

# **HD 2008: “The Milton Wexler Celebration of Life”**

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Forty years ago, Milton Wexler created the Hereditary Disease Foundation and its unique workshop program. Both have become models for tackling difficult scientific problems and have been instrumental in fueling and driving the search for a cure for Huntington's disease (HD). To honor Milton's 100<sup>th</sup> birthday, 350 scientists traveled to Cambridge, MA to attend the "HD2008: The Milton Wexler Celebration of Life."

Milton Wexler would have undoubtedly enjoyed the meeting, the stimulating new findings and the exciting prospects for the near future. The meeting provided a wide-ranging update on what is currently known about HD, as well as new perspectives on what can be done to translate this information into clinical benefits. Participants presented new data, new approaches, and new ideas for future research ranging from the social context of HD to the molecular underpinnings of the disease.

In addition to a wealth of specific data, several bigger picture lessons emerged. Two years ago, participants presented data indicating that intramolecular, intermolecular, intracellular, timing and genetic contexts play key roles in shaping HD pathogenesis (Chicurel, 2006). This year, context emerged as a major theme again, but it was greatly enriched by an increased level of understanding of HD at many levels.

The formation of mutant huntingtin aggregates, for example, was dissected more precisely, with the description of two pathways in competition with each other, separable by specific peptide-based inhibitors. Moreover, the complicated topic of histone deacetylase involvement in HD was brought into focus by new findings specifically implicating HDAC-4 as the major player in HD. And new correlations between neuropathological variations and symptom heterogeneity offered a new understanding of the clinical complexity of HD, as well as the possibility of monitoring different facets of HD and perhaps, in the future, allow the tailoring of treatments to match individuals' specific needs.

Also, this year's meeting was characterized by a divergence of technical advances and a convergence of ideas on several key issues. New tools and approaches were described that are filling in increasingly specific niches in HD research. HD models in a variety of species, including non-human primates, a growing number of candidate biomarkers that promise to substantially improve the efficiency of clinical trials, and specific assays to measure the efficacy of drugs targeting a wide range of pathological mechanisms were discussed.

On the other hand, there was a greater degree of convergence in the results of mechanisms of disease than described in previous meetings. Although there are still a vast number of pathological mechanisms associated with HD, participants discussed ways to face this prioritization challenge and there was an encouraging degree of convergence on some key issues. For example, the promise of targeting mutant huntingtin itself—either pre- or post-translationally—was reinforced by several independent lines of research. In particular, the modulation of cells' clearance mechanisms emerged as an especially exciting area of future research. Moreover, many studies converged on the importance of synaptic alterations and transcriptional dysregulation as key mechanisms of HD pathology. And an increased interest in systems approaches provided hope for a new level of experimental and conceptual integration in the near future.

## **Psychological and social issues**

***N.B. At the time of this meeting, the speaker, for issues of potential employment and insurance discrimination, chose to remain anonymous. Since then, he has bravely made his identity public. We welcome James D'Ambola's courage. Read the full transcript and/or listen James give his talk on the Hereditary Disease Foundation website – [www.hdfoundation.org](http://www.hdfoundation.org)***

### ***Past and present***

As commonly defined in the literature, HD is an autosomal-dominant neurodegenerative disorder characterized by movement abnormalities and cognitive and emotional disturbances. Few realize, however, the complexity of HD's associated psychological and social challenges. Two speakers are struggling with the presence of HD in their families and lives. One, an eloquent man who chose to remain anonymous, and Kevin Baker, a journalist and novelist who has written about HD, helped participants grasp the profound and complex psychological and social challenges of living with HD. The anonymous speaker, for example, described his father's transformation by HD from a loving, communicative, and responsible parent into an irritable, silent man who got himself into debt because of bad business decisions:

“When you add all of these changes up, it's a very ugly picture. HD had cut down a man in his prime, and replaced him with a shell of my father. Piece by piece, HD relentlessly carved him away, until he more resembled a child, than he did a grown man. It was very painful for me to be stuck watching helplessly, as bit by bit he was taken from me. In effect, I had to mourn my father because that man was gone. And yet I still had to see his shell every day. I still had to face the inhuman condition that HD left him in.” He also said, “This is what HD does, as it whittles away a person, it forces impossible choices under the most difficult of conditions.”

The speaker also described his own descent into a long, dark depression after leaving graduate school because of his emerging HD symptoms.

Both emphasized the importance and need for research. D'Ambola concluded his talk: “I am very excited to learn about your latest results and ideas. I thank you all for the work you have already done, and the work you continue to do. From the grad student who contributes various pieces of negative data (like myself), all the way up to the Principal Investigator compiling numerous publications, we are all part of the process that will ultimately produce the cure for HD. Each of us has already contributed our own grains of sand to a pile. When this pile of knowledge gets high enough, it will give us a cure. When that cure comes, as I have no doubt that it will, in the end, we will find that we were all part of one of the most powerful and incredible stories in the history of science.”

Baker emphasized the toxicity of simply contemplating the possibility of succumbing to the disease and helped explain why many patients go into denial, like his mother, who is now in the late stages of the disease but has yet to accept her HD diagnosis. Baker's mother was transformed psychologically by HD—once a kind, intelligent and responsible woman, she became angry and violent, began craving alcohol, and ended up demolishing the life she had worked so hard to build. Baker quoted his mother as once saying, “I have to go through the woods.” Living with HD, Baker noted, is indeed like walking through a very dark and tangled forest.

The social context of HD is also very important in defining HD's consequences. As explained by Alice Wexler, the experience of a disease is shaped not only by symptoms but also by community attitudes, cultural practices, and medical perceptions. Drawing on historical research from her new book *The Woman Who Walked into the Sea*, Wexler provided refreshing evidence that, at least in one case, HD families were respected and integrated into their community. Wexler showed the example of HD families living in East Hampton on Long Island—George Huntington's hometown—in the late eighteenth and nineteenth centuries. In this town, HD families appear to have been well-accepted, in fact, many HD family members were prominent citizens and leaders. Wexler speculates that their acceptance may have been related to their status as descendants of the founders of the town and their similar religious, social, and genetic heritage—in effect, genealogy trumped genetics. The presence of the sympathetic Huntington family of physicians may also have been a factor.

At the same time, Wexler showed how medical publications starting in the 1840s, and especially in the 1930s, promoted the view that HD families were consistently ostracized and disliked by their neighbors and that female sufferers in the past were often persecuted as witches. A Connecticut psychiatrist, P.R. Vessie, especially popularized the myth that several of the early New England witches had actually been women with symptoms of Huntington's, while their allegedly miscreant husbands and sons had also been afflicted with symptoms of this disease.

But according to Wexler, Vessie's grim portrayal appears to be limited and flawed, based on ahistorical assumptions and on genealogies that have long been discredited. Though focused mainly on East Hampton, her research suggested considerable variation among communities in the treatment of families with Huntington's, depending upon length of their residence, the size of the community, religion, ethnicity, and class factors. She also failed to find any link between Huntington's disease and any women accused of witchcraft in early New England, although this myth continues to circulate. She argued that Vessie's influential claims about the witchcraft connections and bad behavior of the earliest persons with Huntington's in America were more a reflection of the eugenic prejudices of the 1930s than of the historical realities of the colonial past, especially since Vessie himself was a proponent of “rigid sterilization.”

According to Wexler, such negative portrayals in the medical literature have contributed to the stigmatization of HD families, which the health care system continues to foster. As noted by Baker, people often feel pressure to conceal their genetic heritage to keep their medical coverage. Indeed the anonymous speaker chose to remain anonymous precisely because of fear of losing his job and, by extension, his health coverage.

