

Hereditary Disease Foundation
Milton Wexler Interdisciplinary Workshop:
"Pipeline, pathogenesis and progress toward a cure for Huntington's disease"

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Abstract

Dr. Nancy Wexler, President of the Hereditary Disease Foundation, welcomed the workshop attendees and told them how hopeful she was for the future of science under President Barack Obama. Over the course of two days of discussion, workshop participants shared data and insight on topics ranging from the clinical aspects of Huntington's disease (HD) to current lead therapeutics in development, to new mechanisms of disease pathogenesis. Several areas were identified that required further research, and tool development, but participants remained excited and optimistic. Dr. Carl Johnson, Executive Director for Science of the Hereditary Disease Foundation, told participants that the challenge was to determine the targets that contributed significantly to the disease in humans; and how to validate these mechanisms and targets; and several ideas were put forward to address this. To start the workshop, participants met and spoke with a caregiver mother, and her daughter who has HD. They gave participants valuable information about their experiences with HD and with the wider HD community. Their frank account revealed the heartbreak and suffering that HD invokes, but also showed the courage and spirit with which both mother and daughter faced HD. Moved by the experience, the participants discussed the areas of research that were most pertinent for development of clinical treatments, including the growing amount of data on non-traditional neuropathological changes in HD, and disease progression in different populations of HD patients. Assay development, both *in vivo* and *in vitro*, and how to increase assay efficiency and sensitivity, were discussed in detail. Participants reported on the development of current lead therapeutics, covering a wide range of targets and technologies. Finally, participants discussed new targets and mechanisms for future development. Participants agreed that greater detail than ever before was now known about potential pathogenic mechanisms, and were very hopeful for the future.

Personal experiences of HD

To begin the workshop, the participants were introduced to a caregiver, and her daughter who has HD, and together they spoke about their experiences with the disease. The caregiver's husband had had HD and her three children were also affected.

The caregiver noted that that the HD community was much smaller than the cancer or AIDS community. In addition, she told participants that denial of the disease existed in many families. Many would rather not acknowledge the consequences of HD. For example, the caregiver told participants that her oldest daughter's first husband had filed for divorce when he sensed that she was developing HD symptoms. The caregiver described how her oldest daughter's children spent less time with their mother now. She said that she thought they were protecting

themselves, but it caused heartbreak for her daughter. Interactions with strangers were also difficult, due to the lack of understanding of HD and HD symptoms. She also said that affected members of her extended family did not go to neurologists when they began to show symptoms, as they did not want to reveal they had the disease. In addition, logistics made it very difficult to become involved in clinical trials and for many families it was difficult to keep up to date with scientific research. This was because many families did not have internet access, due to the extreme financial toll the disease exerted. The caregiver told participants that the only home that would take her son did not have a good reputation and that he had problems with clothes being stolen, and laundry.

The caregiver told participants that her oldest daughter was hopeful of being cured and that her son was in good spirits. Finally though, she was overcome by emotion as she told participants of her youngest daughter's current condition, and how this haunted her. The courageous testimony of the caregiver and her daughter, and their willingness to openly discuss their struggles was a moving start to the 2009 HDF Milton Wexler Interdisciplinary Workshop: "Pipeline, pathogenesis and progress toward a cure for Huntington's disease."

HD and the HD phenotype

Dr. Rosas, Massachusetts General Hospital, brought participants up to date on non-traditional aspects of HD neuropathology and phenotype. She told participants that she saw changes in Stage I and II HD patients' hippocampi that were as dramatic as in Alzheimer's disease. She noted that the motor, sensory, occipital and associated occipital cortices, as well as white matter, were all affected very early. The visual areas were affected even in gene carriers not yet showing clinical symptoms, she said, and she told participants that the temporal nature of neuropathology should be taken into account while treating HD. Dr. Bordelon of UCLA pointed out that the UHDRS could be fine-tuned to reflect the neuroanatomical changes. Rosas noted that HD was very heterogenous in symptom presentation and that she had older patients with similar repeat lengths to younger patients, but that progressed more slowly. Also, symptom progression in two of her patients had slowed down following cancer chemotherapy. Clinical histories and endstage pathology could be used retrospectively to examine disease progression, Drs. DiFiglia (Massachusetts General Hospital and Harvard Medical School) and Chesselet (UCLA) noted. Dr. Wexler observed that progression rates could depend upon the care of the patient. Dr. Hughes, Buck Institute for Age Research, noted that it would be important to determine if late onset patients also had long lived siblings. Dr. Yang, UCLA, added that there were a surprising amount of copy number variations in the normal human population, which could cause the different ages of onset. Dr. Finkbeiner, of the Gladstone Institute, UCSF, pointed out that the mechanisms underlying onset and progression may be different – he said that Dr. Muchowski's data on the kynurenine 3-monooxygenase inhibitors (see Therapeutics in HD; Preclinical Studies) suggested that there were ways to manipulate the processes individually.

