tip to another neuron nearby through a gap between two cells known as the synapse. The interaction between two neurons is strengthened or weakened based on how much stimulating signal the synapse receives over time. This change in strength is known as synaptic plasticity. Synaptic plasticity is critical to learning and forming new memories. Increasing evidence suggests transport of mRNAs to the synapse and local translation of these mRNAs contributes to synaptic plasticity. Following synaptic transmission, proteins within the branches of the neuron need be rapidly replenished. RNA binding proteins not only facilitate the transport of mRNAs but also regulate the efficiency at which mRNAs are decoded by the ribosome to make new proteins where and when they are needed.

HTT protein has a role in RNA transport and translation

My lab has studied transcriptional and post-transcriptional gene regulatory processes in mammalian cells for many years. Because the normal function of HTT and the mechanism by which its mutant counterpart contributes to the pathogenesis of Huntington's disease remains unclear, we began investigating the role of HTT in post-transcriptional gene regulatory pathways. With advanced imaging techniques we determined the location of the normal HTT protein in neurons grown in a culture dish. Strikingly, we discovered that HTT could be found near RNA granules present in neurons. As described above, RNA granules are assemblies of large RNA-protein complexes responsible for transporting mRNA to specific locations in the cell. To determine

Naoko Tanese, PhD
Associate Dean for Biomedical Sciences
Director, Sackler Institute of Graduate Biomedical Sciences

whether HTT influences mRNA localization, we reduced the level of HTT in neurons grown in culture and examined its effect on transport of mRNA. We found that the reduction of HTT in neurons disrupts proper mRNA localization. The result suggests that HTT contributes to the integrity of RNA granules and thereby facilitates mRNA transport.

To understand the cellular processes that HTT is involved in and how they may differ for mutant HTT, we carried out experimental procedures to isolate normal and mutant HTT protein from cells and tissues. We next identified proteins that associate with each HTT form. By identifying the functions of the proteins that co-isolated with HTT, we uncovered a new role for HTT. Analysis of the binding partners of HTT proteins revealed that both normal and mutant HTT interact with proteins involved in RNA processing/metabolism and protein synthesis.

HTT protein associates with its own mRNA

The identification of proteins co-isolated with the HTT protein provided further support to HTT's role in mRNA transport and translation. We next asked whether HTT regulates gene expression post-transcriptionally through interactions with mRNAs and if so, which mRNAs. Towards this end we extracted mRNAs that co-isolated with the HTT protein complex and analysed by direct sequencing, an advanced technology that permits rapid identification of nucleic acids. Surprisingly, we found both HTT protein forms to associate with their own HTT mRNA.

Further examination of the HTT protein's association with its own mRNA revealed that mutant HTT associates with a shorter version of the normal HTT mRNA. The HTT gene encodes a long mRNA transcript. However, the presence of a much smaller mRNA transcript encoding a short amino acid sequence from one end of the HTT protein has been detected in cells harbouring a mutant HTT gene. The short transcript was produced as a result of a post-transcriptional processing step termed mRNA splicing. Splicing is a commonly occurring event, which results in the removal of unnecessary sequences from the primary mRNA sequence to produce mature mRNAs used to make proteins by the ribosome. Normally mRNAs can be spliced in different ways to produce multiple transcripts from one gene. However, in the case of the short HTT transcript, the HTT mRNA has been incorrectly spliced. This mis-splicing event produced a smaller protein consisting of a short amino acid sequence. Significantly, the truncated protein contained the expanded glutamine repeats, which is known to be toxic to cells. The fact that mis-spliced mRNA was detected only in the presence of mutant HTT and not normal HTT suggests the possibility that it may play a role in the disease pathogenesis. Preventing the production of the mis-spliced mRNA would be one way to reduce cell toxicity that contributes to the progression of Huntington' disease.