### ***Future***

Several ways to address psychological and social problems were discussed at the meeting. For example, Baker highlighted the importance of giving HD families access to counseling, as he described how much he benefited from the counseling he received at Columbia after being tested for HD, and wished his mother and sister would have received similar help. In addition, Denise Brocklebank stressed the importance of keeping counseling guidelines current. In particular, Brocklebank presented new information on repeat instability, which she considered should be incorporated into the guidelines. As explained by Brocklebank, it is currently assumed that individuals with 40 CAG repeats or more in one of their huntingtin alleles will develop HD and, although individuals with fewer repeats won't develop the disease, the offspring of those with repeats between 27 and 39 are at risk. This increased risk is thought to be the result of

repeats expanding into the pathological range. However, as noted by Brocklebank, the risk has not been assessed in a statistically significant manner.

To address this issue, Brocklebank and colleagues developed software to trace instability and used it to prospectively examine 647 allele transmissions in the Venezuelan HD kindreds, the largest and most complete set of HD families available. The data revealed that repeats in the 27 to 35 range are highly stable. None of 69 transmitted alleles in this range expanded into a penetrant range. In contrast, 14% of transmitted alleles expanded into the symptomatic range in individuals having 36-39 repeats and 49% of alleles expanded in individuals having 40 repeats or more (28% of these alleles contracted). As noted by Brocklebank, it will be important to inform individuals who are tested of this improved prognosis for individuals in the 27 to 35 repeat range.

To address HD-associated mental issues, Steven Hersch noted that more and better evidence-based, palliative treatments are needed. Hersch pointed out that several symptomatic treatments are currently available, but they are insufficient. Anti-depressants, anti-psychotics, anti-epileptics and drugs for treating obsessive-compulsive disorder, for example, work well for treating HD-associated depression, irritability, psychosis, impulsivity and obsessive thoughts and concerns. Also, nutrition, in particular weight maintenance, has proven helpful for ameliorating both mental and motor symptoms. But treatments for other mental aspects of HD, such as impaired cognitive motor control and the decline of higher brain functions, are sorely needed. Hersch considered that increased research into cognitive enhancers may help provide treatments for the dementia, apathy, language and memory dysfunction associated with HD. In addition, he noted the need for research into drugs that not only improve movement disturbances, such as chorea, dyskinesia, rigidity, and bradykinesia, but drugs that can help improve cognitive motor control, including the regulation of speed, force, planning, and accuracy of movements, as well as the coordination of interactions with one's self and the environment.

## **Eliminating Mutant Huntingtin**

### ***Gene Silencing***

The most straightforward approach to treating HD is to eliminate, or at least reduce, mutant huntingtin. As reported in previous meetings, gene silencing technologies are being recruited to achieve this goal. The approach is particularly attractive because data from several groups, most notably Ai Yamamoto and colleagues, indicate there is a window of plasticity in HD during which knocking down the expression of mutant huntingtin reverses many of its associated pathologies. This year, Beverly Davidson updated participants on the status of these efforts by summarizing a recent HDF workshop on the topic. Davidson explained that important strides have been made using both RNAi technologies (using viral and non-viral delivery methods) and antisense oligonucleotides. Both approaches have been shown to ameliorate pathology and symptoms in animal models of HD.

However, several challenges remain. As described in previous meetings, the question as to whether allele specificity (targeting only the mutant form of huntingtin) will be necessary is still unresolved. Previous studies indicate that normal huntingtin is critical for embryonic development and the survival of neurons in the adult forebrain. Thus, several researchers, including Bill Kaemmerer, Neil Aronin, and Phillip Zamore, are developing allele-specific silencers using heterozygotic single nucleotide polymorphisms (SNPs) to produce siRNAs that distinguish between individuals' huntingtin alleles.

However, if a partial reduction of normal huntingtin is tolerable, strategies that don't distinguish between mutant and wildtype alleles are worth pursuing. In 2006, several participants reported encouraging findings suggesting that reducing wildtype huntingtin by gene silencing is safe. Two years later, the findings have held up, with no ill-effects observed up to 14 months after treatment in rodents. Davidson noted that a 50% reduction in wildtype huntingtin in mice striata is well tolerated. However, experiments in non-human primates have yet to be performed and the effects on other brain regions, particularly the cortex which may be more vulnerable as noted by Tony Reiner, must be evaluated. David Housman suggested searching for individuals with missense or nonsense mutations that knock out one of their huntingtin alleles. He also suggested investigating the complementary question: how much of a reduction in mutant huntingtin is necessary to achieve a significant clinical improvement?

Another safety concern discussed was the irreversibility of silencing treatments. Viral delivery systems result in permanent genetic changes and, as noted by Don Cleveland, oligonucleotides and siRNA molecules can persist within cells for long periods of time. Thus, it will be critical to select very carefully when and where to deliver these treatments. As noted by Davidson, this will be particularly challenging in light of the neuropathological and symptomatic heterogeneity of the disease (See *HD phenotypic heterogeneity*).

### ***Protein Clearance***

Enhancing the elimination of mutant huntingtin protein is another attractive therapeutic strategy. In 2006 only a few presentations addressed this approach, but this year participants considered it a particularly promising area of future research. Because cells possess endogenous mechanisms to eliminate misfolded proteins, it is appealing to attempt to recruit and boost these mechanisms to enhance the natural elimination of mutant huntingtin. Indeed, according to new data presented by Andrey Tsvetkov from Steven Finkbeiner's lab, cells are in fact more efficient at eliminating mutant huntingtin than the wildtype protein. The team used an optical pulse-chase approach in which they fused Dendra-2 to huntingtin exon 1 to measure the half-life of wildtype and mutant huntingtin species. Although the half-lives varied between neuronal types, polyglutamine expansion consistently destabilized huntingtin, decreasing its cellular half-life.

Another finding that makes protein clearance an attractive therapeutic target for HD is that wildtype and mutant huntingtin appear to be cleared with differing dependencies on the ubiquitin-proteasome and autophagy pathways. Although there are still many uncertainties about exactly how they are eliminated, particularly in their soluble forms, there is evidence that the contributions of different clearance mechanisms vary between the two. For example, as noted by David Rubinsztein, when macroautophagy—a bulk degradation pathway for long-lived cytoplasmic proteins, protein complexes, and damaged organelles—is blocked, soluble and aggregated mutant huntingtin accumulate, but wildtype levels remain essentially unchanged. Conversely, as noted by Paolo Paganetti, the inhibition of proteasomes primarily affects the clearance of wildtype huntingtin species.

In addition, modulating autophagy is attractive because it has been implicated in the clearance of a wide range of aggregate-prone proteins associated with several neurodegenerative disorders. For example, Rubinsztein noted that, in addition to mutant huntingtin, mutant ataxin-3 and mutant alpha-synuclein levels can be downregulated by inducing autophagy.

Stimulating autophagy has already proven to ameliorate the HD phenotype in animal models of HD. Using rapamycin, a mTOR kinase inhibitor that enhances autophagy, Rubinsztein observed decreased toxicity, reduced levels of mutant huntingtin in both soluble and aggregate

forms, and a significant improvement of the HD phenotype in flies and mice. In addition, several studies have confirmed that rapamycin is acting specifically on the macroautophagy pathway. Based on these findings, Rubinsztein's group has tested a rapamycin ester with pharmacological properties tailored for clinical use and has confirmed its penetration across the blood-brain barrier in mice, as well as its *in vivo* effects on mTOR and several proteins downstream of mTOR. As noted by Paganetti, determining dosage and frequency of treatment for this type of compound can be readily performed by monitoring the phosphorylation of mTOR targets.

However, because the mTOR pathway is involved in many cellular functions, compounds that affect this pathway often have undesirable side-effects. Thus, Rubinsztein is now searching for alternative inducers of autophagy, such as decreasing intracellular IP<sub>3</sub> levels. The approach is particularly attractive because there are several drugs currently used to treat brain disorders—eg, carbamazepine, valproic acid, and lithium chloride—which decrease IP<sub>3</sub>. The team has also developed a screen to identify new regulators of autophagy using a cell line that expresses mutant alpha-synuclein in an inducible manner. So far, they have identified a few drugs, including clonidine and an L-type calcium channel antagonist which, like IP<sub>3</sub> regulators, induce autophagy in an mTOR-independent manner. The drugs reduced aggregates and toxicity in flies and zebrafish expressing mutant huntingtin.

