HD2018: The Milton Wexler Celebration of Life

August 8-11, 2018
Hereditary Disease Foundation
Royal Sonesta Hotel
Cambridge, MA

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Thursday, August 9th

Personal Perspectives and a Celebration of Hope

The 2018 Milton Wexler Celebration of Life began with an introduction from Nancy Wexler and a moving interview with an individual with HD.

An Introduction from Nancy Wexler

Nancy Wexler opened the 2018 Milton Wexler Celebration of Life with a 50th anniversary message for all participants, expressing her thanks for their continued scientific collaboration towards an end to Huntington's disease. Nancy recalled her mother's family and their belief that only men could inherit the disorder that claimed her mother's father and brothers. As a young woman, her mother's passion for research led her to the Morgan Fly Room at Columbia, where she contributed to genetic studies that were an early precursor to the mapping work that identified the HD gene. But after decades of research, it became clear that HD was an autosomal dominant disorder, meaning that any child of an affected person had a 50/50 chance of inheritance. Nancy's mother was diagnosed in 1968. Soon after, Nancy's father, Milton Wexler, set out to assemble researchers to work towards a cure for HD. As a result, many extraordinary scientific milestones were achieved, including the initiation of a multi-generational study of Venezuelan HD families that led to the discovery of the gene in 1993. Today we are still on a quest to understand why the gene is lethal, but for the first time the gene itself has been targeted, and we are in the midst of promising clinical trials to silence huntingtin. This was a dream of her father's at the end of his life. Nancy concluded by expressing her gratitude towards everyone present for "throwing all of our shoulders against a really hard wall together," an apt metaphor for the collaborative efforts driving remarkable research progress. At the 2018 Milton Wexler Celebration of Life, there was more hope than ever that with persistence, that wall will come down.

Living with Huntington's Disease: Family Perspectives

Wendy is a patient of Dr. Diana Rosas at Massachusetts General Hospital (MGH), and she and her husband Bill graciously took the stage at the start of the conference to speak about how HD has affected their lives. Wendy expressed a sense of being "starstruck" in a room full of gifted and caring people who want to find a solution to this horrible disease, and embarked on her story, guided by Dr. Rosas. She found out she had a CAG repeat expansion within her HD gene in 2001, and will be turning 50 next week. She was adopted at birth, and her adoptive father, a policeman, died on the job when Wendy was 13. Following a self-destructive few years, she got sober and on track, and as a smart and athletic woman was encouraged by her family to attempt an exam to join the police force. She passed the test in 1993 and began a fulfilling and demanding career as a detective, working on sexual assault and later homicide cases, where she was the only woman on the unit. Given her own father's death on the force and the support her family felt

from the other officers at that time, she loved that part of her job was to be there for people at very difficult times.

Wendy was very close with her adoptive mother, and the idea of finding her biological parents did not resonate with her until she was 32 years old. She initially hid it from her mom, but was able to connect with her birth mother, who had never had more children. They rejoiced in little similarities, like how they both loved chocolate chip cookies without the chocolate chips. She learned that her biological father was in a nursing home but not why, and got in touch with her biological grandfather to confirm. She would later learn that her grandfather had carried a CAG repeat mutation just below the pathological range for HD, which had expanded into the disease range when passed to her father. She decided to meet her biological father in 2001. Not knowing that the nursing home was an HD patient unit, and unaware of his movement symptoms, Wendy took along a big vase of flowers that the nurses would not allow her to bring in. She explained that meeting her biological father was a wonderful and awful connection, a feeling of love for someone she had never known and who was very ill. He could barely talk, but through a social worker, he convinced her to get tested for the disease that was taking his life. Wendy opted for anonymous genetic testing at MGH, which Dr. Rosas explained was a huge deal at the time, due to lack of awareness of HD and its challenges. Even today, a test indicating expanded repeats within the HD gene can be prohibitive for obtaining health and life insurance, and the definition of a "preexisting condition" can change in various political climates.

Wendy explained that once she had her results, "everything twisted from there." She was trying to make sense of her life as an athlete and detective, to cope with the weight of the knowledge about her future, and to deal with extra stress at work because of a particularly horrible homicide case. She became very cynical and turned back to alcohol for a time, and made the decision not to have children because of the 50/50 chance of passing on HD. At the same time, she almost immediately started doing research studies, out of hope for a treatment, but mostly because of a great love and trust in Dr. Rosas. Over the intervening years, she began to show symptoms, the most debilitating of which were unrelenting depression, suicidality, and severe OCD about cleanliness and germs that left her feeling housebound and burdensome to her husband. It has been difficult to come to terms with her movement symptoms; she was largely unaware of her chorea until watching her own wedding video, which was otherwise the happiest day of her life. She began having difficulty driving (though she is still driving) and, ever the athlete, running or walking around her community sometimes exposes her to scathing public scrutiny. She is aware of the great strain on her marriage as she continues to have bouts of unpredictable emotions and motor difficulties that make her unable to do household chores without breaking things or injuring herself. She has near-daily bouts of choking, struggles to maintain her weight, and suffers from central sleep apnea and peripheral pain. Dr. Rosas noted that these are overlooked symptoms for many HD patients that the medical and research community should attend to.

Despite these persistent challenges, Wendy has come a very long way and is now managing well on several medications, after a few courses of electroconvulsive therapy. She called her partner a "fixer," and a "vibrant charismatic wonderful man," who "does everything

possible to make my world safe." She's devastated that her cognitive and perceptual issues make it impossible for her to read the book he is about to publish. Bill spoke up briefly about his experience and his emotions, mainly anger at the circumstances. Being a passive and positive person, he finds it frustrating and scary not to know what kind of situation he might find at home, and compared it to living with someone struggling with alcoholism. Watching her comprehend her own decline is very difficult for him, and he wants to shield her from everything and be everything for her: "you want to be doctor and psychiatrist and husband and best friend." It's been isolating without experienced family members and friends – some are in denial, and some, like Wendy's mother, don't understand that the emotional symptoms are disease-related and not a choice or a weakness of character. However, Wendy has recently found some community through a support group. Now that she is venturing outdoors again, Bill loves taking her camping with their RV and just watching her be at peace.

Discovery and Development of the First Huntingtin Lowering Antisense Drug

25 years after the discovery of the HD gene, the first trial of huntingtin-lowering therapy is underway. A session describing the journey from discovery to human efficacy trial was a focal point of the meeting. Dr. Frank Bennett, Senior Vice President of Research at Ionis Pharmaceuticals, spoke about the development and validation of antisense oligonucleotide (ASO) technology to treat HD and other genetic disorders. Dr. Blair Leavitt, clinician and professor at the University of British Columbia, followed with a presentation of the design and results of the Phase 1b/2a clinical trial. Finally, Dr. Omar Khwaja, Global Head of Neuroscience Translational Medicine and Rare Disease at Roche Pharmaceuticals, concluded with a discussion of next steps for Roche with carrying out the next phase of testing of Ionis-HTTRx (RG6042) in a Phase 3 clinical trial called GENERATION-HD1.

Discovery and Preclinical Development of Ionis-HTTRx (RG6042)

Ionis Pharmaceuticals is exploring and modifying antisense oligonucleotide technology for a variety of medical purposes. Some compounds are designed to block or enhance translation, to splice RNA, or to cause transcript degradation. Huntingtin-lowering antisense oligonucleotide (ASO) drug RG6042 (formerly Ionis-HTTRx) is in this latter category, binding to RNA and tricking cells into taking huntingtin out with the trash. Frank Bennett explained that the key to using ASO technology for therapeutics is medicinal chemistry: by modifying an ASO's structure it is possible to increase potency, enhance stability, and mitigate inflammatory responses. Once Ionis had several potent and stable candidates for ASO-based knockdown of the human mutant huntingtin gene, they needed to investigate delivery, distribution, pharmacokinetics, and pharmacodynamics in animal models.

Don Cleveland's laboratory was instrumental at this step, showing that the huntingtin-lowering ASOs could reach the brain when delivered into the cerebral spinal fluid (the fluid that surrounds the brain) in rodents, and was eliminated normally from the body. Immunohistochemistry in non-human primate tissue showed broad distribution with highest

concentration in grey matter and entry into many types of neurons and glia. Deeper structures like the caudate and putamen (particularly vulnerable in HD) had lower concentrations of ASOs, but up to 50 or 75% knockdown could be achieved, and the drug stayed around for 4 to 13 weeks after a single injection. The next step was to choose one of three viable strategies to knock down huntingtin: target the CAG repeat, the mutant allele, or total huntingtin (both mutant and normal huntingtin alleles).

To avoid off-target effects and focus on potentially treating all people with HD, Ionis went with the total huntingtin approach. They then demonstrated that the chosen ASO, IONIS-HTT_{Rx}, could have lasting effects on huntingtin levels and an impact on motor function and survival in mice (Kordasiewicz et al., 2012). Further primate studies demonstrated that huntingtin-lowering in the cerebrospinal fluid (CSF) correlated with the level of knockdown in cortex, and toxicity studies were extremely promising, with up to 15 months of drug showing no negative effects in non-human primates. Through the generous donation of brain tissue from a child who died of Spinal Muscular Atrophy (SMA) after treatment with nusinersen, Ionis learned that their ASO to treat SMA also penetrated to many brain areas in human. After addressing many key questions in animal models, Ionis was ready to move to the clinic.

Ionis-HTTRx (RG6042) Phase 1/2A Clinical Study

Blair Leavitt presented the details of the first successful huntingtin-lowering clinical study in humans, the result of more than a decade of work by the HD research community. Simultaneous to the preclinical studies in mice and primates described by Bennett, other researchers were developing the methodology to measure levels of huntingtin in the CSF, including Amber Southwell and Ed Wild (Southwell et al., 2015; Wild et al., 2015). The correlation between CSF and brain levels of huntingtin meant that CSF could be used as a biomarker, a critical step towards initiating the clinical trial.

The study itself was designed to test safety and tolerability as primary endpoints, with secondary pharmacokinetic and pharmacodynamic endpoints, as well as additional exploratory and target engagement outcomes. Because of the ASO's 4-week onset of action and persistence in the body, participants received monthly intrathecal injections. There were four dosing levels tested in an ascending-dose design, where placebo and the lowest dose were given first, followed by higher and higher doses after safety was ensured. Patients were recruited at 9 sites in Canada, Germany, and the UK. There were no major safety events aside from one hospital stay in the placebo group due to a severe headache that resolved on its own. After the main trial, all participants chose to continue on active open-label drug.

The trial ultimately met its primary endpoint: ASO treatment corresponded with a dose-dependent decrease in CSF levels of mutant huntingtin, bringing levels down to about 40-60%. This decline in CSF levels is predicted to correspond with a 55-85% huntingtin decrease in cortex and a 20-50% decrease in caudate. There were no significant group-wise improvements in any active dose versus placebo, meaning that each small dosing group, when compared to placebo, showed no clinical changes. However, when the full group was analyzed, the degree of lowering correlated with the degree of clinical improvement, especially on the Unified Huntington's

Disease Rating Scale (UHDRS). The trial is ongoing and there have been no safety events for any of the 46 people in the open-label study.

Next Steps for Ionis-HTTRx (RG6042): Natural History Study and GENERATION-HD1

After a successful safety trial, Ionis passed the torch to Roche Pharmaceuticals (known as Genentech in the United States) to carry out a larger efficacy trial of Ionis-HTTRx, which was renamed to RG6042. Omar Khwaja introduced Roche/Genentech, and shared with participants that his career in medicine and genetic diseases was inspired by reading the Double Helix, which described the discovery of DNA and detailed Nancy Wexler's work with HD families in Venezuela.

Huntington's disease is a flagship area of study at Roche, which has a long history of neuroscience research and has been a family-owned company for more than a hundred years. Beyond the ongoing huntingtin-lowering study, one of their main interests is to develop digital tools for measurement of HD symptoms. Wearable sensors and smartphone-enabled assessments could create a richer picture of motor and cognitive symptoms over time and reduce the burden on study participants. For ongoing studies of RG6042, Roche has three main areas of focus. The first is an open-label extension study to assess long-term safety and tolerability, ongoing currently in the 46 original safety trial participants. The second is an upcoming observational, natural history study in which there will be no active treatment, but the goal is to better link mutant huntingtin levels with disease progression. The third is the planned global Phase 3 efficacy study, called GENERATION-HD1, which aims to replicate and extend the safety findings from Phase 1b/2a, and to show efficacy of RG6042 for slowing or reversing symptoms of HD.

Khwaja assured conference attendees that there is an enormous group of more than 70 people at Roche/Genentech working around the clock on the HD study, and this team is growing. He expressed his gratitude for the courage of trial participants, those willing to give their time and risk their own health in search of therapies that may not benefit them directly but may benefit others in the future.