Silencing mutant HTT gene

Although the gene that causes Huntington's disease was discovered more than two decades ago, development of therapies for the disease has been challenging. A therapeutic approach that has shown promise involves reducing the amount of mutant HTT mRNA and protein in affected cells. Gene silencing is a revolutionary technology with broad applications that may be used to treat or cure many diseases. In the case of Huntington's disease, most patients have one normal copy and one mutant copy of the HTT gene. The normal HTT gene is required to maintain healthy neurons. Thus, selective silencing of the mutant HTT gene is preferred. However, this is difficult to achieve because normal and mutant HTT genes are identical in their DNA sequence except for the length of the repeat sequence. However, it has been reported that partial silencing of mutant HTT (accompanied by partial silencing of normal HTT) might be enough to alleviate the toxic effects of the mutant gene in a mouse model of Huntington's diseases. Further, successful alterations of the mutant HTT gene in mice using the CRISPR gene editing system

have been reported. These groundbreaking approaches are being actively pursued in hopes of delaying the onset and progression of this terrible disease.

mRNAs regulated by HTT

In addition to its own mRNA, we know that HTT protein associates with other mRNAs. We think HTT associates with a subset of mRNAs present in neuronal RNA granules. We think HTT regulates transport and local translation of these mRNAs in response to synaptic activity. We hypothesize that HTT may regulate the transport and translation of mRNAs that are vital to the survival of specific neurons. Our goal is to identify additional mRNAs that associate with normal HTT and mutant HTT. The involvement of the HTT protein in post-transcriptional gene regulation could explain the specific pattern of neuronal loss and symptoms seen in Huntington's disease. It is possible that mutant HTT affects select groups of genes/mRNAs more adversely over others. These potential changes are likely small to account for the normal development of affected individuals and delay in symptom appearance. An emerging body of evidence suggests regulated transport and local translation of mRNAs in neurons play a critical role in establishing their connectivity. Our findings implicate the normal HTT in these important dynamic processes in neurons. It is possible that the mutant HTT perturbs them in some way, contributing to the disease pathogenesis.

We have uncovered novel roles for normal and mutant Huntington's disease protein HTT in post-transcriptional gene regulatory pathways in neurons. These novel functions have several implications for the development of Huntington's disease. As discussed at the beginning, mutations in RNA binding proteins have been implicated in several neurodegenerative disorders. These RNA binding proteins normally play a role in mRNA transport, translation of mRNA, and formation of RNA-protein granules. The RNA binding proteins whose mutations have been linked to neurological disorders have been localized to granules found in neurons. Our study is the first to report that HTT plays a similar role in mRNA transport and translation. HTT protein is much larger than RNA binding proteins linked to other diseases. It is possible that HTT has a scaffolding role in RNA transport and translation. Recent studies indicate that mutant RNA binding proteins show altered biophysical properties. They have increased propensity to interact with one another and affect the formation and function of RNA granules. The findings are consistent with the idea that mutant RNA binding proteins found in these granules accumulate and become converted to irreversible toxic aggregates under prolonged stress. We suggest that HTT aggregates may be formed in a similar manner. Development of chemical agents that prevent aggregation or disrupt aggregates may serve to reverse toxicity associated with mutant proteins. Through understanding of how HTT supports neurons with these functions, we hope to reveal effective new targets for therapeutic intervention.

Huntington's disease: Understanding the impact

Jennifer Simpson of the Huntington's Disease Society of America highlights the disease and how there is still a long way to go before it can be truly understood