Rubinsztein noted that all these compounds act on targets in a common cyclic pathway: cAMP regulates IP<sub>3</sub> levels via EPAC and the small GTPase RAP2a, which activate phospholipase C-epsilon. IP<sub>3</sub> mediates calcium release from the endoplasmic reticulum which causes calpain activation. Calpains cleave and activate G<sub>sα</sub>, which then closes the cycle by regulating cAMP levels. In addition to their potential value as individual therapeutic agents, these compounds offer the possibility of regulating autophagy in an additive manner by combining them with mTOR-dependent drugs used in low doses to reduce their negative side-effects.

Additional efforts to identify compounds that boost mutant huntingtin clearance were presented by Paganetti's group. In particular, Andreas Weiss described a high-throughput assay using time-resolved fluorescence resonance energy transfer (TR-FRET) to identify compounds that decrease soluble mutant huntingtin. The assay relies on two anti-huntingtin antibodies, one labeled with a donor fluorophore and the other with an acceptor fluorophore. When the antibodies are brought into close proximity by binding to a huntingtin molecule, excitation of the donor leads to energy transfer to the acceptor, which causes it to emit at its characteristic wavelength. Background fluorescence can be readily separated from the experimental signal because the donor fluorophore is chosen to have a very slow decay rate so the experimental signal is long-lived compared to background signals.

To run the assay, a neuronal cell line that expresses mutant huntingtin inducibly is incubated with test compounds, lysate buffer and antibodies are added, and the resulting fluorescent signal is measured. Using an automated system, Weiss noted they can screen 70,000 compounds a day. Pre-screening of a 10,000 compound library yielded encouraging results indicating the assay is sensitive (femtomolar range), reliable, and reproducible over a large dynamic range. Currently, the team is screening a 1.5 million compound library and has identified 2000 compounds that decrease mutant huntingtin in a dose-dependent manner. The hits are being triaged and genomic screens will be used to identify the compounds' corresponding targets for future chemical optimization. As noted by Weiss and Paganetti, the assay may also have other applications in which monitoring mutant huntingtin levels is useful (e.g., to assess the efficacy of RNAi or chaperone-based treatments, or as a clinical biomarker for disease progression – see *HD Biomarkers*).

The potential value of these efforts is further supported by recent findings indicating how autophagy may be compromised in HD. As noted by Rubinsztein, excitotoxic insults, which have been implicated in the disease process (see *Synaptic Alterations*), elevate intracellular calcium which may then result in reduced autophagy based on the above mentioned cAMP/ IP<sub>3</sub> pathway. In addition, recent data from Scott Zeitlin's lab suggest that wildtype huntingtin may play a regulatory role in autophagy that is impaired by polyglutamine expansion. Zeitlin's team found that deleting the polyglutamine stretch in wildtype huntingtin ( $\Delta$ Q-htt) enhances motor performance and extends lifespan by approximately 18%. In addition, heterozygote mice carrying a mutant huntingtin allele with 140 CAG repeats fare better with a  $\Delta$ Q-htt allele than with regular wildtype huntingtin. Their behavioral phenotype is improved and there's a significant decrease in neuropil aggregates and lipofuscin, an undigestible waste product resulting from organelle autophagy. Monitoring several markers of autophagy, Zeitlin observed that the  $\Delta$ Q-htt heterozygotes appear to have elevated levels of autophagy and, in particular, huntingtin neuropil aggregates are cleared more effectively as indicated by the increased co-localization of p62 with the aggregates. Because the active form of mTOR appears unchanged in these mice, Zeitlin suspects the apparent induction of autophagy is mTOR-independent. Based on early studies with a huntingtin conditional knockout, Zeitlin suspects that wildtype huntingtin is not an autophagy protein per se, but can act as a regulator of the process, perhaps through its role as a scaffolding protein.

To make the most of these converging lines of evidence pointing at autophagy as a promising therapeutic candidate, several open questions must be addressed. Safety issues must be carefully examined considering that targeting a general clearance mechanism in a chronic manner is likely to have side-effects. Yet another consideration is that, as reported by Tsvetkov, different neuronal types appear to process mutant huntingtin differently—e.g., mutant huntingtin has a shorter half-life in cortical cells than in striatal cells. Thus, deciding when, where and how much to modulate autophagy will require careful examination.

Sorting out how different forms and fragments of huntingtin are eliminated and their relative importance will also be important to help focus efforts directed at clearing mutant huntingtin, noted Paganetti. It is possible that, in the long run, targeting huntingtin's post-translational modifications will yield safer, more specific therapies. Joan Steffan, for example, presented data indicating that the phosphorylation of huntingtin by the I $\kappa$ B kinase complex (IKK) influences the protein's clearance. Using phospho- and acetyl-specific huntingtin antibodies, and point mutations that mimic these modifications or lack of them, Steffan monitored the location and clearance of different huntingtin species in cultured cells and striatal slices. In summary, her data suggest that pro-inflammatory stimuli, associated with IKK activation, may induce the phosphorylation (serines 13 and 16) and acetylation (lysine 9) of both wildtype and mutant huntingtin, and promote the proteins' clearance by proteasomes and lysosomes. Steffan suggested that IKK phosphorylation may facilitate clearance by knocking huntingtin off membranes so that it becomes more accessible to cytoplasmic clearance systems. In the later stages of the disease, however, this displacement may be deleterious. With the impairment of lysosomal and proteasomal clearance systems due to aging, and perhaps due to mutant huntingtin's own toxic effects on autophagy and proteasome function, displaced mutant huntingtin can travel into the nucleus where its toxicity may be enhanced by caspase fragmentation, particularly by caspase-6. (IKK may also stimulate caspase cleavage of mutant huntingtin by promoting the degradation of the pro-survival protein Bcl-xL, see *Fragmentation* below).

Steffan is now examining HD mouse brains and William Yang has generated a BAC mouse model with point mutations in serines 13 and 16 which promise to help test Steffan's model in vivo. In addition, Steffan is investigating the role of selective autophagy in the clearance of modified huntingtin. The autophagy proteins LAMP-2A and ATG-7, in addition to ubiquitin ligase CHIP and chaperone Hsc70, appear to be involved.

### **The Different Forms of Huntingtin**

The growing numbers of options for reducing mutant huntingtin protein highlight the need to characterize and understand the origins and effects of its different forms. This understanding may prove useful not only for designing ways to eliminate mutant huntingtin, but for advancing other avenues of therapeutic research. Huntingtin is a large protein that can be modified in many ways, including proteolysis, ubiquitination, SUMOylation, phosphorylation and acetylation. In addition, it can adopt various configurations, including several different aggregate forms. Furthermore, its polyglutamine stretch can vary in length within different brain regions because of somatic repeat expansion. New insights into the form, function, generation, and regulation of these huntingtin forms were presented at the meeting

#### ***Phosphorylation***

Several of huntingtin's functions and effects appear to be regulated by phosphorylation. As previously mentioned, IKK phosphorylation seems to be important in determining huntingtin localization, clearance and toxicity. Moreover, as reported by Frederic Saudou, huntingtin phosphorylation influences axonal transport. Previous findings from Saudou's team indicated that wildtype huntingtin facilitates microtubule-dependent axonal transport, in particular the transport of brain-derived neurotrophic factor (BDNF) which is required for striatal cell survival. This function appears to be mediated by huntingtin's interaction with molecular motors, including dynein, kinesin, and, through its association with huntingtin-associated-protein-1 (HAP1), dynactin. Polyglutamine expansion of huntingtin results in an increased association of huntingtin/HAP1 with dynactin, which leads to detachment of the complex from microtubules, and decreased BDNF transport.