Therapeutics in HD; Preclinical studies

Several targets that had shown beneficial effects when modulated in preclinical trials were discussed. The targets included CoQ10 and idebenone; profilin and Rho kinase inhibition; MLK (and JNK) inhibition; kynurenine 3-monooxygenase (KMO) inhibition; HDAC inhibitors; chaperone induction; reducing aggregation (C28); and knock down of huntingtin

(RNAi and antisense strategies). Several trials used treatments or drugs that are already in use in humans (CoQ10 and idebenone, Rho kinase inhibition and MLK inhibition). Others utilized leads not yet approved for use in humans (KMO inhibitors) and finally, some targets require further lead development (HDAC inhibitors, Celastrol, C28 and knock-down mechanisms).

Dr. Fischbeck, NIH/NINDS, spoke about the mitochondrial cofactor and antioxidant CoEnzyme Q10 (CoQ10) and a similar compound, idebenone. He explained that CoQ10 had a long hydrocarbon side chain, which impeded its CNS absorption, and that idebenone had a shorter side chain, with much better CNS absorption. Idebenone has been used in clinical trials, in Friedrich's ataxia in which it shows neurologic benefit, as well as in stroke (Phase III studies in Europe and US). He noted that idebenone had been tried previously in HD at a low dose, but that they were now using 10 or 15 times that dose, resulting in higher brain levels and good tolerance. Dr. Hickey, UCLA, said that they had tested CoQ10 in CAG140 knock-in mice. There were beneficial effects on behavior, with more beneficial effects at the lower dose but aggregates were not affected. CoQ10 impaired performance of wildtype mice on the rotarod. Dr. Chesselet said that similar impairment by CoQ10 of rotarod performance had been observed by Schilling et al. Dr. Johnson recommended examining several doses of idebenone and CoQ10 and determining effects on survival and cell death. Fischbeck noted it was important to correlate brain levels in mice with human data. Dr. Merry, Thomas Jefferson University, suggested infusing CoQ10 directly into the brain to address the low brain bioavailability, and Dr. Hughes noted that the low brain bioavailability of CoQ10 may underlie its relatively modest effects. Dr. Diamond, UCSF, then talked about Rho kinase inhibitors, which had been discovered in a cell-based aggregation assay. He explained that Rho kinase phosphorylated profilin, which then did not bind huntingtin. Following inhibition of the kinase, huntingtin-bound profilin showed reduced aggregation, possibly through sequestration or stabilization. One inhibitor, Fasidil, was already in the clinic, Diamond noted. His laboratory tested Fasidil and a related compound in different models of HD, with beneficial effects on motor tasks. No effect on aggregates or life span was found, however the concentration reached in brain may not have been enough to affect aggregates. Dr. Fischbeck said that his laboratory had found that profilin was specifically depleted in HD brain, in cell culture and in fly models. Dr. Raymond, University of British Columbia, said that at early stages in the YAC mutant mice, the amount of profilin was not altered, but they had not looked at phosphorylation. Moving to another target, Dr. Thompson, UCI, explained that her laboratory had shown through gene expression profiling in cells, that c-Jun N-terminal kinase (JNK) pathways were activated. She noted that the specific JNK inhibitor SP-600125 was protective in HD models. Based on this work her laboratory went on to investigate the therapeutic potential of CEP1347, a mixed lineage kinase (MLK) inhibitor. CEP1347 acts upstream of JNK, and had been used in a clinical trial for early Parkinson's disease, but in that trial it had not shown efficacy. However, in HD cell and fly models and a small trial in R6/2 mice, Thompson found protective effects. A full trial at Psychogenics is now underway. Dr. Muchowski of the Gladstone Institute, UCSF, noted that several compounds that showed protective effects in mouse models in different laboratories failed to show efficacy at Psychogenics. Participants suggested that this could be related to Psychogenics' environmental enrichment protocols, and thresholds for defining a therapeutic effect.