Development of Therapy for Other Protein Aggregation Disease

Jeff Kelly of the Scripps Research Institute in California delivered a keynote lecture on how his lab's work on the chemistry and biology of protein folding led to a successful clinical trial for a genetic disease called transthyretin (TTR) amyloidosis.

Adapting Proteostasis Chemically and Biologically to Ameliorate Aggregation-Associated Degenerative Diseases

TTR amyloidosis is a disorder in which a protein called transthyretin accumulates in various parts of the heart, nervous system, and eye. This leads to destruction of tissues in the heart as well as in the peripheral and autonomic nervous systems. Like HD, it is autosomal

dominant, with a 50/50 chance of being passed to a child. TTR amyloidosis causes a variety of symptoms including dementia, neurologic episodes, vascular bleeding, and glaucoma.

Kelly emphasized an emerging amyloid hypothesis that differs slightly from the classic idea that aggregated proteins are toxic. The theory is that the *process* of aggregation, rather than a specific form of aggregate, is causative. *Which* type of aggregate is toxic, and *how* they might cause neuron death is still widely debated, and trials to "bust" different types of disease aggregates using antibodies have failed to ameliorate disease or to clarify which type is the right type to target. Kelly's hypothesis is that rather than waiting to treat existing aggregates, we should intervene as early as possible. More than 150 familial mutations are known to make this process move faster, ultimately causing disease. Liver transplantation has been a successful treatment in many cases, and it can extend life by up to 30 years, but it has not been widely accepted because 10% of patients die due to the procedure.

In TTR amyloidosis, the rate-limiting factor for aggregation is how quickly the transthyretin tetramer dissociates into dimers (pairs of protein chains). Certain mutations can alter the energy state of the tetramer, slowing down the rate of dissociation (Hammarstrom et al., 2001; Hammarstrom et al., 2003), and people with these mutations got TTR amyloidosis much later in life. To harness this knowledge for therapeutics, Kelly's group worked on testing small molecule drugs that bind to the native state of the tetramer, to mimic the disease-delaying effect of the mutation. They identified a single molecule that would have this effect (Bulawa et al., 2012), and because there has never been a way to successfully model TTR amyloidosis in animals, they moved straight into humans.

The drug had a high rate of success in a very specific population of people with TTR amyloidosis, slowing or stopping the development of symptoms. Although this particular disease gene is not common in the US, the drug has been approved in many countries around the world. After 5 years of observation, 34% of people in the trial stopped progressing in their disease, and another 34% had a dramatic slowing of symptoms. However, 30% don't respond at all to the drug. A delay of just 18 months in beginning the treatment drops the success rate from 68% response to 46%, so the critical thing is to treat early. Monitoring this progression, and knowing what "early" means, is tricky because there are not yet existing biomarkers that show a response to the medication. This is an important current area of study.

Because the small molecule approach requires one drug for each protein, the process of discovery and application is long and arduous. Kelly's group is also working on how to hasten aggregate breakdown and restore balance by other means, like enhancing autophagy or protein refolding. Through these studies on TTR amyloidosis, the wider neurodegenerative disease research community can learn about aggregate breakdown, and the HD community can derive hope from this story of identified mutation, to biological mechanism, to successful clinical trial.

Huntingtin Structure and Function

Since the discovery of the HD gene, scientists have been trying to visualize the shape of the huntingtin protein and to understand its many functions. However, its enormous size and versatility have made these goals elusive for many decades. For the first time in 2018, scientists were able to capture huntingtin alongside its molecular dance partners. Other labs are elucidating the role of huntingtin in different parts of the cell or in organs outside of the brain.

Cryo-EM Structure of Huntingtin in a Complex with HAP40

The most advanced images of huntingtin to date were captured by a team of German scientists led by Dr. Stefan Kochanek at Ulm University. Kochanek explained that this project has been ongoing for ten years. It was very difficult to capture huntingtin by a common freezing and imaging technique called cryo-electron microscopy (cryo-EM). This is because many different shapes of the huntingtin protein exist, and because it sticks to itself in pairs, triplicates, and larger groups. To overcome this, Kochanek's team created a "trap" using another protein called HAP-40, which sticks tightly to huntingtin and stabilizes it enough to perform structural imaging techniques like cryo-EM. Without HAP-40 there to support it, huntingtin is extremely flexible and wiggly.

Trapping huntingtin with HAP-40 did the trick, enabling the team to construct a high-resolution model of most (72%) of the protein complex, including the N-HEAT, C-HEAT, and bridge domains. Essentially, in its 3D form, huntingtin looks sort of like a pair of headphones, with a big loop that bridges over to a second wheel shape. Unfortunately, the structure of exon-1, the part containing the CAG repeats, was left unresolved, as were many important sites of post-translational modification that influence huntingtin's function, so there's more work to be done. But overall, the Kochanek lab has triumphed in publishing huntingtin's structure after many years of hard work (Guo et al., 2018) and the structure they've revealed supports huntingtin's role as an interaction hub for other proteins.

Cryo-Electron Tomography of mHTT ex vivo and in situ

Dr. Wah Chiu, a biophysicist at Stanford and pioneer of cryo-EM techniques, spoke about another set of experiments to visualize huntingtin by pairing it with a protein called TRiC. TRiC is a chaperone molecule, one that provides favorable conditions for other proteins to fold up properly, nearly 10% of those found in cells. For past and present work on huntingtin and the TRiC chaperonin network, Chiu has collaborated with Leslie Thompson, David Housman, Judith Frydman, and Bill Mobley. His talk covered two stories: the visualization of TRiC, and of mutant huntingtin aggregates in cells.

The cryo-tomography technique they used, unlike standard methods of capturing molecular structure, did not need to use crystallization at all, meaning they're able to look at the structure in different biochemical states. Chiu presented a 3.6 angstrom cryo-EM structure of human TRiC mapped from a variety of angles. The molecules form a barrel shape with stacked rings of 8 protein chains which are structurally similar but not identical. At this resolution, they are able to look at not only the entire tertiary structure, but also at the active sites on the protein,

by binding the chaperonin to a substrate that triggers the protein folding process. In the apo state, where it's unbound, the barrels are uncovered and open. Even though parts of the barrel subunits are disordered, they can trace the backbone and build models of each one, revealing that they have multiple structural conformations and abilities to swing and rotate. Chiu pointed out that this is an excellent reminder that proteins are dynamic and not static.

In a classic experiment from Judith Frydman's lab (Tam et al., 2006), her group showed that increasing concentrations of TRiC inhibit the aggregation of mutant huntingtin, and that they colocalize in cells. Chiu's lab showed via cryo-electron tomography that TRiC sticks to huntingtin fibrils. A deeper analysis showed that there's a density (a protein blob) inside the TRiC barrel, and they propose that TRiC counters huntingtin aggregation through a cap-and-contain type of mechanism, where it binds to the ends of the forming fibrils and engulfs huntingtin within the barrel (Shahmoradian et al., 2013).

The next step was to work out the conditions to plate BAC-HD neurons onto a special surface so that they could use cryo-EM to examine the neurites (neuron outgrowths) and electron tomography to identify different biochemical structures in the tissue, such as microtubules and TRiC molecules. Chiu aims to apply these techniques to the visualization of aggregated huntingtin in neurons of different HD animal models, to examine how the surrounding cellular environment and structures are affected. To this end, his lab is collaborating with Dr. Leslie Thompson's, to examine cells from patients with different polyQ mutations in huntingtin. Using EM imaging and computational software they have been able to identify different deficits in HD patient-derived neurons, such as transport problems, abnormal mitochondria, and aggregates.

New Investigations into Huntingtin's Nuclear Roles

Huntington's disease is thought of primarily as a disorder of the brain. However, because mutant huntingtin protein is produced within every cell of the body, there are widespread signs of disease outside of the nervous system, which has been explored by various researchers over the decades. Dr. Jeff Carroll, an HD advocate and researcher at the University of Washington, is interested in better understanding the role of huntingtin all over the body, and how huntingtin-lowering therapies may affect the body long-term. To study these questions, his lab is using a variety of genetically modified mice to silence the huntingtin gene in different peripheral organs.

Carroll shared three preliminary stories from his lab in order to highlight the projects they are working on and to invite ideas and collaborations. First, the lab is performing large-scale analyses to determine which RNAs and proteins are altered when huntingtin is knocked down. Second, they are extending these analyses to mouse lines in which huntingtin is genetically missing from tissues like fat and liver. Third, they are examining how huntingtin silencing affects DNA repair genes, and specifically somatic CAG repeat expansion, the phenomenon where a person's mutation becomes even larger in certain cells of the body and brain. Altered DNA repair mechanisms and the expansion of CAG repeats in certain tissues during an individual's lifetime are believed to influence age of onset, and these topics are discussed further below as they were explored in great detail during the 2018 HDF conference. Throughout his talk, Carroll strongly emphasized a desire to join forces with other researchers on these questions and beyond.

Datablitz Presentations

The Milton Wexler Celebration of life provides opportunities for graduate and postdoctoral scientists to share their work. The following young researchers were selected to showcase their studies through brief talks because of their impressive poster abstract submissions.

A neuroprotective role for Alfy in Huntington's disease

Katherine Croce, a graduate student in Ai Yamamoto's laboratory at Columbia University, discussed her work on "Alfy," an evolutionarily conserved protein that promotes the selective autophagy of aggregates. At 400 kd, Alfy is enormous, and it contains several domains that allow it to link the cell's basic autophagy machinery for protein clearance with aggregated proteins like mutant huntingtin. Loss of Alfy function leads to loss of aggregate clearance while leaving general autophagy intact, and overexpression of Alfy leads to the clearance of existing aggregated huntingtin protein (Eenjes et al., 2016; Filimonenko et al., 2010). Furthermore, the human GWAS study (which identified genetic modifiers of HD age of onset) uncovered a peak at Alfy's location in the genome. This led to the recent discovery that a mutation in Alfy is associated with a delay in age of HD onset for an average of 8-10 years, and sometimes up to 25 or more.

Katherine's research seeks to better understand why this delay occurs, and she hypothesizes that the Alfy variant leads to higher expression levels or greater bioavailability of Alfy in the brain. Her strategy is to work with two Alfy mouse models, an overexpresser and an Alfy variant knockin mutant, and to cross them with HD mouse lines (N171082Q and ZQ175). So far, she has validated her overexpression model through immunostaining and can rescue embryonic death of Alfy-null mice. As Katherine's graduate work unfolds, it will be interesting to learn how the disease-delaying mutation affects Alfy's function and availability.

Striatal projection neurons require huntingtin for synaptic connectivity and longevity

Long-term removal of huntingtin in the brain is a major concern in upcoming clinical trials, especially in cell populations that are vulnerable in HD. Caley Burrus, a graduate student in Dr. Cagla Eroglu's lab at Duke University, is studying huntingtin's role in striatal neurons that express D1 and D2 receptors. These cells are important within the "direct" and "indirect" striatal circuits that control mood and movement. Burrus created two genetic knockouts, one to delete huntingtin in D1-expressing neurons of the direct pathway, and one to delete huntingtin in D2-expressing neurons of the indirect pathway. She tested motor behavior and synaptic connectivity in these mouse lines at 2 and 10 months of age. Huntingtin knockout within the indirect pathway leads the mice to become hyperactive, but with no major deficits on the Rotarod task. Huntingtin knockout within the direct pathway causes mice to become hypoactive, and this line shows motor impairments on the Rotarod.

Overall, huntingtin deletion in both types of striatal neurons causes abnormal function, neurodegeneration, altered nuclear structure, and behavioral problems. Burrus's results demonstrate the importance and the differing roles of huntingtin in the direct and indirect striatal pathways.

Potent and long-term RNAi-based silencing of huntingtin expression in the CNS

Julia Alterman is a graduate student at the University of Massachusetts Medical School working in the laboratory of Dr. Anastasia Khvorova and collaborating with Dr. Neil Aronin and Dr. Marian DiFiglia. The Khvorova lab works on the design of siRNA drugs to improve their delivery and spread within different tissues. Specifically, Alterman focuses on scaffolds for the delivery of huntingtin-silencing siRNAs to the brain. They currently have an siRNA for the central nervous system that spreads widely throughout the brain within 48 hours of a single injection into the mouse ventricles. When injected bilaterally, the modified siRNA penetrates many anatomical structures, including the thalamus, striatum, medial cortex, and posterior cortex.