New findings by the team now indicate that the phosphorylation of mutant huntingtin at serine 421 can reverse this transport defect and restore BDNF release, implicating it as a potential therapeutic target. Expressing mutant huntingtin species with point mutations mimicking phosphorylation at serine 421, the researchers observed neuroprotection and normalized microtubule-mediated transport in vitro. They also found that IGF-1 increases the velocity of vesicle transport in vitro in a manner dependent on the phosphorylation of serine 421.

To establish the function of wildtype huntingtin phosphorylation, the researchers also examined wildtype huntingtin's binding of molecular motors with and without serine 421 phosphorylation and characterized the movement of vesicles under these distinct conditions. Interestingly, their data indicate that the phosphorylation increases kinesin, but not dynein, binding to microtubules. Consistent with this observation, the researchers observed that when huntingtin serine 421 is phosphorylated, most transport is retrograde, whereas when huntingtin is not phosphorylated, most transport is anterograde. As noted by Saudou, this is the first description of a protein that regulates transport directionality.

Other phosphorylations may also play important roles in defining huntingtin function and toxicity. For example, as described by Ray Truant, huntingtin contains motifs recognized by

protein kinase C and creatine kinase II that mediate *in vivo* phosphorylation as assessed by protein affinity chromatography and mass spectrometry. These modifications appear to regulate nuclear entry, aggregation and toxicity. Truant's group is currently screening compounds in search of regulators of these phosphorylations.

### ***Fragmentation***

Another important post-translational modification is fragmentation. The generation of toxic mutant huntingtin fragments has long been implicated in HD pathogenesis. Yet much remains unknown about the environmental signals and cellular pathways that regulate this process. Data presented by Paul Patterson offer new clues about this regulation, indicating that DNA damage, which is known to occur in HD neurons, stimulates huntingtin cleavage through a pathway regulated by IKK $\beta$ . To dissect this pathway, the researchers relied on pharmacological and genetic regulators (shRNA knock down of IKK $\beta$  and overexpression of Bcl-xL). They used etoposide to induce DNA damage in cultured human neurons and observed that activation of IKK $\beta$  coincides with the induction of caspases 3 and 6 and subsequent proteolysis of Htt. Silencing of IKK $\beta$  expression blocks these events. DNA damage promotes IKK $\beta$ -dependent reduction of Bcl-xL, which may be the underlying cause of caspase activation. IKK $\beta$  phosphorylates Bcl-xL, which is known to promote its degradation. Compensatory expression of Bcl-xL also prevents the activation of caspases and cleavage of Htt by DNA damage. Patterson added that other stresses—such as glutamate-mediated toxicity, aging, stress, inflammation and oxidation—also activate IKK and thus are likely to contribute to huntingtin fragmentation. Moreover, the process may be further enhanced by positive feedback: huntingtin fragments containing exon 1 can activate IKK $\beta$  by binding the IKK $\gamma$  subunit. This can promote persistent IKK $\beta$  activation and accelerate the cleavage of full-length Htt. Also, IKK activation increases interleukin-6 (IL-6), which may lead to enhanced IKK activation due to IL-6's role as a key mediator of inflammation. Patterson is now investigating the effects of IL-6 and IL-1 $\beta$  on the pathway.

To better define the fragments resulting from huntingtin proteolysis, Andrew Tebbenkamp from David Borchelt's lab used antibodies that recognize different epitopes of huntingtin. Confirming previous results from Yvon Trotter's lab, Tebbenkamp identified an N-terminal fragment (cp-A) associated with nuclear pathological aggregates and established it as being N-terminal to amino acid 115. To define the fragment's cleavage sequence, the researchers systematically replaced ten amino acids at a time in the vicinity of amino acid 115 and confirmed that the proteolytic site is between amino acids 105 and 114, as previously described. In addition, they observed that the charges of adjacent amino acids (85-115) are also important for protease recognition.

To assess whether cleavage of huntingtin occurs as a cascade in which one proteolytic fragment serves as a substrate for another, Tebbenkamp also examined the cleavage of huntingtin fragments generated by calpain and caspases which terminate between amino acids 450 and 600. He found that, although these fragments are capable of generating cp-A and cp-B (a fragment spanning amino acids 146 to 214), they do so very inefficiently. Also, these cleavages do not occur in a manner dependent on polyglutamine length. Thus, caspase and calpain fragments do not appear to be precursors of cp-A and cp-B. Future studies should help determine whether cp-A and cp-B are generated directly from full-length huntingtin or whether they arise from other fragments. Tebbenkamp also emphasized the importance of determining the relative toxicities of the different fragments.

## ***Aggregation***

Aggregation is another post-translational process that importantly affects mutant huntingtin's function and toxicity. As described by Paul Muchowski, huntingtin is a dynamic protein that can assume different conformations and form many different kinds of aggregates. Several studies have described mutant huntingtin filaments and oligomeric structures, but Muchowski added there are many other configurations that can be observed with the three-dimensional capabilities of atomic force microscopy, including annular and ring-like structures. The particular aggregate forms observed depend on the conformation of individual huntingtin molecules, as well as their concentration.

Muchowski noted that antibodies are a powerful tool to begin dissecting the structures of these aggregates and their effects on cellular function, as well as to develop therapeutic strategies against specific aggregate forms and perhaps even track disease progression. Working with antibodies developed by Paul Patterson and Steven Finkbeiner, Muchowski has observed that antibodies can effectively differentiate between many huntingtin forms and have correspondingly distinct effects on aggregation and toxicity. Consistent with data from Patterson's lab, Muchowski reported that even antibodies that ostensibly recognize the same epitope (e.g., MW1, MW3, 3B5H10 etc. which all recognize the poly-glutamine tract), can recognize different populations of aggregates located in different subcellular locations. In addition, these antibodies have widely varying effects on aggregation. Structural analyses of some of the antibodies bound to their antigens have revealed that the polyglutamine epitope is recognized in different configurations by different antibodies. For example, the polyglutamine stretch appears to be in a beta-sheet compact configuration when bound to the 3B5H10 antibody, but not when bound to the MW1 antibody. Interestingly, the two antibodies are drastically different: while MW1 enhances the process of aggregation, 3B5H10 completely arrests it.

As noted in previous meetings, the therapeutic possibilities of using antibodies, or intracellular antibodies known as intrabodies, to target toxic huntingtin forms are attractive. Several antibodies, including MW7, have been found to decrease cell toxicity and some are being used to develop therapeutic agents. The 3B5H10 antibody, for example, has been found to stabilize monomers, prevent aggregation and dissolve fibrils, implying that monomers and fibrils exist in an equilibrium which can be manipulated with antibodies. Muchowski cautioned, however, that *in vivo* studies will be very important to determine which antibodies have true therapeutic potential. Given the many forms of huntingtin aggregates, simple aggregation inhibitors may not work. Also, it will be important to distinguish causality from correlation. For example, although 3B5H10 staining is predictive of striatal cell death as reported by Finkbeiner, the toxicity of the antibody's target remains unknown. The 3B5H10 antibody may be recognizing a toxic precursor, rather than the toxic species itself.

One intrabody that appears to have beneficial effects *in vivo* was described by Xiao-Jiang Li. His team used an adenovirus vector to express an intrabody that preferentially binds to mutant huntingtin (derived from the mEM48 monoclonal antibody) in the striata of R6/2 and N171-82Q mice. Bilateral expression of the construct resulted in a dramatic reduction of neuropil aggregates and a significant improvement in rotarod performance. The findings illustrate the potential for using intrabodies to ameliorate the HD phenotype, as well as intrabodies' potential as a tool to help differentiate huntingtin's toxicity in the cytoplasm versus the nucleus, since intrabodies have access only to the cytoplasm (see below).