Dr. Muchowski then told participants of his progress with KMO inhibitors. His laboratory had found that if KMO was knocked out, huntingtin toxicity in yeast was reduced. KMO is in the

tryptophan pathway, which contains metabolites including the toxins quinolinic acid (QA) and 3-hydroxykynurenine (3-HK), he said. In R6/2 mice, they had found beneficial effects of KMO inhibition on a number of measurements of symptom progression, including life span; and neuropathological markers had correlated with KMO activity. *In vitro*, microglial inflammatory function remained unaffected by the treatment. Several KMO inhibitors had been developed for use in the clinic he said, however brain bioavailability was low. He had newer agents that showed beneficial effects in preclinical trials, but they still required pharmacokinetic and pharmacodynamic development. Dr. Davidson of the University of Iowa, noted that the microglial effect was intriguing in light of Dr. Don Cleveland's work where he affected glial expression of superoxide dismutase (SOD) in models of Amyotrophic Lateral Sclerosis and impacted progression of symptoms, but not age at onset.

Moving on to HDAC inhibitors, Dr. Hughes said that his laboratory had found a strong protective effect of HDAC 4 knock down on mutant huntingtin toxicity *in vitro*. HDAC 4 knockdown also slowed aggregate formation and overexpression of HDAC 4 accelerated aggregate formation, he said. However, the deacetylase activity of HDAC 4 is very low, he noted. He recalled that Dr. Gillian Bates had shown that knock-down of HDAC 4 was protective in R6/2 mice. Dr. Steffan, UCI, said that she had examined HDAC 4 separately, to look at post translational modification of huntingtin and found that overexpression of HDAC 4 increased SUMOylation of mutant huntingtin. At late disease stages, this could be detrimental and cause build up of SUMOylated proteins. She said that this agreed with Hughes's data, because knockdown of HDAC 4 would be protective in an end stage model. Dr. Johnson highlighted the importance of understanding the targets and isoforms important for pathogenesis in the HDAC pathways. Dr. Morimoto, Northwestern University, then brought participants up to date on celastrol development. Celastrol is an activator of the heat shock response and by binding to SH groups on proteins, it activates the oxidative stress response; mimicking low level protein damage. Celastrol is toxic however, and so Dr. Johnson surmised that more chemistry was required and Morimoto said that they were at the lead development stage. He said that he was looking for better activity. Dr. Housman, MIT, also noted that better chemistry was required in the case of C28, a compound selected for development from huntingtin aggregation cell-based assays. This compound had shown modest but significant beneficial effects *in vivo* he said. Dr. Muchowski noted that C28 was very close structurally to one of the KMO inhibitors although there were differences in the behavioral readouts that C28 affected, in comparison to the KMO inhibitors. The discussion then moved to RNAi and antisense knockdown strategies. Dr. Davidson said that the major question was if one could reduce both the mutant and wildtype alleles and still have beneficial effects. Following a single bilateral administration they had observed 70% knock down without any short term deleterious effects. She also said that Dr. Nicole Deglon had now examined brains from rats and primates for up to 12 months, with 80% knock down and no cell loss. With regard to mutant allele silencing, she noted that there were up to 5 SNPs that may represent 65-80% of HD patients and she is currently generating siRNAs to animals that will express these SNPs, to examine allele specific knock down. Moving onto antisense oligonucleotides (ASOs), she said that Dr. Cleveland's laboratory is currently examining the effects of peripheral administration of ASOs in R6/2 mice, with no deleterious effects thus far. Dr. Hickey said that at UCLA they have used ASOs targeted to the C terminus of the mouse sequence, in CAG140 knock-in mice. They had observed dose dependent knock down of mRNA and ISIS Inc. had shown that this knock down remained for at least 4 weeks following termination of infusion. Dr. Chesselet said that this

could translate to intermittent dosing. Davidson pointed out that it appeared that ASOs may be more active in glia. Chesselet remarked that the large knockdown of huntingtin expression was unlikely to be solely attributed to glia, and DiFiglia noted that huntingtin expression in neurons was greater than in glia.

Developing better targets

Dr. Wilson, University of Texas at San Antonio, told participants that analysis of protein networks could be used to the advantage of HD research, particularly in light of the complexity of the pathways involved. He said there were qualitative aspects of networks that could be used to predict their behavior, which could then be used in modeling the disease. Dr. Yang said this would be very important in order to prioritize the nodes that were important. Dr. Johnson reminded participants that the bottleneck in HD therapeutic development was the large number of targets and putative mechanisms. Dr. Johnson pointed out that many genes that were protective in HD worms overlapped with the genes that increased lifespan in aging screens in invertebrates. The challenge was to determine which contributed significantly to the human disease. It might not be possible to address all important areas of dysregulation if one treats too far downstream, he said. Yang suggested examining the importance of targets by overexpressing them and then knocking them out, in the context mutant huntingtin expression. However, he added this was not the same as pharmacological validation, which allowed for more range in target manipulation. Chesselet observed that many treatments improved several behaviors in R6/2 mice, but Dr. Housman added that the genetic targets of many of these treatments were not clear and Chesselet noted that it was unlikely to be one target.