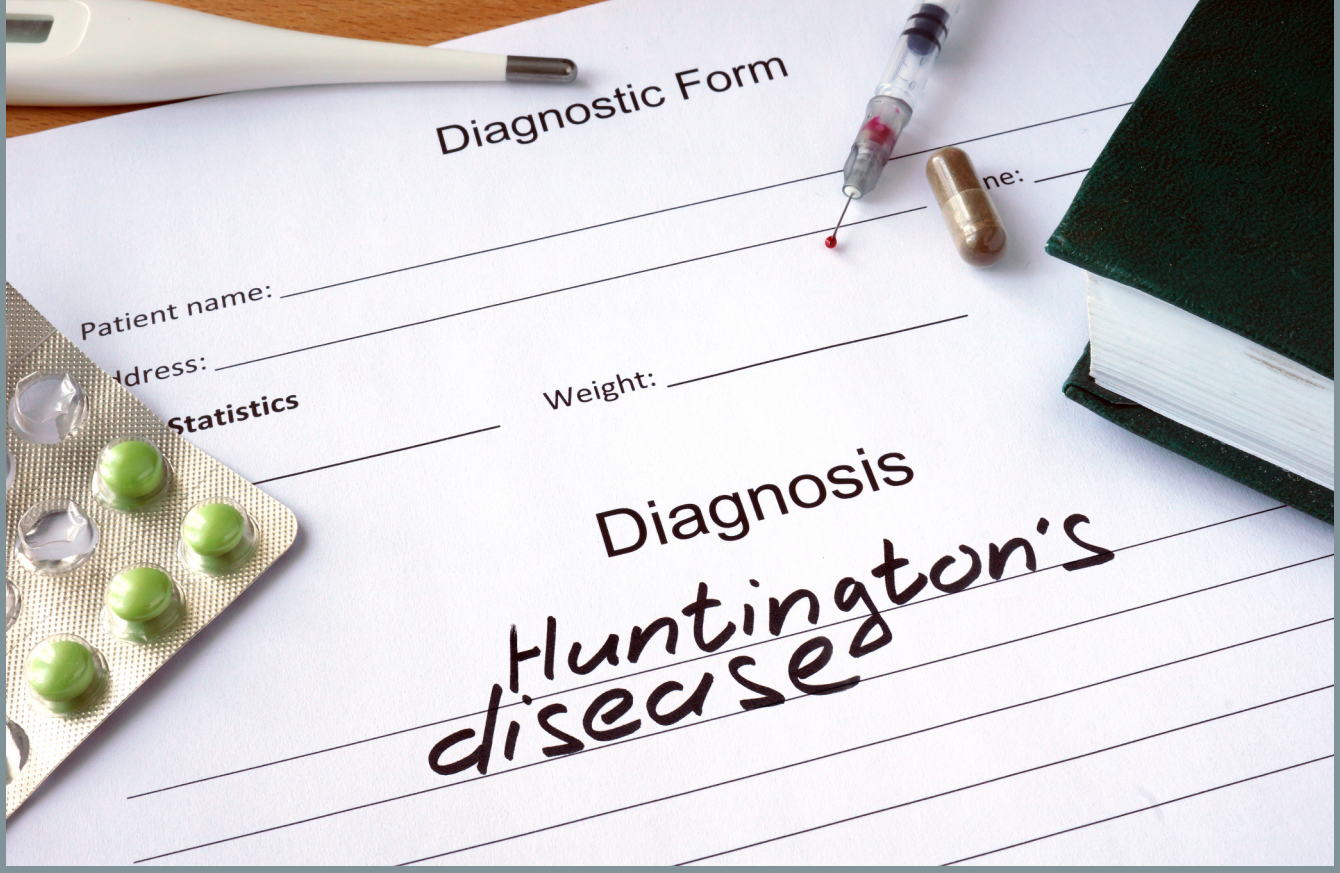
Since the discovery of the gene that causes Huntington's disease in 1993, exponential progress has been made in elucidating the true scope of Huntington's disease, but there are still miles to go to truly understand the impact of symptoms of HD families. Huntington's disease (HD), is an autosomal dominant neurological disease caused by an expanded CAG repeat in the Huntingtin gene. The disease is characterised by progressive functional decline and motor, psychiatric and cognitive symptoms, in addition to weight loss, sleep disturbances and dysregulation of the autonomic nervous system1. Each child of a person who carries the gene mutation that causes HD has a 50% chance of inheriting the faulty gene.

"Although we currently have no cure for this disease, we do have the ability to allow individuals to access treatments to help them manage this debilitating illness. While we work on a cure and find hope for tomorrow, we have to ensure that families affected by HD can access the help they need today."

Cognitive and behavioral symptoms are most impactful to HD patients and families

In preparation for a Patient Focused Drug Development meeting with the U.S. Food and Drug Administration, the Huntington's Disease Society of America (HDSA) surveyed the HD community in the U.S. on topics related to HD symptoms and treatments. Between two surveys, more than 3,600 responses were collected from individuals affected by HD, Juvenile Huntington's disease (JHD) and caregivers for those with HD and JHD. In reviewing the data collected, clear trends began to emerge between caregivers and HD/JHD patients alike. Caregivers responded most frequently that chorea was the most impactful symptom of HD (30%), but in aggregate, behavioral and cognitive symptoms were reported as the most impactful to their lives by more than 50% of both caregivers and HD/JHD patients2.