Important strides have also been made in understanding the process of huntingtin aggregation. Summarizing several studies in his lab, Ron Wetzel described two major

aggregation pathways in competition with each other, and explained how polyglutamine expansions can facilitate aggregation directly, as well as through the destabilization of adjacent huntingtin domains. The first pathway, described almost ten years ago, is mediated by simple polyglutamine sequences. It is a nucleation-dependent process, involving a monomeric nucleus and cyclic rounds of elongation leading to the generation of amyloid-like aggregates.

In contrast, the second pathway depends on the 17 amino acids in huntingtin's amino terminus and is more complicated, involving intermediate structures. Because there is no energetic barrier to overcome initially, the aggregation process starts off very quickly in a nucleation-independent manner. A transition phase follows, characterized by the formation of oligomers harboring N-termini at their core, as revealed by the N-termini becoming increasingly resistant to trypsin cleavage. At this point, a new, more rapid phase of aggregation is nucleated, characterized by the formation of amyloid-like aggregates. In this new class of aggregates, the polyglutamine stretches move into the core, as assessed by a gradual decrease in MW1 staining, and the amino terminus also acquires a more rigid structure. As a mature beta-sheet-rich amyloid structure emerges, the aggregates continue to grow in the normal amyloid fashion, by monomer addition (monomer's still make up 80% of the population at the time nucleation occurs). Although the second pathway is mediated by huntingtin's amino-terminus, it is influenced early on by the polyglutamine stretch. Based on previous reports indicating that the polyglutamine stretch can destabilize or disrupt adjacent domains, Wetzel hypothesized that it might help unfold the amino terminus and facilitate aggregation. To test this idea, Wetzel measured the distance between the first and seventeenth amino acid in exon 1 molecules using FRET. As predicted, the FRET distance increased with polyglutamine repeat length. Based on these studies, together with nuclear magnetic resonance analyses and computer simulations, Wetzel reported that the amino terminus appears to normally exist in a compact "coil" state that has no stable structure, resembling a fluid "molton globule" of flickering structural elements. With longer polyglutamine repeats, this compact structure is disrupted allowing the formation of oligomers. (It is important to note, however, that the amino-terminus has also been reported to adopt an alpha-helix structure under some circumstances in vivo, as noted by Ray Truant. In this configuration, it appears to promote the aggregation of small huntingtin fragments and is important for the localization of the full-length protein to the endoplasmic reticulum.)

Distinct peptides can selectively inhibit each of the two pathways: a polyglutamine sequence perforated by prolines inhibits the first pathway, and an N-terminus peptide comprised of huntingtin's first 17 amino acids (NT-17) blocks the second. As expected, the inhibitors have additive effects when mixed together and applied to mutant exon 1. Wetzel also has evidence supporting the existence of two aggregation pathways in vivo. Using PC12 cells expressing mutant huntingtin exon 1 fused with green fluorescent protein, Wetzel's team observed two populations of aggregates that differ in their dependence on microtubule integrity for their formation and stability. Large, cytoplasmic aggregates depend on microtubule integrity, while punctate nuclear aggregates and small cytoplasmic aggregation foci that seed amyloid fibril growth, appear to be formed via separate, microtubule-independent pathways. Wetzel speculates that the microtubule-dependent aggregates may represent products resulting from the aggregation pathway mediated by huntingtin's amino terminus, while the others are products of the pathway mediated by polyglutamine. Interestingly, when the microtubule-dependent pathway is blocked using a microtubule depolymerizing agent, a sharp increase in the other aggregates is observed, suggesting the two pathways are in competition with each other.

Another distinction between aggregate forms is their subcellular distribution. Nuclear inclusions have long been associated with HD pathology, and new data presented by Brian Wilburn from William Yang's lab bolstered their potential importance. Wilburn and colleagues generated a transgenic BAC mouse model of Huntington's disease-like 2 (HDL2), a disorder which may help identify HD mechanisms of pathology because it is both clinically and pathologically very similar to HD, but is caused by a different mutation and a different gene. The HDL2 mutation is a CTG repeat expansion within a variably spliced exon of junctophilin-3, a junctional membrane complex protein involved in the regulation of calcium flux. As pointed out by Russ Margolis's team, multiple pathways may lead to HDL2 pathogenesis, including loss of function of the junctophilin-3 protein, toxicity of the sense RNA strand, and/or toxicity of the antisense strand, either via its altered regulation of sense strand expression or because of its translation into an expanded polyglutamine protein. In the BAC model, Wilburn presented evidence for two possible pathogenic mechanisms, toxic CUG RNA and a novel antisense CAG transcript encoding a toxic polyglutamine protein. Given the clinical similarity of HD and HDL2, this dual pathology may be particularly relevant to disease development (It is important to note, however, that Margolis's team has found evidence for RNA toxicity in HDL2 tissues, but no evidence for polyglutamine expanded proteins. See below for more information.)

Despite the focus of many studies on nuclear inclusions, cytoplasmic aggregates also seem to play an important role in HD pathology, as noted by Xiao-Jiang Li. All mouse models of HD have neuropil aggregates, and analysis of the first transgenic monkey model of HD (see *Large animal models*) revealed abundant neuropil aggregates in swelling neuronal processes. As mentioned above, Li used a mEM48 intrabody to target neuropil aggregates specifically. Using subcellular fractionation analyses, he observed a reduction in synaptic huntingtin accumulation and an increase in huntingtin fragments, suggesting that the intrabody promotes degradation. And as previously mentioned, when the adenovirus was injected bilaterally, rotarod performance improved significantly. Reasoning that neuropil aggregates may cause at least some of their damage by disrupting the ubiquitin-proteasome system (UPS), Li used GFPu constructs to monitor UPS function in neurites. To target GFP to synapses, he fused synaptic proteins (e.g., SNAP-25, PSD95) and GFP coding sequences, and placed the GFPu/RFP reporter constructs under the control of a synapsin promoter. When the researchers expressed the construct in primary neurons from an HD knock-in mouse they observed an age-dependent increase in GFP fluorescence, reflecting a progressive decline in UPS function.

It is also important to consider, that not all aspects of HD pathology may be mediated by one or more forms of mutant huntingtin protein. As noted by Dobrila Rudnicki, it is possible that sense and/or antisense transcripts of the mutant huntingtin gene may also cause toxicity. Rudnicki noted that data from Margolis's lab indicate that toxicity of RNA transcripts may contribute to pathogenesis of HDL2. Rudnicki has previously shown that, in addition to protein inclusions, HDL2 neurons contain RNA foci similar to those described in myotonic dystrophy. Overexpression of expanded CUG repeat-containing junctophilin-3 transcripts lacking translation initiation sites leads to RNA foci formation and toxicity in non-neuronal, neuronal-like and neuronal cells.

Based on these findings in HDL2 and given the great clinical and pathological similarity between HDL2 and HD, Russ Margolis' team has started to explore the role of RNA toxicity in HD. They have observed that overexpression of huntingtin exon 1 constructs, containing an early stop codon before the CAG repeat leads to repeat length-dependent toxicity in cultured cells. Furthermore, Wonjae Chung, a graduate student in Margolis' lab, has detected antisense HD

locus-specific transcripts that span the CAG/CTG repeat region. One of the transcripts, ASHD-2, is expressed selectively in the brain and is decreased in HD cortex. As explained by Rudnicki, antisense transcripts with expanded repeats could interact abnormally with RNA-binding proteins, disrupt the regulation of sense strand production, and/or have other, more direct toxic effects. Rudnicki and Margolis teams plan to quantitatively examine and compare the expression of huntingtin sense and antisense transcripts in different control vs. HD brain regions; explore the role of RNA toxicity in cell and animal models of HD; assess whether eventual regulation of sense strand expression by antisense transcripts occurs in *cis* or *trans*; and search for novel huntingtin RNA-binding proteins that may be involved in the disease pathogenesis.