Developing better tools

The group then discussed assay development, and in particular how to assay live cells *in vitro* and *in vivo*, as treatment proceeded. The group agreed that this area required better tools. For *in vitro* systems, Dr. Finkbeiner said that he was using antibodies targeted to different huntingtin aggregate conformers to predict cell fate, with the limitation that this was not yet possible in live cells. Dr. Muchowski added that they didn't have the technology to analyze those small conformers *in vivo*. Dr. Fischbeck added that Dr. Bordelon was getting at this with her PET imaging of aggregation in patients. Dr. Chesselet said that transcriptional dysregulation was present well before cell death, at times of early behavioral dysfunction. Dr. Raymond noted that they had found very early changes, including sensitivity to QA toxicity and increased NDMA receptor currents, in the YAC transgenic mice. Dr. Yang added that in the BAC HD mice, early electrophysiological changes were also present. Dr. Yamamoto, Columbia University, said that in the HD94 reversible mutant mice, hippocampal LTP was an early beneficial effect of knock down of transcription of the mutant fragment. Dr. Housman observed that some of these changes could be amenable to imaging and Dr. Diamond said that a mouse with several reporter genes could be very beneficial.

Chesselet noted that reduced climbing was one of the earliest phenotypes in HD mice, and it seemed to be resistant to therapeutic intervention, suggesting it had a high threshold. It was important to use behavioral tests that were early in onset and difficult to improve, she said. This could help to narrow down the long list of therapeutics. Diamond said he uses the retina as a system to analyze therapeutics, using ERGs and visual acuity and that the use of the internal

control eye reduced variability and vastly increased the power to detect treatment effects. Muchowski noted that it was unclear how the behavioral readouts correlated with changes in neuropathology and Yang said that for mice, this could be addressed by regional expression of huntingtin. He said that when he switched off mutant huntingtin in cortical pyramidal neurons in his BAC HD mice, he observed partial improvements in psychiatric dysfunction (forced swimming and light dark box), in rotarod deficits and in the number of striatal dark neurons. The group also spoke about the changes in peripheral tissues and Housman suggested overexpressing, and also knocking out, suppressors of mutant huntingtin toxicity in the peripheral tissues, in parallel to brain tissue, in order to validate the peripheral tissues as biomarkers.

Key pathways of toxicity

Several mechanisms of toxicity were then discussed in detail, including interference with the transcriptional coactivator PGC1alpha; synaptic activity, receptor localization and circuitry in the striatum; phosphorylation of S13 and S16 from the N terminal 17 amino acids of huntingtin; and the biophysical properties of huntingtin. Participants noted that some of these mechanisms (PGC1alpha activity and synaptic activity) could be targeted by drugs already in use in humans.

Dr. LaSpada (University of Washington) spoke to participants about PGC1alpha, a transcriptional coactivator. His laboratory had found that HD mice did not thermoregulate properly, due to interference with the activity of PGC1alpha. He is using an inducible PGC1alpha mouse, crossbred to N171 TG mice, to now examine this in greater detail. Upon induction of PGC1alpha, there was a significant improvement of the neurological phenotype in the mice, with no effect on survival. LaSpada said that overexpression of PGC1alpha in the brain eradicated aggregates. He said that they had not expected this latter effect, but that energy status was improved and this was important for protein clearance. LaSpada's group has now identified PPARdelta as an interactor, which is highly expressed in brain. They have found significant repression of PPARdelta transcriptional activation in StHdhQ111 cells. He said that the repression correlated with polyglutamine length and dose, and he noted that activators of PPARdelta were currently in use in the clinic. The discussion then moved to Dr. Raymond who spoke about her work on excitotoxicity and the vulnerability of striatal neurons to death in HD. The activity of NR2B containing NMDA receptors was increased in neurons from newborn YAC 128 mice, she said. She also described how the rate of new receptor insertion was faster in mutant neurons versus wildtype neurons. Forward trafficking brought more receptors to the membrane, but to outside the synapse; and this imbalance remained at late stages of disease she noted. At early stages of disease, when more corticostriatal glutamate is released, these extrasynaptic receptors could activate calpain and other proteases, inducing compensatory responses. She noted that memantine could block extrasynaptic receptors. Ideally Memantine, given early, would prevent the compensatory changes which could be detrimental to the cell in the longer term. Memantine has now been examined in YAC128 transgenic mice and Dr. Wexler pointed out that it was important to utilize the data from preclinical trials in mice to better inform the clinical trials in patients.