Huntington's disease has long been classified as a movement disorder, though prodromal features encompass cognitive and behavioral symptoms of HD3. Although classified as "prodromal", the cognitive and behavioral symptoms of HD are major elements of the disease and its impact on the individual and their families. Cognitive and behavioral symptoms can manifest as much as a decade before motor symptoms develop, and as a result often go undiagnosed as symptoms of HD. It is not uncommon for individuals with HD to be misdiagnosed with a variety of psychiatric disorders before being correctly diagnosed with HD at the onset of motor



symptoms. Delayed diagnosis may unfairly disadvantage people with HD and cognitive-behavioral symptoms, especially in terms of accessing the kind of care and benefits people with HD really need to best manage the progression of their disease4.

For Huntington's disease patients, treatment options are lacking

In the world of HD, treatment options are few and far between. As of the publication of this article, only two medications exist that are FDA approved for the treatment of HD, and both treat chorea symptoms associated with the disease. Currently, there are no disease-modifying treatments or cures. When surveyed on availability and efficacy of current treatments for cognitive symptoms of HD, more than 80% of respondents noted that they or their loved one were not taking any kind of medication for symptoms like deterioration of memory and thinking2. For behavioral symptoms like anxiety, depression and irritability, individuals responded most frequently that they or their loved one was not taking any kind of medication to treat those symptoms2. The lack of treatment options, especially for cognitive symptoms of HD, stands in stark contrast to the impact those symptoms have on the lives of people with Huntington's disease.

Access to care early on is critical to managing Huntington's disease

As patients with HD become symptomatic, it is key that those individuals have access to comprehensive care with doctors who are knowledgeable in HD. HD patients in early to middle stages of the disease need coordinated multidisciplinary healthcare services, including assessment of cognitive function and counselling by (neuro) psychologists,

rehabilitation programmes, active physiotherapeutic interventions, speech therapist training and occupational therapy5. Lack of access to care for families with HD means unmanaged or poorly managed symptoms, higher rates of caregiver burnout, potential unnecessary hospitalisations and early entry into long-term care facilities. With access to specialists knowledgeable in HD, families can avoid unnecessary additional emotional and financial burdens. In the U.S., HDSA has created a clinical care model through the Center of Excellence programme, awarding grants to HD clinics around the country to provide an all-in-one service center for families affected by Huntington's disease. HDSA currently funds 41 Centers of Excellence around the U.S.

"In the world of HD, treatment options are few and far between. As of the publication of this article, only two medications exist that are FDA approved for the treatment of HD, and both treat chorea symptoms associated with the disease. Currently, there are no disease-modifying treatments or cures."

Expanded access to government programmes can help HD families

Individuals with HD access multiple forms of governmental support as their disease progresses. Because HD symptom onset commonly occurs during prime working years, many families are devastated financially, and need to rely on programmes like Social Security Disability Income (SSDI), Medicaid and Medicare. Utilising these programmes can help families access professionals like neurologists, neuropsychiatrists, speech therapists and physical therapists. Expansion of Medicaid programmes has resulted in a reduction of unmet need for mental health services, in addition to positive impacts on the budgets of states that expanded Medicaid as states no longer needed to use some of their general funds to pay for behavioral health treatment for the uninsured6. Individuals with HD are included amongst those who have benefitted from the expansion of programmes like Medicaid, and would further benefit from expedited access to Medicare through the Social Security Disability Income programme. HDSA has been advocating, alongside the HD community, for a waiver of the two-year Medicare waiting period for individuals who are disabled by Huntington's disease and utilising the SSDI programme. The Huntington's disease Parity act of 2017 is a bi-partisan solution to an HD shaped hole in the social safety net. It is one step of many to help ensure access to important behavioral health services and specialist neurologists who can assist families maintain quality of life for folks with HD for as long as possible. Although we currently have no cure for this disease, we do have the ability to allow individuals to access treatments to help them manage this debilitating illness. While we work on a cure and find hope for tomorrow, we have to ensure that families affected by HD can access the help they need today. ■