### ***Somatic CAG Expansion***

Another source of different mutant huntingtin forms is CAG repeat somatic expansion. CAG repeats have been reported to increase, particularly in the striatum, with increasing age. But the mechanisms underlying these changes are not well understood. Summarizing recent studies, Karine Merienne noted that this process has been linked to oxidative DNA damage. In particular, repeat instability is significantly reduced in mice deficient for *Ogg-1*, an enzyme involved in repairing oxidative DNA damage. However, the knocking out of *Ogg-1* does not entirely eliminate CAG instability, nor does it explain the process's age-dependence, and striatal vulnerability. To address the role of DNA oxidation in the process's age-dependence and tissue-selectivity, using HD transgenic mice, Merienne and colleagues examined the extent of oxidative DNA damage at different stages and across brain regions with contrasting degrees of somatic instability. Their results indicate that DNA damage is globally increased in HD striatum and cortex when compared to control mice, but is surprisingly higher in the cerebellum, a brain region with little somatic instability, than in the striatum and cortex. They further show that DNA damage is abnormally high in CAG-expanded loci at both early and late stages of disease and in both the striatum and the cerebellum, relatively unstable and stable regions respectively. Merienne and colleagues conclude that DNA damage is necessary but not sufficient to trigger somatic instability.

To begin to dissect the molecular underpinnings of these observations, Merienne and colleagues examined repair proteins that are regulated in a tissue and age-dependent manner. Their data indicate that basic excision repair (BER) enzymes are deregulated in HD mouse brains and, importantly, are differently expressed in the striatum and cerebellum. In particular, the levels of APE-1 and FEN-1 endonucleases are higher in the cerebellum, suggesting that repair kinetics is different between both brain regions. Describing her current model, Merienne explained that oxidative DNA damage is high in CAG-expanded loci, even in young animals, and probably cannot be properly repaired because of the secondary structure of expanded CAG regions, which results in low accessibility for repair enzymes. Furthermore, when the enzymes finally gain access, because of the dynamic nature of CAG-expanded secondary structures they cause expansions in unstable tissues like the striatum where the stoichiometry of enzymes favors expansion over correct repair. Modulating AP endonuclease 1 activity, which coordinates various steps of BER, may help reduce somatic instability. As described by Vanita Chopra, however, other mechanisms may also contribute to oxidative DNA damage. In particular, redox-active metals in the nucleus, such as copper, may be important (see ***Mechanisms of transcriptional dysregulation***).

## **Synaptic Alterations**

Although not as straightforward as targeting mutant huntingtin, or one of its various forms, several approaches that focus on the downstream effects of the mutant protein are yielding encouraging results. One of the fundamental consequences of HD is a breakdown in the normal communication between the cortex and striatum. Examining the electrophysiology, cell biology and biochemistry of synaptic function in HD is thus helping illuminate this aspect of the disease and providing new candidate targets for drug development.

As described by George Rebec, information processing in the cortico-striatal synapse is profoundly altered in HD. Rebec has used chronically implanted microwires to record the activities of neurons in the cortex and striatum of awake, behaving mice. Whereas in normal animals, he observes a high degree of correlated firing in both the striatum and cortex, in HD mice coincident firing is extremely low. In addition, when Rebec has recorded from the striatum and cortex simultaneously, he has similarly seen a high correlation of firing between the two brain regions in wildtype mice (approximately 40%) and a very low correlation in HD mice (approximately 5%).

The data support previous findings from Michael Levine's group who have conducted whole-cell patch recordings of mouse brain slices and found that the striatum becomes disconnected from the cortex in HD mice. As presented by Carlos Cepeda, Levine's group has observed biphasic changes in synaptic activity that progressively lead to disconnection. In the early stages of disease, very large amplitude synaptic events and high frequency bursts of activity are observed in striatal cells. However, as the disease progresses and the animals become symptomatic, there's a progressive reduction in the frequency of spontaneous excitatory postsynaptic currents (EPSCs), accompanied by a thinning of dendrites and a loss of dendritic spines. In addition, others have reported presynaptic biphasic changes characterized by an initial increase in the rate of glutamate release followed by a decrease.

As noted by Cepeda, the therapeutic implications of these findings are significant. In the early stages of HD, reducing cortical hyperexcitability is expected to have clinical benefits while, conversely, in the later stages, bolstering activity is expected to be beneficial. Consistent with these expectations, Cepeda and colleagues observed a reduction in EPSC frequency and in the amplitude of large synaptic events when they administered riluzole, a sodium channel blocker, to slices from mice in the early stages of HD. Cepeda also tested the effects of a cannabinoid receptor type 1 (CB1) agonist in early HD, based on data indicating that CB1 receptors are reduced early on in HD. Surprisingly, the agonist increased EPSC frequency in HD striatal neurons, but decreased it in wildtype neurons. Thus, CB1 agonists might be beneficial for the late, rather than early, stages of HD. An agonist for the adenosine type 2A receptor (A2A-R) also proved capable of increasing spontaneous EPSC frequency, as well as EPSC amplitudes (however, modulating A2A-Rs can have unexpected consequences because presynaptic and postsynaptic A2A-Rs can mediate opposing effects). Based on previous findings indicating that voluntary exercise enhances long-term potentiation, Cepeda also tested the effects of placing running wheels in the cages of mice with advanced HD. There was no measurable effect. However, this may be due to R6/2 mice's disinclination to exercise.

Additional and more specific targets for therapeutic intervention are also emerging from several advances in the cellular and molecular understanding of HD-associated synaptic alterations. For example, Austen Milnerwood presented his and others' data from Lynn Raymond's lab indicating that extrasynaptic NMDA receptors are elevated in striatal HD cells.

Previous findings from the Raymond lab, revealed elevated NMDA receptor-evoked currents in presymptomatic mouse models of HD. However, the expression levels of the receptors were unchanged and spontaneous NMDA currents appeared similar to those of control cells. Based on studies indicating that the localization of NMDA receptors is key to their function—synaptic receptors promote antioxidant protection, LTP, and BDNF transcription, whereas extrasynaptic receptors have deleterious effects on transcription and activate cell death pathways—the researchers investigated the distribution of NMDA receptors in HD cells. Analyzing synaptosome fractions, Milnerwood and colleagues found increased extrasynaptic, but not synaptic, NMDA receptors in HD striatal cells. Furthermore, they observed increased extrasynaptic signaling that correlates with CAG repeat length and is dependent on the cleavage of mutant huntingtin by caspase-6, a protease that has been proposed to play a key role in HD pathology.

Several new therapeutic candidates are suggested by these findings. For example, as noted by Milnerwood, the NMDA receptor antagonist memantine may be beneficial because it preferentially reduces the activities of extrasynaptic receptors. Downstream consequences, such as transcriptional disruptions mediated by extrasynaptic NMDA receptor activation, may also be targeted. In addition, proteins involved in receptor localization such as PSD95 or GIPC—a protein that associates preferentially with extrasynaptic NMDA receptors and may play a role in their organization and trafficking—are new potential targets, as well as palmitoylation which is involved in the localization of several receptors.

Other receptor localization abnormalities, due to impaired membrane recycling, may also contribute to HD neuropathology. As noted by Xueyi Li, previous studies have shown that HD fibroblasts have an abundance of large, elongated vesicles and a reduced number of small vesicles, suggesting a disruption in generation of vesicles from recycling endosomes. To probe the mechanistic underpinnings of this alteration, Li examined the activity of rab11, a guanosine triphosphatase that regulates membrane dynamics at recycling endosomes. His team found that mutant huntingtin inhibits rab11 activity. Moreover, using immunofluorescence and biochemical analyses, Li observed that kalirin, a guanine nucleotide exchange factor, interacts with huntingtin in a manner, unexpectedly independent of huntingtin-associated protein 1 (HAP-1) although HAP1 interacts with both kalirin and huntingtin. Whereas wildtype huntingtin stimulates the catalytic activity of kalirin on rab11, mutant huntingtin reduces it. As noted by Li, several receptors and transporters, including cannabinoid receptors, transferrin receptors, and glutamate/cysteine transporters, are recycled in a rab11-dependent manner. Expression of a dominant-active rab11 in primary HD cortical neurons restored the reduced activity of the EAAC1/EAAT-3 glutamate and cysteine transporter. Thus, HD pathogenesis may include deficits in the recycling of membrane proteins. To test this hypothesis in vivo, Li and colleagues are generating HD mice that express dominant-active rab11 in the brain, and plan to assess its effects on neuroprotection and survival.