Alterman shared recent evidence that with this version of the drug, they can achieve potent and long-term silencing of huntingtin for up to 6 months in wild-type mice, and have so far shown similar potency and duration up to 3 months in the BAC-deltaN17 HD mouse model. This effect is dose-dependent: the higher the level of siRNA, the better the silencing. They have now worked out dosage and performed these experiments in 4 non-human primates, in which they see very good distribution throughout the brain and silencing in the entire cortex, hippocampus, caudate and putamen (the only regions tested). This is a remarkable achievement compared with results from other delivery methods. These experiments are an exciting demonstration of a potentially viable new approach to huntingtin-lowering in the human brain.

Novel computational approaches for signal extraction from striatal multi-color photometry recordings and evaluating high-throughput approach avoidance learning applied to HD mouse model

Alexander Friedman, a research scientist at MIT, is an electrophysiologist using cutting-edge techniques to better understand which networks of brain cells underlie behavioral changes in HD. He has developed an interpretable and robust new behavioral learning task and paired it with measurements of the electrical impulses between cells, using a new brain implant that can record from hundreds of neurons at a time. Friedman shared findings that aged ZQ175 HD mice have measurable difficulty learning and changing tasks, due primarily to a lack of motivation. These behavioral changes were linked to deficits in the connectivity between cortical and striatal cells. This type of emerging technology for assessing networks of neurons is extremely novel, and this work is the first time it has been tested in an HD model.

Friday, August 10th

Therapeutic Approaches

Huntingtin-lowering was a hot topic in 2018, but researchers don't have all their eggs in one basket. Speakers in this session shared additional therapeutic approaches, like high-efficiency screens of existing drugs to protect brain cells, the design of small molecules to change the behavior of RNA and even DNA, and a planned re-trial of an existing drug that has improved the lives of patients with kidney disease.

N6-Furfuryladenine is Protective in HD Models by Signaling Htt Phosphorylation

Dr. Ray Truant has spent a decade researching huntingtin's function as an oxidative stress response protein. One major recent observation in his lab is that huntingtin's response to oxidative stress involves relocation: the protein moves from organelles in the cytoplasm to the nucleus (DiGiovanni et al., 2016). For this translocation response, huntingtin requires a small chemical addition, in a process known as phosphorylation. However, the addition of this small protein tag to mutant huntingtin is hindered by the expanded polyQ sequence on mutant huntingtin. This means that in HD, huntingtin is hypo-phosphorylated and can't properly perform certain functions including the relocation response to oxidative stress. Therefore, the Truant lab has recently focused on methods of restoring phosphorylation to huntingtin as a therapeutic avenue.

One approach to this problem is to perform unbiased high content analysis screening of bioactive compounds, to uncover drugs that could phosphorylate huntingtin. Essentially this means treating HD cells with hundreds and thousands of existing drugs to see if any could restore the tagging process. Random image capture of these cells and machine sorting are used to give an unbiased view of which compounds affect phosphorylation of the N17 region of huntingtin. Truant's group can then map out groups of compounds that affect the system in different ways.

One compound that arose in this unbiased scanning process is called N6-furfuryladenine (N6FFA). It's actually a plant cytokine that people have been aware of for about 50 years. In humans, it's also the product of base excision repair of oxidized DNA, meaning that it's naturally produced when our cells mend DNA damage. N6FFA is not a kinase inhibitor, an oxidant, or an element of the protein clearance pathway; it is a pro-compound salvaged to a triphosphate. It is used as a phosphate donor by kinases including casein kinase 2 (CK2), which can phosphorylate huntingtin N17. Basically, Truant's group showed that N6FFA can act as a source for the extra chemical addition that mutant huntingtin needs to function better (Bowie et al., 2018).

To test whether it could be therapeutic, Truant's team injected N6FFA into the YAC128 mouse model of HD. They found that this treatment improved body weight and motor abnormalities. Low dose oral treatment did not result in similar phenotypic improvements, but it did lower levels of mutant huntingtin protein in the cortex. Furthermore, they found that N6FFA signaling occurs at sites of DNA damage: it colocalizes at speckles with DNA repair protein ATM, and when DNA damage was induced, they saw increased levels of N17 huntingtin

phosphorylation. Truant's overall model is that under conditions of DNA damage and low-ATP stress, N6FFA is salvaged to provide a triphosphate substrate (KTP) that signals CK2 to phosphorylate huntingtin. In HD, the signal between CK2 and huntingtin is not occurring at an efficient rate, and N6FFa can basically lend a hand to add the chemical tag that allows huntingtin to do its job more smoothly. Moving forward, the Truant lab is addressing a number of ongoing questions around reactive oxygen stress, DNA and RNA repair.

Design and Study of Small Molecules Targeting RNA Repeats

Dr. Matt Disney is an RNA chemical biologist, a researcher at the Scripps institute who studies how RNA folds into different structures and how it can be drugged. In recent years Disney has applied his expertise to better understand the structure of huntingtin RNA, including the CAG repeats. This approach is of great interest to the HD field, because it could reveal ways to interfere with huntingtin RNA using small molecule drugs. Whereas the promising ASO approaches in current clinical trials require invasive injections, it would be ideal to design an orally available small molecule to attack huntingtin RNA. Disney's lab focuses on rational, predictable approaches to find structures and sequences in RNA to target.

RNA forms characteristic 3D structures like loops, bulges, and "pseudo-knots," all motifs that could potentially bind small molecules. Previous work from computational biologists like Turner, Zuker, Tinoco, Mathews, and others has generated models that can predict how RNA sequences will fold up into 3D shapes. Disney combines these models with a knowledge of RNA network hubs, and then uses high-throughput drug screens to check which ones could actually bind and act as predicted. The lab does this by categorizing RNAs in a motif library, and identifying small molecules that bind to different RNA structures. There are nearly half a million entries in this online library, which can be used to probe interaction landscapes and determine all the RNA structures that could bind to a particular small molecule, with high or low affinity. They have used this method to identify small molecules that bind to myotonic dystrophy proteins, and they are now trying to advance their top targets towards therapeutics. They have validated the activity of these small molecules, which can inhibit protein binding, activate translation, or degrade mRNAs.

The key distinguishing factor from ASO approaches is that small molecules bind to a structured rather than an unstructured region of RNA. In type 1 myotonic dystrophy, a CUG repeat disorder of the RNA, Disney's team is trying to rescue a splicing deficit. To validate how selectively they are able to target a particular RNA in a cell or organism, they developed an experimental system called Chem-CLIP, which enables them to check that the drug is chemically stuck to the RNA it is meant to target. They are also working on mapping the exact binding sites, which has never before been achieved for small molecules targeting RNA. For myotonic dystrophy, they used these methods to show that their small molecule drug only interacts with the mutant allele of the RNA it was designed for (Rzuczek et al., 2017).

A recent study by the group, in which this small molecule was delivered to a myotonic dystrophy mouse model, describes improved splicing deficits, restoration of functional protein ClCn1, and improvement of phenotype and transcriptomic deficits, without affecting the healthy set of RNAs in the body overall. The Disney lab is also working on methods for tricking a repeating

disease RNA into synthesizing its own inhibitor, and making fluorescent sensors to check target engagement by microscopy. These precise new approaches open the door for HD researchers to collaborate and focus on huntingtin mRNA.

Slipped-CAG DNA-binding small molecule induces contractions of expanded CAG repeat tracts *in vivo*

Huntington's disease age of onset is linked with the length of a person's CAG repeat expansion: the longer the repeat, the earlier the likelihood of developing symptoms. Although there is variability, the therapeutic strategy of removing even a single repeat could theoretically delay onset for a few years. Dr. Christopher Pearson, a senior scientist at Toronto's Hospital for Sick Children, has studied the mechanism of somatic repeat expansion for over 2 decades, where individual cells in the brain and body develop even longer CAG repeats than the size inherited from the parents. In the striatum, more than 50% of cells develop somatic expansions, and cells with very large expansions are more likely to die (Kennedy et al., 2003). In 2010, the Pearson team urged that the scientific community focus upon repeat instability not only as the basis for human diseases but as a potential target for therapy for HD and other repeat diseases (Lopez Castel et al., 2010). The importance of DNA repair mechanisms in somatic repeat expansions have been recently revealed by several groups and consortia in genome-wide association studies (GWAS) of human DNA (Bettencourt et al., 2016; Genetic Modifiers of Huntington's Disease, 2015; Moss et al., 2017). Essentially those studies suggested that variants in DNA repair genes are major modifiers of both the age-of-onset and progression of HD. In 2013, Dr. Pearson's lab showed that naturally occurring variants in the murine DNA mismatch repair gene called *Msh3* can influence these somatic CAG expansions in HD mice (Tome et al., 2013). Strikingly, the *Msh3* gene variants regulate the MSH3 protein levels, where higher levels are associated with greater CAG expansions, and lower MSH3 protein levels are associated with lower CAG expansions. These murine observations may explain the human GWAS results.

Pearson explained that one of the causes of these expansions is slipped-DNA structures formed by the CAG/CTG repeats, and likened the shifted strands to a zipper not lining up properly. Slipped-DNAs are mutagenic intermediates of repeat expansions. Our cells can mutations through DNA repair, and Pearson's lab studies the repair genes that actively drive or protect against CAG instability. The hypothesis in the field is that when repair of one strand happens more efficiently than for the other strand, there is a bias towards the retention of looping structures in the DNA, ultimately leading to the expansion of a triplet tract. In mice that don't have the gene MSH3, there is a lower instance of somatic and transmitted CAG repeat expansions, and this delays the aggregation of mutant huntingtin.

Dr. Pearson presented an unpublished project where his team can modulate the levels of somatic CAG expansions. To combat the somatic expansion of CAGs, Pearson's lab designed a small molecule that binds specifically to long CAG slip-out DNAs. The small-molecule has so far been safe to use in cells. In HD patient cells with a CAG expansion of 180 or 43, the drug causes the long repeats to contract (although occasionally there are some expansions as well). Additionally, the drug is specific for the unstable expanded repeats, rather than for the non-

expanded allele, and cell division is not important for its mechanism of action. They tested the compound in 8-week-old R6/2 mice by infusing it into the striatum. When delivered four times over four weeks, there is a dramatic reduction of somatic CAG repeat expansions, without any cell death or neurogenesis. This is a promising therapeutic strategy that will require more exploration around the mechanism of action before it can be considered for human use.

TTI-0102, A Cysteamine Precursor for HD

Cysteamine is a drug with several indications and many mechanisms of action, including the successful treatment of cystinosis, the accumulation of the amino acid cystine in organs like the kidney. For cystinosis, patients need to take high doses of cysteamine and receive constant exposure; even missing a few hours can be very damaging to the kidneys. Patrice Rioux, the CEO of Thiogenesis Therapeutics, spoke about the potential to use cysteamine in HD, with the idea of promoting cellular and mitochondrial health and reduction of cell death. It has previously been evaluated clinically as a treatment for HD, but without major success thus far. However, with a redesigned trial and a new formulation, Rioux believes it could show promise for HD patients.

The design of the previous Raptor trial of cysteamine for HD was unconventional. For 18 months, participants received either 600 mg cysteamine twice a day or a placebo, and then for an additional 18 months, all participants received the drug. The dosing could not go higher, because the drug's odor of sulfur becomes so intense that the study can't be blinded. During the trial, participants were evaluated for movement and psychiatric symptoms at 0, 12,18,24, and 36 months. It was a small trial, and patients were allowed to be on other medications, including tetrabenazine. Although the drug didn't lead to any improvements overall, if they analyzed only people *not* taking tetrabenazine, there was a slowing of progression of motor symptoms (post-hoc analysis, p=0.03). Some participants experienced stomach-related side effects, but there was no significant difference in adverse events between the drug and placebo groups. People who took the drug for a full 36 months had an *almost* statistically significant improvement in total functional capacity (a measure of ability to function day-to-day) and some improvement on the UHDRS independence scale. However, the primary endpoint, UHDRS, was not met for all patients, and Raptor has not published these results.

Because the drug did show some promise, there may be another trial with a new formulation, if they can work it out properly. Essentially the compound has been redesigned, so that instead of being pure cysteamine, it is a cysteamine prodrug that gets broken down into cysteamine when it is consumed. A new trial would include 240 patients with stage 1 having three arms, placebo, 1200 mg of drug, or 1800 mg of drug, for the first 18 months, and then the second stage would involve another 18 months of treatment using the best dose from stage 1. There are reportedly previous trial participants who are strong advocates for the drug, and with testing there may be potential for this new cysteamine precursor to have therapeutic value.

Influencing Age of Onset

Recent years in the HD research community have seen a strong focus on the mystery of Huntington's disease age of onset. Large-scale human genomic studies have identified genes that influence the timing of symptom development, assays are being developed to guide trials of disease modifying drugs, and efforts have turned towards understanding and preventing somatic repeat expansions as a source of accelerated onset. During this session, five speakers touched upon topics relevant to onset of HD.