Then Drs. Yang and Steffan spoke to participants about the S13 and S16 sites in N terminal huntingtin. Dr. Yang described how he had generated mice with S-A (serine – alanine; phosphoresistant) or S-D (serine – aspartate; phosphomimetic) mutations. He noted that both

constructs rescued huntingtin knock-out embryonic lethality. The phosphoresistant mice showed deficits similar to BAC HD mutant mice. In contrast, he said that phosphomimetic mice showed no deficit in behavior, and no brain atrophy. Experiments such as these were critical to determine pathogenic nodes he said, and could be used for other sites along the protein, for example the S421 phosphorylation site or polyproline region. In her laboratory, Steffan had found that phosphorylation of the S13 and S16 sites regulated posttranslational modification of huntingtin. She said that the mutant form was not phosphorylated as well as the wildtype form, so it was not cleared as rapidly as the wildtype form. I κ B kinase (IKK) phosphorylates S13 and is activated in R6/2 mice, she noted, but it is not very efficient and may actually allow protein build up. Dr. Hughes said that it would be interesting to look at both young and old animals in clearance paradigms, as aging was detrimental to clearance systems. Dr. Yamamoto said that in the presence of mutant huntingtin, the proteasome or lysosomal machinery appeared to function normally. Yang said they had not yet examined turnover in the phosphomimetic or phosphoresistant mice. Drs. Thompson and Yang acknowledged that the right reagents, which work *in vivo* for mice and eventually for human research, would be critical for this kind of research.

Dr. Wetzel, University of Pittsburgh, spoke about the work that his laboratory was doing on the biophysical properties of short sequences of huntingtin. He said that the N terminal 17 amino acids of huntingtin form a compact coil; however expanded polyglutamine destabilizes the coil, which then aggregates very efficiently to form oligomers. He said that the polyproline region of exon 1 had a modest effect on aggregation whereas the N terminal 17 amino acids of exon 1 had a large accelerating effect on aggregation, whether it was N terminal to or C terminal to the first 17 amino acids of huntingtin. By altering the kinetics of aggregation he said, one could effect how the monomer is dealt with, because it is present for longer periods in time. Wetzel also noted that the role of HEAT repeats was to interact with other proteins, but in huntingtin, they could sequester and bind the N terminus, which could be prevented by huntingtin cleavage. Muchowski said that under atomic force microscopy, whether using exon1 or shorter sequences, the oligomers were spherical and variable in size but approximately 20-50nm in diameter. He added however, that that A β could form a whole range of different oligomers that were easily immunologically discriminated in a mouse, which nevertheless looked similar in EM and AFM. Muchowski said that he was now examining if caspase fragments and full length huntingtin aggregated in the same way as smaller fragments. He said the cleavage pattern of huntingtin in human HD patients was critically important in view of the shortstop mice, and the caspase 6 cleavage site. Dr. DiFiglia noted that she had been unable to find the caspase 6 cleavage product in control and HD fibroblasts, in which there was activated caspase 6. However, DiFiglia acknowledged that the fragment may not be stable and further analysis was required. She also noted the importance of electron microscopy for analysis of aggregate morphology and described how she had observed similar aggregate morphologies in cells overexpressing mutant huntingtin and in patient tissue.

Conclusion

The participants agreed that a lot of very challenging issues had been discussed. Dr. DiFiglia pointed out that workshop participants were pushing the limits of knowledge and the questions were becoming more difficult to answer, which she said was the right place to be. Many action points had been raised and they included: the need to study the families of late-onset HD

patients; to study CAG repeat length in patients by age strata; to examine cancer incidence in HD; to examine KMO activity in HD patient microglia; to test C28 in KMO assays; to complete preclinical trials for several agents in additional preclinical trials in full length models; to understand more fully the targets and mechanisms in the HDAC pathways; to understand the correlation of behavioral changes to neuropathological changes in the mouse models; to develop tools for live *in vivo* and *in vitro* monitoring of huntingtin aggregate intermediates and neuronal dysfunction; and to examine the structure of huntingtin itself. Participants agreed that many of these issues were not easy to address. However, it was confirmed that much progress had already been made and participants were excited and inspired by the extent and breadth of the topics and issues that had been broached, and the advancements that had been made in many areas.