Transporter abnormalities were also examined by Paul Rosenberg who described HD-associated defects in the activity of EAAC1, the neuronal glutamate transporter. Rosenberg used striatal cell lines derived from HD knockin mice and control mice to assess how the expression and function of glutamate transporters is affected by mutant huntingtin. Consistent with previous studies reporting HD-associated reductions in the mRNA and protein of the glutamate transporter GLT1, Rosenberg observed a decrease in the expression in the HD cells of the GLT1b, the only GLT1 variant found in these cell lines. Surprisingly, however, he observed a four-fold increase in glutamate uptake. Monitoring uptake under different conditions with different uptake

inhibitors, Rosenberg determined that the transporter responsible for the increased activity in HD cells is EAAC1, a neuronal transporter involved in clearing synaptic glutamate, as well as in providing substrate for the synthesis of GABA. Initially, Rosenberg suspected involvement of the PI3K-Akt signaling pathway because Akt has been shown to regulate the trafficking of EAAC1. Inhibitors of PI3-kinase did block the upregulation of glutamate uptake in the HD cells. However, phospho-Akt immunoreactivity was found to be decreased in the mutant cells. Thus, the abnormality may reside in the PI3 kinase pathway upstream of Akt, and Rosenberg plans to characterize the abnormality in future experiments. In addition, he plans to validate the EAAC1 abnormality in primary cells and animal models of HD.

Targeting glutamate transporter deficiencies in HD has therapeutic potential, as illustrated by experiments performed by Rebec and colleagues. Rebec's team discovered that ascorbate striatal levels, which correlate with glutamate uptake, are decreased in HD as assessed by voltametry measurements in behaving R6/2 mice. In addition, after waking, HD ascorbate levels in the striatum rise much more slowly than those of wildtype animals. Ascorbate is an anti-oxidant that protects cells against free radicals, but it is also released in high concentrations in the striatum in response to cortical activation and is closely correlated with glutamate uptake. After noticing that treating R6/2 mice with very high doses of ascorbate resulted in robust behavioral improvements—including better climbing, locomotion, and turning—Rebec and colleagues tested whether an activator of GLT1 might have similar effects. They treated R6/2 mice with the beta-lactam antibiotic ceftriaxone which activates GLT1 and then used microdialysis to measure glutamate levels in vivo. Their data indicate that glutamate uptake in R6/2 brains, which is decreased compared to wildtype mice, can be normalized by ceftriaxone. In addition, ceftriaxone treatment improves clasping, climbing and turning behaviors.

It is important to note that therapeutic candidates that target HD synaptic alterations need not act on neurons directly. GLT1, for example, is expressed most abundantly in glial, not neuronal, cells. Another example of a promising glial target is the microglial kynurenine pathway of tryptophan degradation. Paul Muchowski noted that a genetic screen for suppressors of HD toxicity in yeast yielded several components of this pathway. Furthermore, Paolo Guidetti had found that metabolites of the kynurenine pathway—including the excitotoxins kynurenate and quinolinate, and the free radical generator 3-hydroxykynurenine—are elevated in HD mice brains and in the neostriatum and neocortex of HD patients at early stages of disease.

Muchowski's current model is that mutant huntingtin expression results in the activation of the kynurenine pathway which affects microglial health and/or affects neurons by inducing microglia to secrete neurotoxic metabolites. Searching for compounds that inhibit the kynurenine pathway, Muchowski has identified a small molecule which, when chemically modified, can penetrate the blood-brain barrier and inhibit the pathway. The inhibitor increases R6/2 survival by almost 40% and results in modest behavioral improvements when placed in the animals' food. Confirming that the compound is affecting its intended target, Muchowski has observed a dose-dependent decrease in the pathway's toxic metabolites in striatum and cortex, but not serum. Muchowski explained that the inhibitor appears to reduce microglial activation, but not directly. The inhibitor's effects on reducing kynurenine metabolites seem to improve neuronal health (as suggested by an increase in the expression of synaptophysin and c-fos), and thus induce neurons to signal microglia to suppress activation.

Alterations in components of the protein turnover machinery of cells might also contribute to synaptic HD pathology. As previously noted, Xiao-Jiang Li and colleagues observed an HD-associated progressive decline in UPS function, specifically at synaptic sites

(see **Aggregation**). In addition, the hyperexcitability observed in the early stages of HD may cause excitotoxicity which, in turn, may decrease autophagy (see **Protein Clearance**). Moreover, Saudou's findings indicate that axonal transport is disrupted by mutant huntingtin (see **Phosphorylation**).

### **Transcriptional Dysregulation**

Transcriptional dysregulation also emerged as a promising candidate for focusing research and therapeutic efforts. It has long been known that several transcriptional abnormalities are associated with HD and that mutant huntingtin affects transcription. In addition, as noted by Dmitri Krainc, many CAG diseases—including spinocerebellar ataxias type 1, 3, 7 and 17—involve transcriptional dysregulation. New studies presented at the meeting, provided information on the mechanisms underlying HD transcriptional dysregulation, as well as new candidates for clinical applications.

#### ***Mechanisms of transcriptional dysregulation***

As previously mentioned, HD is associated with oxidative DNA damage. In addition to its potential effects on CAG repeat instability, oxidative damage may have effects on transcription. As noted by Chopra, oxidation may affect the binding of transcription factors, as well as affect chromatin remodeling. To test this hypothesis, her team used formamidopyrimidine DNA glycosylase (Fpg), an endoglycosidase that cleaves DNA at oxidation sites coupled with PCR to survey the distribution of HD-associated oxidative damage. In R6/2 cortices and total striatal homogenates, Chopra observed a global increase in promoter oxidation, but failed to find a correlation between the oxidation levels of specific promoters and the transcriptional status of their corresponding genes. However, focusing specifically on medium spiny neurons using laser capture microdissection, the researchers found significant increases in the oxidation levels of D2 dopamine receptor and DARP-32 promoters which correlate with major transcriptional deficiencies in both of these genes. Furthermore, they observed increased oxidation of the BDNF promoter in total HD brain which also corresponds to observed decreases in BDNF transcription.

In search of the mechanisms underlying this oxidative damage, Chopra examined the activities and expression levels of various DNA repair enzymes. The results indicated that the activity of most enzymes in HD, including *Ogg-1*, is very similar to that observed in wildtype brains. Furthermore, no change in any of the BER enzymes was observed, except for DNA polymerase  $\beta$  which was found to be downregulated in the striata and cortices of R6/2 mice (see also **Somatic CAG Expansion**). However, Chopra reported increased levels of redox active metals in HD brains. In particular, she found increased copper binding to genomic DNA. Chopra is now examining copper's role in HD-associated DNA oxidation, and eventually plans to evaluate its effects on transcription. In addition, she hopes to extend her studies to other models of HD and monitor how the effects she's observed change over time.