Genetic Modifiers of HD Age of Onset in the Venezuelan Kindreds

Large-scale studies of human DNA samples have identified tiny changes that can affect the age at which HD symptoms arise, known as genetic modifiers. Dr. Chris Ng, a postdoc at MIT in the laboratory of Dr. David Housman, spoke about the search for and characterization of these modifiers. HD researchers are fortunate to have 23 years of clinical data and samples from families in the Lake Maracaibo region of Venezuela. This rich resource includes 20,000 neurological and 8,000 cognitive exams, along with samples of blood from the same individuals, and in some cases, brain tissue. A decade of work within this data, including genome-wide association studies (GWAS), has revealed single-nucleotide polymorphisms (SNPs, one-letter DNA changes) in several genes that modify HD onset (Gayan et al., 2008; Genetic Modifiers of Huntington's Disease, 2015). These genes include FAN1, CNTN6, PCDHG, and Alfy.

A common problem with GWAS "hits" is that it is difficult to interpret the function of these SNPs when they occur in non-coding regions of the genome – bits of DNA that will never become proteins. To address this, Ng is improving on the fine-mapping of modifiers through increased marker density using sequencing and imputation. Basically, he is using novel techniques that create more genetic sign-posts to better hone in on minuscule changes. He is able to analyze 1000 genomes at a time and to combine different sets of human cohorts. These methods generate "clusters" of genes with different functions that are important for age of onset, and the team is collaborating with other laboratories (Such as Tom Maniatis at Columbia) to validate these findings in mouse models or through genetic knockout of clusters. They have found, for example, that their hit CNTN6 is upregulated in R6/1 mice, and that RRM2B expression decreases in the cortex.

The peak found near FAN1 is the most significant, but there are both positive and negative modifiers here (earlier and later symptom onset). Ng is working to validate these modifiers in animal models and collaborating with labs like Leslie Thomson's that can work with patient iPSC-derived neurons, so that they can begin to think about how to design targeted therapies. This may require some innovative approaches, because straightforward techniques such as delivering extra FAN1 can actually be lethal. For example, examining the DNA and its protein packaging, together known as chromatin, it seems that a non-coding RNA may regulate FAN1 expression. If this is the case, it could potentially be targeted with an ASO to increase FAN1 expression. Overall, this work will certainly help to identify and to gain a better understanding of the DNA regions that seem to regulate age of onset of HD symptoms.

Development of an Investigational Assay to Determine Htt Single Nucleotide Polymorphisms (SNP) Linked to the Expanded CAG Repeats in Huntington Disease

The vast majority of individuals with HD inherit one unaffected and one mutant copy of the huntingtin gene, and therefore their cells contain both healthy and toxic forms of the protein. Wave Life Sciences is developing ASOs that selectively target mutant huntingtin, while leaving the normal huntingtin relatively intact. Wave's chemistry also controls the chirality of the nucleic acid backbone, meaning that they can ensure the exact shape of the ASO by controlling the orientation of the non-bridging sulfur atoms in phosphorothioate oligonucleotides. Wave currently has two experimental drugs that are being tested in Phase 1b/2a clinical trials. Wave's approach differs from the total huntingtin-lowering approach favored by Genentech/Roche, and requires methods to identify and hone in on the mutant copy of the huntingtin gene. By finding tiny DNA changes (SNPs) that occur only on the mutant allele, they can design ASOs to lower the amount of toxic huntingtin protein and preserve the normal protein. Dr. Jaya Goyal from Wave spoke about the development of assays that will allow them to identify patients for this selective targeting.

Wave's investigational therapies are targeting 2 different SNPs that are associated with the mutant huntingtin allele. Previous research has shown, that together, these 2 SNPs are found in up to 70% of HD patients; with an additional third SNP, the percentage of covered HD patients would increase to 80% (Skotte et al., 2014, Pfister et al., 2009). They designed an ASO to target each SNP, and performed in vitro studies to confirm selective knockdown of the mutant huntingtin protein. They are now carrying out two simultaneous Phase 1b/2a trials, known as PRECISION-HD1 and PRECISION-HD2. The trials are designed to test safety and tolerability, as well as pharmacokinetics and pharmacodynamics, of the ASOs in participants with stage 1-2 HD. The studies will recruit 50 participants each, ages 25-65. As part of the assessment of eligibility for participation in the studies, Wave must check that patients have the SNPs of interest, and confirm that the CAG repeat expansion and SNP are on the same allele of the huntingtin gene. To ensure very high confidence, they have used PacBio long range sequencing that uses single-molecule real time (SMRT) sequencing. They validated their results in collaboration with the large private HD research foundation CHDI, and found 100% concordance.

Goyal explained that Wave paid special attention to verifying the specifics of every step of this assay, from extracting the RNA, through freeze-thaw stability, to confirm the phasing of the SNP. This fastidiousness serves to provide a high level of confidence that they are making the right eligibility call, so that potential participants can be assigned to the correct trial or to neither trial if genetically ineligible. To confirm the frequency of these SNPs, they conducted an observational research study in which they tested 203 patients across 7 sites in the U.S. Now the PRECISION-HD1 and PRECISION-HD2 clinical trials are underway.

Somatic CAG Instability as a Driver of Huntington's Disease Pathogenesis

Dr. Vanessa Wheeler's team at Massachusetts General Hospital is focused on somatic CAG repeat expansion and its influence on disease onset. During her presentation, she recalled that Peggy Shelbourne was the first researcher to demonstrate massive CAG repeat expansions in

individual cells of the human brain (Kennedy et al., 2003) and to hypothesize that cells with large expansions became vulnerable and died. Wheeler's lab has examined the brains of people with HD who had unexpectedly early or late onset of symptoms, and found evidence of a role for somatic repeat expansion in age of onset: later onset of symptoms tended to associate with less repeat expansion in the cortex, while earlier onset is associated with more expansion (Swami et al., 2009). Dr. Wheeler's group is now doing further patient-based studies to uncover modifiers of instability and to understand the tissue specificity of CAG repeat expansion. Examining multiple brain and peripheral tissues in three human samples has shown that variability between individuals is reflected in multiple tissues.

As was also discussed by Chris Pearson, mismatch repair genes can drive somatic CAG repeat expansion, with knockout of *Msh2*, *Msh3*, *Mlh1*, and *Mlh3* eliminating somatic expansions (Dragileva et al., 2009; Kovalenko et al., 2012; Pinto et al., 2013). The importance of these genes for age of onset comes from GWAS studies in humans. Thus, the prevailing hypothesis is that the DNA repair genes are working at the level of the repeat tract itself to modify disease.

To investigate this further, in collaboration with CHDI, Wheeler's team has studied "interrupted" repeat knock-in mice in which CAG alternates with CAA. The interrupted mouse lines still produce the amino acid glutamine, but the repeat is stabilized. An extremely thorough behavioral battery analysis performed by a company called Psychogenics revealed that the interrupted mice, whether they had 45, 80, or 105 repeats, had phenotypes that were slower to develop than the mice with equivalent numbers of pure CAG repeats. Furthermore, crossing interrupted repeat mice with an *Mlh1* knockout mouse did not improve nuclear huntingtin accumulation, although this is improved by *Mlh1* knockout in pure CAG repeat mice. These observations support the idea that the instability of the repeat itself is what's driving disease.

Wheeler's next area of interest is understanding exactly what kind DNA repair mechanism is driving this somatic repeat instability and suggests that instability may arise by a non-standard DNA repair mechanism. To investigate this, Dr. Wheeler and colleague Dr. Ricardo Mouro Pinto, have developed a knockout system using CRISPR-Cas9 and AAV viral delivery. They are first testing genes of interest through AAV-based targeting of the liver, another area of pronounced somatic repeat instability, and have shown that knockout of certain *Msh* and *Mlh* genes suppress instability, whereas knockout of *Fan1* causes increased instability. Moving forward they are working on identifying novel modifiers of repeat instability in mice.

Through inference from studies in mice and preliminary human data, Wheeler proposes that some people are slow expanders, versus fast expanders, due to their starting CAG length as well as other modifiers. All in all, the Wheeler lab is gathering strong evidence that somatic repeat expansion could be a useful therapeutic target.

The Role of DNA mismatch Repair in Huntington's Disease

Dr. Guo-Min Li of UT Southwestern Medical Center is focused on understanding the role of DNA mismatch repair in HD. This repair system ensures replication fidelity, correcting both base-base mismatches and DNA loop-outs generated during DNA replication. Key components of this system include a set of proteins called MutS and MutL, which have a variety of subtypes. For

example, MutSα (made up of subunits MSH2 and MSH6) recognizes base-base mispairs, and MutSβ (MSH2-MSH3) recognizes insertion-deletion loop-outs. MutSα and MutSβ share the common subunits of MSH2, which were discussed extensively during the conference as genetic modifiers of HD. MutSα is expressed at 10 times the amount of MutSβ, but when MSH3 is overexpressed, the balance shifts towards expression of MutSβ. This shift was found to promote CAG repeat instability in HD animal models (Manley et al., 1999; van den Broek et al., 2002).

The existing hypothesis in the field was that MutSβ led to instability of the CAG tract by sticking to and stabilizing a common hairpin structure formed by CAG repeats, which inhibits hairpin removal by a DNA repair machinery (Owen et al., 2005), such as the DNA hairpin repair system (Hou et al., 2009; Panigrahi et al., 2005). However, Li's lab did not find this to be the case (Tian et al., 2009). Instead, they hypothesize that MutSβ facilitates error-prone DNA synthesis. They reason that to have expansion occur, new DNA must be synthesized. They believe that DNA damage-caused strand breaks and a replication/DNA synthesis intermediate within the repeats can trigger repeats to form a hairpin in the nascent DNA strand, which is then preserved by the binding of MutSβ during DNA synthesis and effectively used as a primer by an error-prone DNA polymerase, leading to CAG repeat expansion. To test this, Li's group created a substrate and synthesis assay with a long CAG repeat structure in the presence of MutSβ, and then determined whether the hairpin was removed or preserved.

Ultimately, they found that a translesion DNA polymerase called DNA polymerase β (polβ), which is also an essential factor in base excision repair, is the culprit. The binding of MutSβ to the hairpin recruits polβ to the complex. Polβ then uses the hairpin as a primer to add several nucleotides to the 3′ -end of the hairpin, which further stabilizes the hairpin structure in the nascent DNA strand, resulting in hairpin retention and repeat expansion. The more MutSβ present, the more hairpins are retained. Li's lab also performed imaging and biochemical assays, both *in vitro* and *in vivo*, to show that MutSβ and polβ interact with each other (Guo et al., 2017; Guo et al., 2016). To understand whether MutSβ is the cause of oxidative stress-induced CAG repeat expansion, they treated cells with hydrogen peroxide, and found that this stimulated MSH3 expression and indeed led to a very rapid lengthening of the repeat tract. Conversely, cells containing a defective form of MutSβ in which the amino acid critical for ATP binding is mutated (E976A) show CAG repeat contraction (Keogh et al., 2017). Taken together, these findings suggest that MutSβ could be a good therapeutic target in HD.

Datablitz: Genetic variation in MSH3 that lowers its expression, ameliorates disease course and limits repeat expansion in HD and myotonic dystrophy type 1

Michael Flower, a PhD student in Sarah Tabrizi's lab at University College London, is working in collaboration with Dr. Darren Monckton of the University of Glasgow to study MSH3, a gene discussed extensively during the conference as a driver of repeat instability in HD. He echoed previous speakers, presenting evidence that genetic variations in MSH3 reduce somatic repeat expansion in myotonic dystrophy patients, and are associated with slower progression of symptoms in HD. Flower is trying to better characterize the genetic region encompassing disease-modifying mutations in exon 1 of MSH3. His particular focus is a SNP called rs557874766, which

occurs within a 9 base-pair sequence that repeats (imperfectly) within MSH3. To understand why MSH3 mutations can have such a positive impact on the course of disease, Flower analyzed the combined data from 218 patients in the TRACK-HD observational study with data from 255 patients involved in a myotonic dystrophy (MD) study. Within these cohorts, he studied different MSH3 variants and how they correlate with the amount of disease repeat expansion, clinical features and disease progression.