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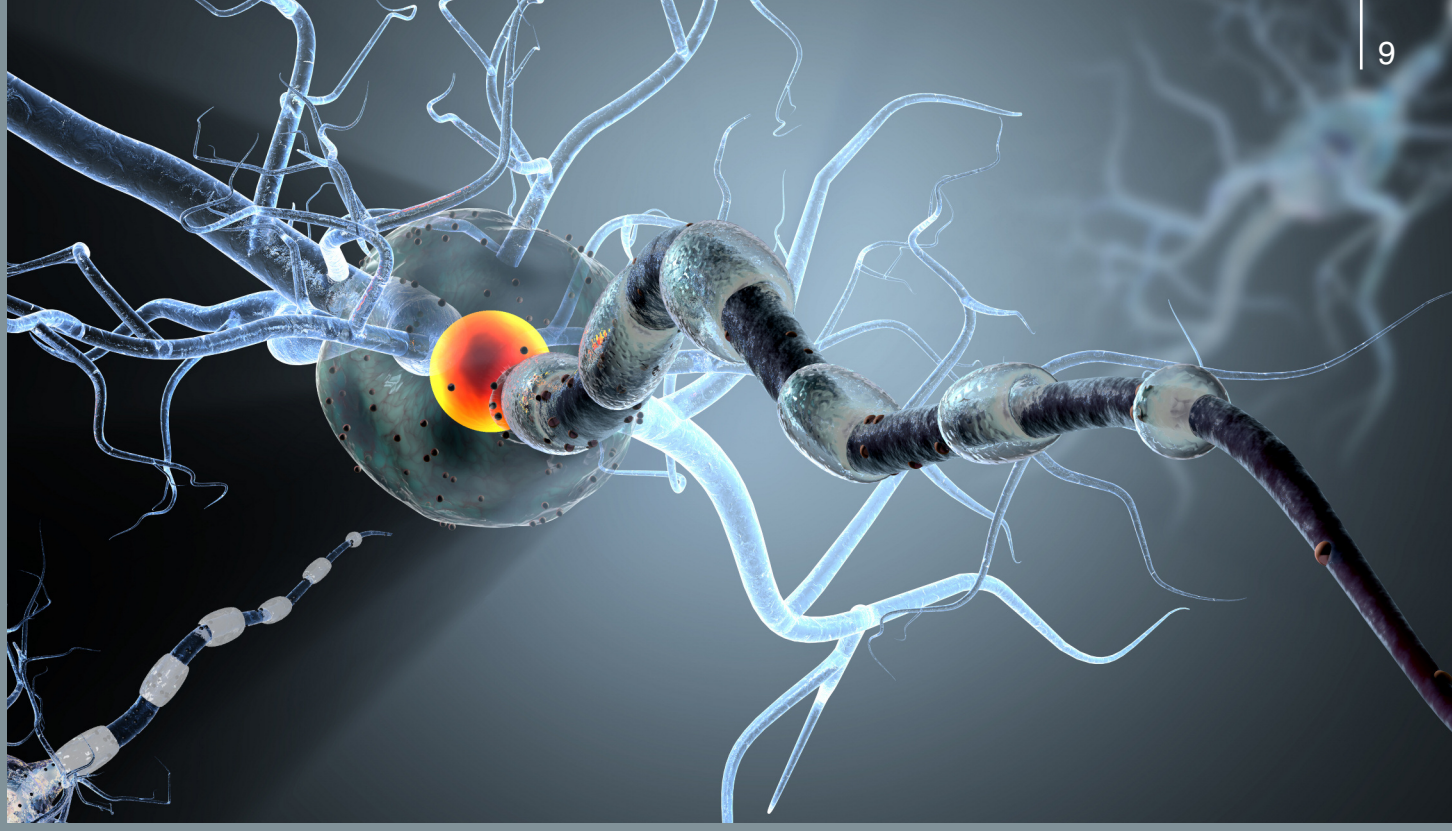

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Huntington's Disease Society of America

Jennifer Simpson
Manager of Advocacy and Youth Programmes
Huntington's Disease Society of America
www.hdsa.org
www.twitter.com/HDSA

Naoko Tanese, PhD
Associate Dean for Biomedical Sciences
Director, Sackler Institute of Graduate Biomedical Sciences

New York University School of Medicine
Tel: +1 212 263 8945
tanesn01@med.nyu.edu
https://med.nyu.edu/faculty/naoko-tanese
http://sackler.med.nyu.edu

NYU School of Medicine