The reference sequence for MSH around SNP rs557874766 contains 6 sets of imperfect 9 base-pair repeats – that's what most people have. This genetic region is poorly conserved in mammals: mice have 2 repeats, gorillas have 3, chimpanzees have 4-5, and humans can have a range. Within the HD and MD cohorts, Flower found 15 different variants of the 9-base-pair repeat sequence, from 3 repeats up to 9. The most common variations they found were 6 repeats (60% of people), then 3 repeats (25% of people). In HD patients, the 3 repeat allele was associated with slower progression, later onset, and slower rate of somatic repeat expansion. Those homozygous for the 3-repeat allele had lower levels of MSH3 RNA in the blood. Taking this further, a collaborator in Cardiff, Dr. Peter Holmans, did a transcription-wide association study (TWAS) and found that increased MSH3 expression was associated with early onset and fast progression. Other GWAS hits, FAN1 and DSHR, came up in this search as well. Flower concludes that reducing levels of MSH3 may have therapeutic potential in repeat expansion diseases.

Other Neurodegenerative Diseases

The Hereditary Disease Foundation supports and provides a forum for discussion not only of Huntington's disease research, but other inherited CAG repeat disorders and additional rare familial diseases. This year's conference included research on spinocerebellar ataxia, XDP (a rare X-linked Parkinsonian syndrome found in people of Filipino descent), spinobulbar muscular atrophy, cancer, and the fatal childhood disorder spinal muscular atrophy. Work in these fields informs the greater body of knowledge about the degenerating brain, while scientific and medical successes provide new approaches and continued hope amongst researchers and disease communities with shared goals.

SCA1 - A Portal to PolyQ Pathogenesis and Therapy

Dr. Harry Orr of the University of Minnesota has devoted his career to studying spinocerebellar ataxia type 1 (SCA1), an inherited disorder caused by an expanded CAG repeat tract on the short arm of chromosome 6. SCA1 causes adult-onset limb ataxia and gait abnormalities, and like HD, is progressive and lethal after 10-15 years. It affects the cerebellum, pons, and medulla, and its major neuropathological feature is loss of Purkinje cells in the cerebellum. Many transgenic models have been created, primarily using the PCP2 promotor to drive expression of the mutant protein in Purkinje cells (Burright et al., 1995).

The ataxin-1 protein interacts with RNA as well as with transcriptional regulators, and the end of the protein (c-terminus) contains its most important stretch. Orr's work has helped to identify many important ataxin-1 motifs and interactors over the years. The interaction of ataxin-

1 with a protein called capicua is absolutely critical to pathogenesis and cell death, and S776 phosphorylation also plays an important role. Orr has developed a working model, in which ataxin-1 shuttles back and forth into the nucleus and interacts with capicua, which brings it to sites of transcription on the DNA. The end result is a big change in gene expression in a network they have named the "magenta gene network," leading ultimately to Purkinje cell disease. Using a statistical method called weighted gene coexpression network analysis (WGCNA), Orr's group has organized the cerebellar transcriptome into networks, and they have found that many signaling pathways are impacted by mutant ataxin-1 (Ingram et al., 2016).

More recently, an ASO has been developed by Ionis Pharmaceuticals that can lower the amount of ataxin-1 protein, similar to the htt-lowering ASO. A single intraventricular injection leads to improvement on motor tasks and extended survival of 154Q ataxin-1 knock-in mice. Interestingly, three injections at 5, 13, and 21 weeks stabilize the motor phenotype, but multiple deliveries don't extend survival longer than the single injection. Orr believes that in addition to brain cell vulnerability, there is also a peripheral component to SCA1. The ASO is effective at reducing differentially expressed genes in the cerebellum, but the pons and medulla are not as effectively corrected. There is a lot of overlap between the magenta model in the 82Q mouse, and the network that can be corrected in the 154Q KI mouse. In triple-injected mice, there is a reduction of ataxin-1 in cerebellar cortex and some deep nuclei, but no change in capicua-regulated genes. Ongoing work in Orr's lab involves testing the ASO in next-generation ataxin-1 mice, and attempting to move the ASOs into people.

A Retrotransposon-Associated DNA Repeat Expansion as the Cause of X-Linked Dystonia-Parkinsonism

X-Linked Dystonia-Parkinsonism (XDP) is a poorly studied disorder, an isolated genetic disease found on an island in the Philippines. It is X-linked, occurring only in males, and has features of both dystonia (stiffness) and Parkinsonism. It usually manifests in a person's mid 30s to 40s, beginning with lower limb dystonia, progressing to head, neck, and jaw problems, with eventual loss of speech and swallowing, and death 15-20 years after diagnosis. Dr. Cris Bragg from Massachusetts General Hospital spoke about the identification of a DNA repeat expansion that may be the cause of XDP. The XDP brain shows lesions in the striatum and cortex, similar in appearance to those of Huntington's disease, with medium spiny neuron loss. However, because there have been so few post-mortem brains collected, the neuropathology is largely unknown.

Previous genetic studies showed 7 different genetic markers for the disease in all patients, involving deletion and insertion of a mobile DNA element (Kawarai et al., 2017). One huge open question in this understudied disorder is to understand which of these genetic markers is functionally driving the disease process. Certain protein complexes involved in initiation of transcription were implicated, but there were literally no hypotheses in the field. Bragg's group and collaborators have been trying to build the XDP haplotype from the ground up. They sequenced the genomes of 800 people of Filipino descent, in both the Philippines and America, and the vast majority of people with XDP actually have up to 54 specific genetic variants. The net effect of this work was to narrow the causative region down to a gene called TAF1, which is

important for regulating the activity of most genes in the body. Within TAF1, they have now identified 13 variants, and are attempting to functionally characterize the XDP locus.

Bragg's lab is building up methodologies and tools to continue studying this genetic locus. They have created iPSCs, made NSCs, done direct conversion to other cell types as well, and were able to assemble the structure of TAF1. They have identified some transcriptional abnormalities and were able to use CRISPR to excise aberrant DNA and rescue affected cells. They have also performed genome-wide transcriptional profiling and identified a number of stress pathways that are initial clues to pursue regarding pathogenesis. Now they are examining a set of retrotransposons that are associated with XDP, with a hexamer repeat whose length is inversely correlated with age of onset (Aneichyk et al., 2018; Bragg et al., 2017). They are unstable and can expand and contract. Bragg thinks it's a speedbump in a large intron that could disrupt the way the gene is working. These are promising first steps towards developing treatments for a rare and devastating family disease.

Spinraza - The First Approved Drug for Spinal Muscular Atrophy

Spinal muscular atrophy (SMA) was discovered by Werdnig and Hoffmann in the 1800s, and the causative gene was identified in 1995. SMA occurs in 1/10,000 newborns, and today there are 35,000 patients in the US, Europe, and Japan. There are four types of SMA: Type 1 patients show infantile onset and usually die before 1 year, Type 2 has an onset of around 6 months with death in early adulthood, Type 3 patients exhibit later onset and close to normal life expectancy, and Type 4 patients show adult onset.

Dr. Adrian Krainer from Cold Spring Harbor Laboratory (CSHL) spoke about the first approved drug for SMA and its implications for Huntington's disease and related disorders. There is no repeat expansion in SMA, but instead there's a genetic duplication that comprises the gene coding for SMN protein, which plays a role in axonal transport and assembly of RNA splicing complexes. In patients SMN1 is mutated or missing, and SMN2 replaces it, sometimes with 2 and sometimes with 3-4 copies, with additional copies leading to less severe forms of the disease. The key difference between SMN1 and SMN2 genes is a single nucleotide in exon-7, which causes 80-90% of the mRNA to be truncated due to exon 7 skipping (Lorson et al., 1999; Monani et al., 1999). Therefore, CSHL and Ionis chose to focus on increasing the efficiency of exon-7 inclusion to reduce disease severity.

They did this using an antisense oligonucleotide (ASO) strategy, blocking repressor binding to restore the balance of exon inclusion. Dr. Yimin Hua at CSHL, in collaboration with Dr. Frank Bennett, tested many ASO designs in vitro, and the most unique and effective of these was chosen to be the drug Spinraza (nusinersen). In mice, Spinraza increased SMN protein levels, lengthened the mean survival, and preserved motor neuron architecture and motor function. After safety and delivery testing in primates, Ionis initiated a human trial and passed the torch to Biogen.

In this pivotal trial, children diagnosed with SMA received an intrathecal (spinal) injection every 4 months, and Spinraza was so successful that the trial terminated early, followed by rapid approval. Now, pre- or post-symptomatic treatment is leading to longer lives and improvements

over time. Dr. Krainer shared several videos of families whose babies and children are ongoing success stories of increased event-free living and overall survival. This is an extremely hopeful story for the HD field, because the Ionis (now Roche) ASO for HD uses a similar backbone and holds similar promise.

Phase separation of low complexity sequence domains in health and disease

For 30 years, low-complexity (LC) protein sequences have been a biological enigma. Steven McKnight of UT Southwestern studies these so-called "gibberish domains" in proteins that don't fold predictably and have unknown function. McKnight explained that LC domains are functionally critical, but if a protein has no 3D shape, it's extremely difficult to figure out how it works. Neuronal mRNA transport granules, also known as p-bodies, don't have a membrane around them, and yet they are organized. McKnight related this to LC domains in that they adopt a structure on a totally different basis from our classic concept of cellular organization.

One protein with an important LC domain is the FUS/EWS/TAF15 protein, which was originally identified in the context of cancer because it can undergo cancer-causing fusion events. However, the part of the protein that actually causes cancer has an unknown function. The FUS protein's sequence is known, but only 4 amino acids make up 83% of it, which is why there's very little structure and folding. In studying such LC sequences within the FUs protein, McKnight's group made a very useful experimental observation: LC sequences can transform from liquid-like droplets into a hydrogel state (Han et al., 2012; Kato et al., 2012). They used this knowledge to design gel-binding biochemical and RNA-seq assays that allowed them to work out which domains are important for allowing the cell to organize granules that retain particular mRNAs.

Visually, the hydrogels themselves were filled with unbranched polymers that resembled amyloids, but the LC domain polymers melted upon dilution, suggesting that they are reversible and do not maintain stability like prions. Understanding why required structural biology techniques – but x-ray crystallography and standard spectroscopy techniques would not work on unstructured proteins, so McKnight's team used a segmental labeling technique – labeling the front end of a protein in one bacterium, and the back end in another. In this way, they were able to uncover the structure of the FUS LC domain (Murray et al., 2017; Murray et al., 2018).

Ultimately, the difference between an LC domain and a prion is hydrophobic amino acids. There's only a single, weakly hydrophobic proline, in the structure, which means that there isn't glue to stick it all together. Furthermore, the cellular structures they're part of are also very labile. In LC domains and disease proteins, the stability versus flexibility comes down to particular amino acids that shift the balance of repulsive versus attractive forces. Essentially, these forces can drive the organization of LC domain polymers, and this is a novel way to think about a variety of biological structures. Although this research doesn't directly concern the huntingtin protein, these techniques and hypotheses could be extremely useful in better understanding the enormous, floppy huntingtin protein and how structural changes due to the low complexity of the glutamine tract might affect its many cellular functions.

Datablitz: Role of USP7 in the pathogenicity of SBMA

Spinobulbar muscular atrophy (SBMA) is the result of a CAG repeat expansion within the androgen receptor gene. Once testosterone binds to the androgen receptor (AR), it moves to the nucleus to regulate transcription. When the AR is mutated in SBMA, the lengthy protein tends to misfold, form aggregates, and cause toxicity and cell death. Dr. Anna Pluciennik, a research instructor at Thomas Jefferson University, explained that there are several hypotheses as to why this process is pathogenic. The mutant protein undergoes altered post-transcriptional modifications and may interact differently with its normal partners or disrupt the cell's protein folding machinery.

Pluciennik's work is focused on examining the effect of the polyQ mutation on the protein-protein interaction network. They use a simple pull-down assay and combine this with SILAC-based quantitative proteomics to measure the amount of protein partners of the normal and mutant protein. Using a cell model of SBMA they can recapitulate certain aspects of disease toxicity and aggregation, and measure soluble and insoluble protein by seeing how fast it migrates through a gel. Their proteomics assays revealed sets of proteins that interact either with the mutant or with the normal AR protein.

One of their top hits is the de-ubiquitinase USP7, which helps to stabilize proteins so they don't get dismantled by the cell. In all of their experiments, USP7 is enriched with soluble androgen receptor, and they have confirmed this in a variety of ways. They also find that over-expression of USP7 increases aggregation of the androgen receptor, whereas partial knockdown decreases aggregation. The addition of extra USP7 is beneficial because it increases ubiquitination of AR and leads to the toxic aggregates being broken down. These over-expression effects are abolished with a mutation at C223S, which drives ubiquitinase activity. They conclude that the AR protein is a substrate for de-ubiquitination by USP7, and are finding now that USP7 knockdown also rescues toxicity and reverses disease phenotypes in fly models. This could be a potential pathway for reducing the toxicity of mutant androgen receptor in a therapeutic manner.

Cutting Edge Topics

Each year the Milton Wexler Celebration of Life includes presentations on a variety of unconventional ideas and novel concepts that could feed the clinical pipeline for HD and other disorders. This year we heard about the potentially fragmented RNA message for mutant huntingtin, the effects of HD on the heart, the search for a blood-based biomarker in Taiwan, and a non-invasive way to deliver gene therapies from the bloodstream right into the brain.

The Incomplete Splicing of the Huntingtin Transcript

At a size of 67 exons and 180 kd, the HD gene and protein are enormous, and there are many different sized proteolytic fragments (pieces of the protein) that can exist in the brains of HD mouse models. Dr. Gill Bates's lab at UCL has identified 14 protein fragments the smallest of

which is an exon-1 huntingtin (HTT) protein, that is known to aggregate readily and be extremely toxic (Landles et al., 2010).

The Bates lab, in collaboration with David Housman's team, has shown that this exon 1 HTT protein is generated by incomplete RNA splicing and is produced in all knock-in mouse models of HD in many brain regions and peripheral tissues (Sathasivam et al., 2013). Furthermore, the level of incomplete splicing increases with the length of the CAG repeat, and the exon-1 fragments generated are pathogenic and prone to aggregation. More recently, they extended these studies to human tissue, examining 6 JHD cases, 12 adult onset HD cases, and 4 unaffected brains (Neueder et al., 2017). In JHD brains, they found conclusive evidence of incomplete splicing, whereas the levels between adult onset and HD were relatively comparable. They propose that toxicity occurs at the protein level and that the level of exon 1 HTT protein in a given cell would increase with levels of somatic instability.

Bates' team is now working on the mechanism of incomplete splicing. They hypothesize that a protein called SRSF6 binds to the CAG tract and recruits a splicing complex to the incorrect location, resulting in incomplete splicing. Andreas Neueder created a minigene cellular system to study how different elements of intron 1 contribute to incomplete splicing, creating short, medium, and long versions with varying CAG repeats. With the short and medium constructs, he saw no change in the level of splicing as CAG increased, but with the long construct, there was a massive increase in the levels of incomplete splicing as CAG increased. He defined the intron 1 sequences necessary for incomplete splicing and showed that this could be modulated by increasing or decreasing the levels of SRSF6 (Neueder et al., 2018). This minigene system has been converted into an assay to screen for small molecules that could enhance splicing and reduce the level of the exon 1 HTT protein.

In collaboration with Marie Francoise Chesselet, the lab is examining this *in vivo* in CAG140 and *Hdh*Q150 homozygotes bred onto the same C57BL/6J strain background, and they find that the level of incomplete splicing correlates with phenotype severity. They have established mouse embryonic fibroblasts (MEFs) from zQ175 knock-in mice. In these cells, approximately 70% of mutant huntingtin is incompletely spliced, making them a useful system for screening and prioritizing agents that lower the levels of huntingtin mRNAs.

Molecular Mechanisms Underlying Heart Dysfunction in HD

The HD mutation affects every cell in the body, but most HD research focuses on the vulnerable cells of the brain. However, there are many peripheral effects of huntingtin; for example, in people with HD, heart disease is the second leading cause of death. Individuals with HD can also have smaller hearts, carotid artery wall thickening, autonomic nervous system abnormalities, and abnormal EKG patterns. Dr. Dan Child, a medical student at the Children's Hospital of Philadelphia, studies HD's effects on the heart. His research focuses involve heart symptoms in humans, the role of mutant HTT in heart, the molecular mechanisms underlying heart phenotypes, and how these mechanisms contribute to pathology.

Many of the human features of HD and heart disease have been modeled in animals. For example, HD mice have smaller hearts (Child et al., 2018), and there are deficits in the mTORC1

pathway, a master regulator of growth and development. When the heart becomes stressed, it usually adapts through growth, but in HD mice, when the heart is stressed with a drug called isoprenaline, it is unable to compensate, leading to a harder working heart, more fibrosis, and earlier death. This suggests that in HD patients, heart disease could be having more extreme effects. Child would like to test this in human heart tissue from HD patients, and is actively searching for more samples.

Oligomeric mutant Huntingtin in the blood as a potential HD biomarker

Researchers at the HDF meeting hailed from all over the globe, and this year Dr. Yijuang Chern spoke about studying HD in Taiwan. She noted that it's very difficult to convince patients in Taiwan to donate CSF samples, so her team is particularly interested in developing an assay to measure mutant huntingtin in blood. The assay uses a novel antibody against a huntingtin protein fragment, and they have validated it using dot blot, Western blot, and immunohistochemistry. It's useful for studying huntingtin accumulation in mouse models, and detects aggregates better than the classic antibody EM48 in 4-week-old R6/2 mice.

Using an immunomagnetic reduction (IMR) assay, Chern's lab tested the antibody's ability to detect mutant huntingtin in samples of HD patient blood. In premanifest HD patients, they were surprised to see a much higher signal than in HD patients. Chern suspects that presymptomatic patients have more oligomeric huntingtin in their plasma. Patient EEG activity appears to correlate with levels of huntingtin in blood. Using statistical and machine learning techniques, they are able to distinguish controls from HD patients. Furthermore, the antibody assay they developed correlates with UHDRS score and disease duration. During the Q+A after this talk, researchers working on the CLARITY CSF collection project were eager to set up a collaboration where they would deliver human CSF samples so that this novel antibody could be tested further.

Noninvasive CNS-wide Gene Delivery with Engineered AAVs: Implications for HD

As gene editing technology continues to improve and evolve, delivery of drugs to the brain remains an important focus for therapeutic development in the HD field. Dr. Ben Deverman from the Broad Institute shared his work on harmless viruses called AAVs that can cross the blood-brain barrier and carry a drug to the brain (Chan et al., 2017). This is exciting because it means that they could potentially be delivered orally or by IV.

The advantage of AAVs is that they are non-pathogenic, safe, and can be customized for a variety of purposes. The capsid (outer shell) can be engineered for tissue or even cell-type specificity, to send them straight to a certain location in the body or brain. This engineering is accomplished through "directive evolution." This means that they begin with many thousands of virus types in a single animal, and see which viruses do what they want, such as transducing a particular type of cell like an astrocyte, or crossing the blood-brain barrier. Then they can selectively amplify those useful viruses by isolating them from the whole brain. Using this two-step amplification method, called CREATE, they were able to create a capsid that crossed over to the brain from the blood 100 times better than their original virus.

The Deverman lab is using various methods to restrict selectivity even further, like adding gene regulatory elements and incorporating miRNA binding sites. They can even use similar viral methods to visualize many neurons at once in great detail, like a "genetic Golgi" stain. They are currently collaborating with CHDI to explore virally-delivered zinc finger nucleases to target long CAG repeats and lower huntingtin. Deverman has introduced a wide array of novel viral methodologies to the HD community that he and others are applying to the development of genetic therapies.

Saturday, August 11th

Systems Biology

Systems biology is a branch of science that uses large biological data sets to computationally and mathematically model the phenomena happening inside of our bodies and brains. A growing body of HD research is exploring how large-scale data sets related to the expression of RNA and proteins during the course of HD can be mined for information that points to potential therapeutic targets.

Applications of deep learning and AI to understanding causes and finding treatments for HD

Deep learning and artificial intelligence methods allow computers to acquire diverse skills that can be applied broadly for tasks like object recognition and cancer diagnostics. Dr. Steven Finkbeiner's lab uses these methods to extract unbiased information from experiments in cells, enabling deeper analyses of data and aiding discovery of disease causes and targets (Christiansen et al., 2018). They have applied unbiased computer-based screening to identify neurons in a dish without immunostaining, or to predict when a cell is dying. This involves a genetically encoded calcium indicator and a database of 23,000 examples to train the computer on what it looks like when a cell is in the process of dying (Jeremy Linsley). After training, the computer was able to predict impending cell death with 99% accuracy, whereas humans can only get up to 80%.

Finkbeiner suggests that this technology could be used to move the field of neurodegenerative disease research towards a deep and varied definition of health and disease. Instead of a single endpoint, a healthy or a sick cell could be defined using multiple parameters. With an unbiased approach, it would be possible to perform large-scale drug studies in cells to determine which compounds cause a shift towards health. Or perhaps these techniques could be applied to stratify patients in a way that predicts their disease course or treatment response, generating a more predictive clinical pipeline.

The Finkbeiner lab has applied this technology towards "diagnosis" of spinal muscular atrophy (SMA) in a dish. They are also involved in Answer ALS, a collective project to create iPS cells lines from 1000 ALS patients, all of whom have robust clinical data. This will make it possible to analyze and train neural networks on a large dataset that can be matched with clinical outcomes. Additional ideas came up during the discussion following this talk. For example, AI

could be used to analyze video for more accurate diagnosis of HD and other diseases, or to analyze patients' cells as they simultaneously participate in clinical trials. In summary, deep learning is useful for running experiments, mining data, and drawing conclusions more accurately than most humans can!

Genome-wide CNS Screening to Identify *In vivo* Modifiers of Mutant Huntingtin Toxicity

Recent work in Dr. Myriam Heiman's lab at MIT has turned to genetic screening in mice, using viral manipulation of thousands of brain cells at once to discover which genes have the greatest potential to rescue cell death in HD. The technique involves injecting a genome-wide library of shRNAs, in the hundreds of thousands, into the HD mouse brain, where each shRNA infects a different cell and turns off a different gene. Later they examine the brain tissue to determine whether each shRNA is still there. The absence of an shRNA means the cell has died (so that gene made the effects of HD worse), while the presence of an shRNA means the cell has survived (so that gene could be protective).

They found several enhancers and suppressors of mutant huntingtin toxicity, corresponding to genes involved in chaperone function, oxidative stress, and cell death. They chose a few to validate further, including Nme1, a metastasis-suppressing gene. There is a small molecule Nme1 enhancer being tested in the cancer field, raising hopes that this method could point to viable existing therapies for treating HD.

HD, a matter of neuronal identity loss: role of epigenetic mechanisms

The HD brain undergoes widespread transcriptional changes, meaning that the disease causes different RNAs to be expressed at lower or higher levels than normal. Transcriptional changes in HD are progressive and most extensive in the striatum, the tissue preferentially affected, suggesting a causal relationship between transcriptional dysregulation and disease progression. The mechanism behind these changes, however, remains unknown. Dr. Karine Merienne from CNRS in Strasbourg, France spoke about the role of epigenetics in this process. Epigenetics refers to the study of the mechanisms that cause chromatin modifications, without changes in the DNA sequence. Epigenetic regulations are crucial during development and cellular differentiation, where they play a key role in the acquisition and maintenance of cell-type-specific identities. A more recent definition of epigenetics also includes chromatin changes that register, signal or perpetuate activity states, including neuronal activity states. In fact, epigenetic regulations have proven essential to the dynamic regulation of neuronal activity as well as processes such as learning and memory. Epigenetic mechanisms comprise histone modifications, which control the degree of compaction of chromatin structure. For instance, histone acetylation at gene regulatory regions, including promoters and/or enhancers (e.g. regulatory regions distal to promoters) promotes a relaxed state of chromatin, which facilitates the recruitment of transcriptional machinery and, in turn, transcription.

Using ChIP-sequencing (a genome-wide technique) on the striatum of HD mice (R6/1 and Q140 knock-in mice) and HD patients, Dr. Merienne's lab investigated epigenetic changes induced by the HD mutation. In particular, they focused on acetylation at lysine 27 of histone 3

(H3K27ac). H3K27ac is a mark enriched at transcriptionally active promoters and enhancers. Their results show that in the striatum of HD mice and HD patients, H3K27ac is selectively decreased at broad neuronal enhancers (so-called neuronal super-enhancers), controlling the expression of neuronal identity genes. Decreased H3K27ac at neuronal super-enhancers is an early event, correlating with down-regulation of their target genes (e.g. neuronal identity genes). Moreover, enhancer RNAs, a class of long non-coding RNAs transcribed from active enhancers and positively regulating their target genes, were also reduced in HD mouse striatum, particularly when originating from neuronal super-enhancers. These data indicate that down-regulation of neuronal identity genes in HD striatum results from impaired regulation of the activity of neuronal super-enhancers. They also used 4C-seq experiments to investigate chromatin looping on the striatum of R6/1 mice. Their 4C-seq data suggest that promoter-enhancer interactions may be impaired at super-enhancer-regulated genes in HD striatum.

Finally, to further explore the relationship between altered epigenetic and transcriptional regulations and behavioral deficits in HD, they trained 14 week-old R6/1 (and WT) mice in striatum-dependent learning task. R6/1 mice were impaired in this task. Their results indicate that learning-induced transcriptome and epigenome of R6/1 striatum are compromised, supporting the view of causal relationship between transcriptional, epigenetic and behavioral alterations in HD.

Dynamics of biological resilience systems in Huntington's disease

Neurons are incredibly resilient, and in the early stages of Huntington's disease, they are able to compensate quite efficiently. However, in situations of aging, toxicity, and disease, cellular compensation may be inefficient. Dr. Christian Neri's lab, at INSERM in Paris, is interested in cell survival systems in HD and the dynamic connections between cellular compensation and vulnerability, with the goal of promoting healthy neurons and glia. To this end, his lab uses machine learning techniques to understand how changes in gene expression affect larger genetic networks in HD. One technique in particular is called weighted edge network analysis (WENA), and it uses data from mice to see which genes are dysregulated and which interact during aging and disease. Neri's lab analyzes mice of different ages up to 10 months (around middle-age for a mouse).

The lab's analyses show that several stress response genes are central to the evolution of HD, indicating a mix of protective and pathological responses. In C. elegans (simple worms) with the human HD gene, they have examined which genes have protective, pathological, or no effect on touch response (Farina et al., 2017; Lejeune et al., 2012; Parker et al., 2005; Parker et al., 2012; Tourette et al., 2014; Vazquez-Manrique et al., 2016). They also revisited the role of miRNA regulation in HD knock-in mice and they precisely mapped how miRNA expression profiles affect their target mRNAs. On a global level, this analysis reveals that miRNA regulation poorly explains the dynamics of gene deregulation in the cortex and striatum of HD knock-in mice, with a couple of exceptions related to neuronal integrity, neurotransmission and cell survival.

To explore the dynamic connections between cellular vulnerability and cellular compensation, Neri's team is looking at what changes occur very early in development, during

neuronal differentiation, through a collaboration with Dr. Lisa Ellerby. This is where FOXO3, a transcriptional regulator, comes in as a protective factor – its activity is altered by wnt receptor ryk signaling and its targets can be reprogrammed to modulate response to cell stress induced by the HD gene. This means that FOXO3 could be protective in early human development. Finally, the lab is also studying DNA repair. In fact, human HD NSCs show more DNA breaks than control, and the kinetics of repair are altered. Neri's working model for promoting cellular compensation in HD is that neurons may be mounting a cell stress response that is critical for survival, but this response needs to be stimulated to remain efficient over time. Early developmental compensation is achieved through a response that is ultimately controlled by FOXO3. From a therapeutic perspective, Neri's proposed strategy is to help vulnerable cells compensate by targeting the stress response mechanisms that are engaged by FOXO3 in HD, and ultimately promote their resilience.

High-Throughput Functional Analysis Distinguishes Pathogenic, Nonpathogenic, and Compensatory Transcriptional Changes in Huntington's Disease

There are many transcriptional (RNA), proteomic (protein), and metabolic changes associated with Huntington's disease, and it can be difficult to understand whether these changes are pathogenic (disease-causing) or compensatory (disease-fighting). Dr. Juan Botas at Baylor College of Medicine studies whether these genetic alterations are causative versus protective by studying fruit flies. They manipulate potentially influential genes identified in mouse and human screens, and pair these manipulations with a simple fly climbing behavior assay.

The lab has focused on 312 "hits" from these screens and has worked to validate their importance for disease (Al-Ramahi et al., 2018). One example is a gene called CACNA1B, which is expressed at lower than normal levels in the brains of people with HD. When the expression of this gene is diminished in HD flies, the flies improve on their climbing ability, meaning that the lowering of CACNA1B must be compensatory. This observation can be followed up with statistical methods to determine which compensatory response pathways are disease modifiers, using techniques of network integration and pathway extension. For pathogenic genes, when the human condition is mimicked, the HD flies do worse behaviorally, and those genes driving pathogenesis can then be mapped as well.

Botas and his team have identified a general correlation between disease progression and downregulation of the GPCR/PLC/PKC signaling pathway. In collaboration with Dr. Boxun Lu, the next step is to validate these modifiers in HD patient iPSC-derived neurons. This involves using siRNAs to knock down different genes, followed by measuring cell health and death in the absence of particular ones. Manipulating some of their original 312 "hits" as well as additional genes identified through pathway mapping can also modulate mutant huntingtin levels, through activation of autophagy pathways. Using these methods, the Botas lab continues to integrate molecular and cellular data from models and humans with fast, in-depth analyses, to understand and target pathogenic and compensatory pathways in HD.

Cells and Circuits

The majority of HD research focuses on how HD damages neurons, but many other cells in the brain may contribute to the course of the disease. Two talks focused on the role of the brain's support cells, and another covered a lifelong effort to understand how particular brain circuits map to distinct HD symptoms.

Astrocytes in Huntington's Disease: An Analysis in BACHD Mice

Michelle Gray's lab is studying the role of supportive brain cells called astrocytes and how they are affected by or influence pathogenesis. Astrocytes participate in a variety of important functions, including energy metabolism, ion homeostasis, immune defense, synapse formation, neurotransmission and uptake, neurogenesis, blood flow regulation, and sleep. In HD, there is a lot of evidence that astrocytes behave abnormally, but it's unclear whether mutant huntingtin in astrocytes actually drives disease.

To explore this, Gray's team used the BAC-HD mouse model and created genetic crosses that ultimately reduced the amount of mutant huntingtin in astrocytes by 40% (Wood et al., 2018). They then tested the behavior and brain chemistry of these mice to see if astrocyte mutant huntingtin contributes to disease. Decreasing mutant huntingtin in astrocytes indeed had a modest benefit, leading to slight improvements at older ages in tasks measuring movement and depression. It also led to an increase in brain weight and postsynaptic protein levels, as well as rescuing electrophysiological changes in neuronal signaling. However, there appears to be no difference in the health of the astrocytes or the level of astrocyte proteins, with the exception of increased expression of a protein called αB-crystallin. Gray concludes that astrocytes expressing mutant huntingtin likely contribute to disease progression but do not initiate disease pathogenesis.

The lab is also exploring the widely-debated theory that glia communicate chemically with neurons. Interestingly, when the Gray lab genetically blocks vesicular fusion in mice, they see behavioral improvements. Essentially this means that stopping mutant huntingtin-laden astrocytes from communicating with neurons is protective, suggesting that there is chemical cross-talk between these cells. A discussion following Gray's talk involved some speculation about the recent clinical failure of laquinimod, a drug which purportedly had the effect of "calming" astrocytes. However, the mechanisms may be completely different than those under investigation by the Gray lab. Ultimately, astrocytes may be a more important player in HD than we suspect.

Glial Pathology and Treatment Potential in HD

One of the earliest signs of HD in the brain, even before symptoms develop, is hypomyelination – loss of the insulation on the "white matter" tracts that connect neurons to one another. This is a sign of glial pathology, since it is the brain's support cells that help to make up this insulation. Dr. Steve Goldman's lab, at the University of Rochester, has been developing glial progenitor cells (GPCs) for use in model systems and as therapeutic agents. These cells are

the developmental precursors to the brain's support cells and can grow and integrate into existing networks.

These GPCs are produced and isolated from human embryonic stem cells. To study HD, the lab compares GPCs derived from embryonic stem cells expressing mutant or normal huntingtin, and grafts them into mouse brains. This is one way to develop HD models, and to understand whether these types of transplants might have therapeutic value. The Goldman lab's analysis of the brains shows that the human cells can truly grow and incorporate into working networks of brain cells. When normal mice are implanted with HD GPCs, the mice perform worse on movement tests. In the striatum, these cells and networks become more "excitable," with less input required to achieve firing thresholds. Ultimately the striatum becomes too active, as occurs in HD.

The team performed RNA-seq analyses and found that the HD grafts caused genes to be differentially regulated in the striatum. One group of downregulated genes are involved in glial differentiation, while another set suppressed potassium channel signaling. They have also built a computational model to try and explain these changes. They believe that hypomyelination may begin with a gene called olig2, which helps to choose the cell's fate and push it towards becoming an oligodendrocyte (which myelinate neurons). When it is downregulated during differentiation, other genes called sox10 and MYRF also go down, and because of their importance for myelination, neurons become hypomyelinated. When Goldman's lab grafts human HD glia into the mouse brain, their development and myelination are slowed, but this can be rescued by overexpressing Sox10 or MYRF.

Grafting of human HD GPCs into mouse brain also creates problems with support cells called astrocytes, causing them to have delayed differentiation and maturation, and this affects their appearance and function later in life. Their structure is abnormal, with big gaps in their fiber arrays. Since these astrocytes help to manage the spaces in which neuronal communication occurs, some synapses end up uncovered and improperly managed, or the neural architecture grows strangely to adapt to the weird shape of the astrocytes. Goldman's team also analyzed protocadherins, proteins that define how cells contact and stick to one another, and found that these genes and their upstream regulators were disrupted in the astrocytes of brains with grafted HD GPCs.

From a therapeutic angle, Goldman is interested in whether HD phenotypes can be rescued by implanting normal GPCs into an HD brain. When they grafted normal human GPCs into very young R6/2 mouse brain, they were able to rescue potassium signaling deficits, delay motor behaviors, and extend survival (Benraiss et al., 2016). A more comprehensive behavioral analysis system also revealed improvements in movement and cognition in these HD mice. Adult symptomatic mice also showed improvements with GPC implants. The mechanism of these improvements appears to be frank replacement of diseased HD glia by normal glia, with reconstitution of normal synaptic anatomy and neurotransmitter uptake. On that basis, Goldman is trying to move this technique forward as a potential therapeutic strategy.

HD and the Human Brain: A Research Journey in Partnership with Families

Sir Richard Faull, director of the Centre for Brain Research at the University of Auckland, has built an extraordinary career studying neurodegenerative diseases, and delivered a talk on his historical and recent work. Faull emphasized the HDF and the wider research community's ultimate goal of helping families with Huntington's disease, and described his initial encounters with families and medical texts that led him to work in this field. Since the 1980s he has championed the formation of a vast interconnected network of HD families, scientists, and clinicians in New Zealand, enabling the establishment of an extensive and well-preserved human HD brain bank. This collection holds great meaning both for the HD research community and for families who feel that their donations help their loved ones to live on and to benefit future generations.

Faull's work has spanned many topics over the decades, from early descriptions of pathology, to identification of NADPH diaphorase loss in HD (Morton et al., 1993), to observations about cannabinoid and GABA receptor loss (Tippett et al., 2007). More recently his lab has focused on combining the rich clinical patient records with post-mortem observations to determine how patterns of cell loss in HD correlate with different symptoms. For example, cases with selective striosome (striatal patch) degeneration correlate with a strong mood phenotype, whereas cases with less prominent striosome degeneration correlate with a strong motor and moderate cognitive phenotype.

Through a double-blind, ten-year study of cell counts in many samples, his lab determined that patients with movement symptoms have massive loss of cells in motor cortex, and those with mood issues show loss of cells in the cingulate cortex. They have been able to correlate loss of many cell populations, including various types of interneurons, with reported clinical signs and symptoms. This painstaking mapping work continues to require the collaborative efforts of researchers and families all over the world.

Systems Biology Datablitz Presentations

Saturday's datablitz session featured short talks from students and postdoctoral researchers working on systems biology, different ways of statistically and computationally modeling biological systems. Topics ranged from synaptic loss and the secretome to localization and seeding of mutant huntingtin.

Microglia Mediate Early Loss of Specific Synaptic Connections in HD

Huntington's disease has a well-defined pathological course within the basal ganglia, but whether later and more widespread cell loss has a developmental basis is not fully understood. Daniel Wilton, a postdoctoral researcher in the laboratory of Beth Stevens at Boston Children's Hospital, is studying the timing and mechanisms of early synapse loss in HD. He explained that synaptic loss is a normal developmental process, as synapses are pruned to preserve the most relevant connections. This involves an immune process known as the complement system, in

which proteins called C1q and C3 localize to developing synapses, and then these complement-tagged synapses are recognized by microglia and destroyed (Liddelow et al., 2017).

Dr. Wilton proposes that synapse loss in HD, observed in both mouse models and humans, could be due to aberrant actions of the complement system. To address this he used AAV-based labeling (harmless, color-coding viruses) to visualize projections between the cortex and striatum in zQ175 mice. He found that microglia more often engulf and destroy synapses in HD brains than in normal mouse brains. Furthermore, genetic removal of complement receptors is a way to eliminate the search-and-destroy system, reducing corticostriatal synapse loss. To use this knowledge therapeutically, Dr. Wilton worked with Annexon Biosciences to create a novel C1q blocking antibody. When delivered to HD mice, the complement level decreased, and there was more preservation of corticostriatal synapses and brain structures. In one model, this led to a partial rescue of movement symptoms, indicating that attacking the complement system may be a viable approach to therapeutics for HD.

Identification of potential therapeutics from the secretome: ECM expression and function in HD iPSC-derived cell models

The extracellular matrix (ECM) is a network of secreted molecules that supports surrounding cells. Dr. Sarah Hernandez, working in Dr. Leslie Thompson's lab in UC Irvine, is studying how the ECM could be targeted therapeutically in HD. Because it's easy to access this biochemical scaffold, the ECM is highly druggable, and there's evidence that it is disrupted in HD. For example, an enzyme that breaks down proteins called MMP10 is upregulated in HD, and this can cleave huntingtin into smaller, more harmful fragments. When MMP10 is knocked down, this can increase the survival of HDQ111 cells (Miller et al., 2010). Furthermore, the HD iPSC consortium showed in 2012 that HD neural progenitor cells have decreased cellular adhesion, forming clumps less efficiently (Consortium, 2012).

Recent work from the Huntington's Disease Stem Cell Consortium suggests that ECM deficits could be modeled in HD. Dr. Hernandez is pursuing this type of technique to ask whether modulating ECM signaling could correct deficits in HD iPSC-derived neurons. She performed an RNAseq analysis which revealed dysregulation of ECM proteins, and is now collaborating with Dr. Paul Gershon to ask whether vulnerable striatal neurons have an altered secretome, and whether this is linked to observed changes in the length of neurites. In fact, medium spiny neurons show HD-related secretome differences, including a 9-fold change in neuroserpin, which plays a role in neuronal growth. Dr. Hernandez suggests that normalizing the amount of secreted neuroserpin could increase the amount of neurotrophic factors in HD brain, with potential therapeutic benefit. Moving forward she will continue to validate these molecular ECM targets, and to test them in animals.

Nuclear localization of Htt mRNA is specific to cells of neuronal origin

Mutant huntingtin mRNA is the focus of many huntingtin-lowering therapeutic strategies, but the actual role of the mRNA itself in toxicity (versus the protein) is poorly understood. Dr. Marie Didiot, at the University of Massachusetts Medical School, studies the localization and

metabolism of huntingtin mRNA (*HTT*). To detect, visualize and quantify *HTT* mRNA, she uses an advanced in situ hybridization method called RNAscope. In cells of neuronal origin, a large fraction of *HTT* mRNA is detected in the nucleus, but in non-neuronal cells like primary fibroblasts or HeLa cells it's mostly detected in the cytoplasm. Additionally, CAG repeat expansion impacts *HTT* mRNA subcellular localization in two ways: *1)* as the CAG length increases, there is more nuclear retention of mutant huntingtin in patient-derived primary fibroblasts and HD mouse models, and *2)* mutant huntingtin mRNA is found to form aggregates in the nucleus in B97-ΔN17 and in YAC128 HD mice. These aggregates appear very close to the nuclear membrane. Further directions involve exploring how nuclear deposits of huntingtin mRNA contribute to disease pathogenesis.

Mutant HTT seeding activity: a marker of disease progression and neurotoxicity in HD models

The toxicity of different lengths, locations, formations, and types of mutant huntingtin have been debated for many years. Anne Ast, a PhD candidate in the laboratory of Erich Wanker in Berlin, studies a non-physical aspect of huntingtin's toxicity: seeding activity. The team has developed a new type of experiment to visualize and measure the ability of different huntingtin fragments to self-propagate into larger, ordered structures. This technique is known as a FRET-based aggregate seeding assay (FRASE), and the central premise is that the faster and larger the huntingtin aggregates build, the brighter the fluorescence shines. Through assays of recombinant mutant huntingtin in a test tube, a "seeding curve" can be developed for different lengths and types of huntingtin. Starting with more seeds, or a higher concentration, shifts the formation of aggregates earlier (Ast et al., 2018).

Brain homogenate (essentially liquefied tissue) also shows seeding activity that can be detected even in pre-symptomatic HD mice, and this activity increases with disease progression. In R6/2 mice, seeding activity was detectable as early as 2 weeks old, or even in newborns when the tissue was prepared with sonification, a technique to process the tissue very finely. Ast investigated traditionally "soluble" and "insoluble" fractions of brain tissue lysate, and predominantly observed seeding activity in the soluble fractions. To visualize this phenomenon, she used immuno electron microscopy, and found tiny fibrils that appear to be responsible for the seeding.

In an HD fly model, it was seeding activity, rather than the amount of large aggregates, that correlated with reduced lifespan. Using an inducible genetics system, even a short pulse of mutant huntingtin expression caused flies to die early, and when the lifespan was short, seeding activity was high. All of these observations together suggest to Ast and the Wanker laboratory that the ability of mutant huntingtin fibrils to seed determines its toxicity. The group is currently extending this work into human brain tissue.

Building Better Models

Very soon after the discovery of the HD gene, researchers began to develop animal models to drive our understanding of Huntington's pathogenesis. 25 years later, the field continues to innovate, updating observations on standard models and developing new ones. From the classic R6/2 to novel mice, sheep, and a new pig model, this year's meeting highlighted our reliance on a wide array of animals for insights on human disease.

Modeling the Effects of Reducing Huntingtin Levels Using LacO-Modified HD Knock-In Mice

Dr. Scott Zeitlin's lab at the University of Virginia uses elegant genetic techniques to build many valuable HD models. Current questions in the lab revolve around huntingtin lowering. Lab members are working with regulatable huntingtin mice, in which the HD gene can be switched on and off. This allows the team to ask whether reduction of huntingtin is more effective at early ages, whether lowering normal huntingtin has adverse outcomes, and whether stopping mutant huntingtin lowering has therapeutic consequences.

One new mouse model uses a LacO system, which allows Htt to be turned on and off all over the body using antibiotics in the drinking water. This leads to about 50-60% reduction of huntingtin expression. Heterozygotes and homozygotes can be used to repress both forms of huntingtin, or just the mutant form. In young mice, repressing huntingtin delays a variety of symptoms, including the formation of aggregates, weight changes, and some motor and cognitive deficits. This approach is effective, whether total or only mutant huntingtin is repressed – and the earlier in life the gene is turned off, the better the outcomes. Conversely, if mutant huntingtin is suddenly introduced later in life, the mice far much worse than those who have had the gene on since birth. Zeitlin suspects that this is because their cells haven't had time to compensate.

The lab is also pairing these behavioral tasks with transcriptomic (RNA) analysis. Many genes are upregulated or downregulated in the cortex and striatum, but the most significant change was in MAPK signaling pathways. However, 30 days versus six months of mutant huntingtin repression had similar effects on the transcriptome, suggesting that both could have therapeutic value. A GAGE analysis showed that the widespread changes during repression are reversed during de-repression (turning the huntingtin gene back on). Furthermore, suppression of total versus mutant huntingtin had similar transcriptomic effects, with the exception of a small set of genes, and we don't know yet whether these lead to changes in behavior. These data are helping to reveal how different types of protocols in future clinical trials might affect pathological and symptomatic changes in response to huntingtin-lowering therapies.

Pig Models of Polyglutamine Diseases

Xiao-Jiang Li's laboratory has recently developed a pig model of Huntington's disease. The rationale for this project is that the size and development of large animal models is much closer to that of humans than mice. The pig has similar brain ridges and size, as well as structure and anatomy, and a closer disease pathogenesis. Additionally, pigs breed fast, by 6 months of age, with large litters, and a knock-in (KI) approach to creating genetic models is feasible.

Li's lab has recently characterized these HD pigs (Yan et al., 2018), which develop chorea, unlike many mouse models. They first created a 105Q/60Q N-terminal fragment model, but these animals die soon after birth. They instead used CRISPR on fetal fibroblast cells, did somatic nuclear transfer, and produced F1 and F2 generations to generate the KI pig. The mutation is now germline transmissible (it can be passed down through normal breeding), and the phenotypes they observe are stable across generations.

Similar to humans and mouse models, these pigs accumulate mutant huntingtin in the brain. They develop chorea or movement problems, as well as issues with the diaphragm muscles that can sometimes lead to death. Li's lab developed a pig treadmill assessment, on which the HD KI performs very poorly. These pigs also have reduced brain size, enlarged lateral ventricles, and loss of striatal neurons, similar to humans with HD. There are additional changes observed in the neurons and glia, and degeneration of axons in the striatum and globus pallidus. In summary, the KI HD pig is a novel model that accurately recapitulates many aspects of human HD. The lab is also working on pigs that model spinocerebellar ataxia type 3, and these animals exhibit ataxia, gait issues, and Purkinje cell loss.

What To Do When an HD Animal Model Takes an Unexpected Turn

Professor Jenny Morton has worked for many years with the classic R6/2 mouse model, and has also championed the development of HD sheep. At this year's HDF meeting, she shared some surprising observations and interpretations from past and recent work with these models.

The R6/2 was the first mouse model of HD, and it has been highly useful for its rapid behavioral decline and extensive pathology. However, it exhibits massive CAG expansion, fast onset, and a lack of neurodegeneration. Recently, Morton's lab found one R6/2 mouse that has a spontaneously contracted (shorter) repeat of 50 CAGs, right in the human adult range. Its siblings and sire have 250 repeats, but its offspring have 50. This mouse developed extensive pathology by the very old age of 3 years, but did not get sick. Its HD offspring are all fertile, don't develop disease, live longer, perform better on motor tasks, and breed better than wild type mice.

Concurrently, there is a surprising lack of phenotype in HD transgenic sheep; although many metabolic changes have been identified, the first sign of motor phenotype is that they stumble over obstacles – but not until seven years old, around middle age. These sheep and mouse observations have led Morton to think about the ways in which mutant huntingtin may actually confer a pre-morbid advantage on a mouse (or human). Years ago, she demonstrated that these mice perform better cognitively at a young age. Many other HD models show advantages as well: resistance to neurotoxins, longer lives, and longer reproductive periods.

This phenomenon occurs in a variety of adult-onset neurodegenerative diseases, and is an example of the phenomenon of antagonistic pleiotropy, when a mutation has both a beneficial and a detrimental effect on an organism's fitness (Carter and Nguyen, 2011). People with HD have lower incidences of cancer, increased fecundity, and higher IQ up to 41 repeats (Albin and Young, 1988; Lee et al., 2018; Murmann et al., 2018; Thion and Humbert, 2018). All of these

observations contribute to a larger body of knowledge indicating that there may be an evolutionary advantage to carrying a longer CAG repeat within the huntingtin gene.

Closing Keynote: New Tools to Study HD Pathogenesis and Therapeutics

Dr. X. William Yang of UCLA delivered a closing keynote that focused on three new tools developed in his laboratory to study HD. Three overarching questions govern his work: 1) Why is HD an age-related disorder? 2) Why are certain types of neurons so vulnerable? And 3) What is the role of misfolding and aggregation of huntingtin?

The first tool, called the BAC-CAG mouse model, was designed to address questions about the tendency for the CAG repeat mutation to become longer in different cells and tissues during aging. This phenomenon is known as somatic repeat expansion. It means that if a person has 42 CAG repeats in their huntingtin gene, there could be individual cells in their liver or brain in which the HD gene expands up to 45, 60, or even 100 CAG repeats. An emerging hypothesis is that this process could cause earlier onset of symptoms, so efforts are underway to understand how it happens and how it might be stopped. The BAC-CAG mouse can model this repeat instability in an age-dependent way: striatum shows increasing repeat expansion during aging. Furthermore, RNA expression analysis of these mice corresponds with findings in the Q140 and Q175 mouse lines. This model could be useful to the community for studying the instability of CAG repeats and how this phenomenon is affected by other genetic modifiers revealed in human and mouse studies.

Yang's second tool is a method of studying specific cell types in the brain by using sparse labeling, so that only a small percentage (1-5%) of neurons "light up," making their structure visible in lots of beautiful detail. This is a way for researchers to "see the trees, not just the forest," because instead of a dense tangle of nerve branches, individual cells can be distinguished visually. Using selective genetic techniques, Dr. Yang's group is able to make different populations of cells shine, making it possible to map the health and disease of spines and synapses. Then different statistical methods can be applied to such findings, to make predictions about the whole brain.

The third new tool is an assay to quantify and characterize "seedable" mutant huntingtin – the tiny clumps of toxic protein that attract each other to form larger masses. Yang's lab developed a high-throughput method for detecting mutant huntingtin seeding. They have found that HD patient CSF has seeding capabilities that are dependent upon the advancement of clinical disease stage, and the neuropathological grade, and that this seeding can be blocked with the heat shock protein DNAJB6 or mitigated by the addition of mutant Huntingtin antibodies.

These three tools are designed to address some of the most pressing questions in HD research, and Yang expressed his interest in collaborations with other researchers to continue exploring and making use of these resources.

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