Participants also discussed ways in which mutant huntingtin may affect transcription directly. Krainc reviewed data indicating that huntingtin interacts with several transcription proteins, including components of the basal transcription machinery TAF<sub>II25</sub> and TFIIF, the transcriptional regulator CBP, and transcription factors Sp1 and REST. In addition, Caroline Benn presented data indicating that huntingtin may affect transcription by binding to DNA directly. Benn and colleagues observed that huntingtin associates with DNA in vitro and in vivo

using mobility shift assays, chromosome immunoprecipitation (ChIP) assays, and “ChIP on chip” assays. Results from the latter experiments revealed that huntingtin occupies gene promoters in vivo and that the wildtype and mutant proteins have different distributions. The data also revealed that huntingtin binds a wide range of genomic targets, approximately 2% of all targets included in the DNA chip, with no apparent correlation with HD expression profiles. However, as noted by Benn, a correlation could be missed because standard DNA chips include only proximal promoter areas. To address this question, Benn subcloned and sequenced all the DNA fragments pulled down by the ChIP experiments. Interestingly, most were repetitive DNA, intergenic, and intronic sequences.

In search of more specific binding targets and to assess whether huntingtin binds to DNA directly, Benn and colleagues used a novel approach involving the incubation of a GST-fused huntingtin construct (GST-exon 1) with an array of transcription factor consensus binding sequences. The results indicated that huntingtin binds to many transcription consensus sequences, and mutant huntingtin binds even more, in a manner consistent with HD expression profiles. Furthermore, DNA immunoprecipitation experiments, in which a GST-exon 1 was incubated with naked DNA, confirmed these findings and revealed that the interaction is dependent on polyglutamine length.

As noted by Ray Truant, there are yet other ways in which mutant huntingtin may affect transcription (see *HD Biomarkers*). Truant reported evidence from his lab, using 3D and 4D optical tracking of living cells, indicates that huntingtin translocates into the nucleus in response to stress where it attaches to actin rods and affects the exchange of profilin and cofilin on actin filaments. Whereas wildtype huntingtin helps mediate the clearance of the rods, the mutant protein stabilizes the rods leading to increased levels of cofilin, an inability of mutant cells to survive oxidative stress, and probably transcriptional alterations. The minimal protein domains required for this activity include huntingtin’s nuclear localization signal, its polyproline profilin-binding motifs and several spectrin and actin binding motifs.

### ***The biological meaning of HD transcriptional alterations***

Although the data described above strongly implicate mutant huntingtin in the mediation of transcriptional dysregulation, the causes underlying specific expression abnormalities are difficult to pinpoint. Indeed, many of HD’s expression alterations are probably not directly caused by mutant huntingtin. For example, as pointed out by Robert Hughes, the striatal transcriptional profiles of BDNF knockout mice are very similar to those of HD patients, even more so than the profiles of HD mouse models, indicating that mutant huntingtin is not required for mediating many of the transcriptional alterations associated with HD. Andy Strand, who reported these results, acknowledged this apparent paradox and added that the expression profiles associated with HD represent an integrated composite of many processes occurring within the diseased organism, including cascades of downstream changes caused by primary transcriptional disruptions, non-specific alterations associated with cell injury and death, and compensatory changes. One approach to begin sorting out which transcriptional changes are most biologically relevant to HD is to compare the expression profiles associated with different striatal and cortical pathologies. Strand noted his team has been pursuing this strategy by examining the transcriptional effects of various striatal disruptions, such as chemical models of mitochondrial dysfunction and a PGC-1 $\alpha$  knockout mouse.

As noted by Paganetti, another complication associated with discerning the biological significance of HD transcriptional alterations is that the importance of any particular change is

not a simple function of its magnitude—small variations in some cellular components may have major effects on cell function, whereas large variations in others may have only minor consequences. And of course, transcriptional alterations change over time so that expression profiles are merely snapshots of a dynamic, ever-changing disease landscape. Ultimately, transcriptional dysregulation must be linked to functionality and specific mechanisms, noted Paganetti, and the challenge will be to identify the changes that are most relevant to the disease process.

### ***The clinical implications of HD transcriptional alterations***

Although the biological meaning of many of the transcriptional alterations associated with HD remains uncertain, several researchers have begun to reap potential clinical benefits from their study. For example, based on observations that HD is associated with a decrease in the expression of genes involved in cholesterol synthesis, Marta Valenza has identified 24S-hydroxycholesterol, a cholesterol derivative produced by striatal and cortical neurons that cross the blood brain barrier, as a promising candidate biomarker of early stages of disease (see ***HD Biomarkers***).

New candidate targets have also emerged from the study of HD transcriptional dysregulation. A particularly exciting result is the identification of the histone deacetylase (HDAC) type 4 as a promising therapeutic target. Previous studies had suggested that HD transcriptional abnormalities might be at least partially corrected by increasing the association of acetylated histones with downregulated genes using HDAC inhibitors. However, it was unclear which of the several HDAC subtypes were relevant to HD. Now, using two independent genetic approaches, Gill Bates' and Bob Hughes' labs have converged on HDAC4 as a key target for therapeutic inhibition. Bates's team performed a series of genetic crosses between HDAC knockout strains and R6/2 mice and found that knocking down HDAC4, but not other HDACs, resulted in improved motor behavior as assessed by rotarod performance. R6/2 mice with reduced HDAC4 levels were significantly delayed in their motor decline—at twelve weeks of age they performed as well as eight-week-old R6/2 mice with normal HDAC levels. The researchers also observed a 50% improvement in aggregate levels at 9 weeks of age. Furthermore, in contrast to the general HDAC inhibitor suberoylanilide hydroxamic acid (SAHA) shown by Bates to improve the HD phenotype, knocking down HDAC4 did not have any obvious toxic effects. Mice who receive chronic SAHA treatments lose weight dramatically, whereas the HDAC4 knockouts maintained their weight without showing any ill-effects.

Consistent with these findings, John Miller from the Hughes lab reported the identification of HDAC4 as a robust toxicity modifier in a screen of HD cells expressing siRNAs against different HDAC subtypes. Toxicity was measured as the induction of apoptosis in mouse HD cells as assessed by caspase-3 activation. Miller also observed a correlation between aggregate load and HDAC4 expression in HEK293T cells expressing mutant or wildtype huntingtin as assessed by the Wanker filter trap method.

To investigate the transcriptional effects of HDAC4 that may be specifically relevant to HD, Miller compared the transcriptional profiles of HD cells to those of HDAC4 knockdown cells and found a significant degree of overlap. In addition, he observed that several HDAC4 responsive genes act as suppressors of huntingtin toxicity when inhibited with RNAi. Bates's team also performed transcriptional analyses, assessing the effects of HDAC4 knockdown on the expression of a few genes that have been clearly associated with HD pathology. In some cases, the downregulation of HDAC4 appeared to help normalize expression levels (e.g., the expression

of BDNF in the cortex was increased). However, in other cases, HDAC4 inhibition had no effect, or even a slightly negative effect (e.g., the expression of DARPP-32, CB1 receptor, pro-enkephalin, and the dopamine receptor 2 in the striatum remained low). Thus, HDAC4 inhibition corrects some, but not all HD-associated transcriptional disruptions.

Understanding the cellular regulation and localization of HDAC4 should help accelerate its potential development as a therapeutic target. As noted by Bates, immunostaining of HDAC4 reveals it is mostly found in the nuclei of wildtype and R6/2 neurons. Furthermore, based on data from immunoprecipitations, immunolocalizations, and Western blots, Miller noted that HDAC4 appears to interact with huntingtin within aggregates and modulates their formation. HDAC4 is a member of the HDAC 2A class of HDACs which shuttle between the nucleus and cytoplasm. As noted by Bates, phosphorylation is known to regulate this shuttling and there may be other such regulators worth investigating. It will also be interesting to examine HDAC4's interaction with HDAC3 because the two proteins are often found in complexes together. Attempts by Miller to knock down HDAC3 resulted in toxicity, but Beth Thomas noted her team observed an amelioration of HD symptoms in R6/2 mice after administering a pharmacological inhibitor specific for HDAC3.

Another way in which investigating the transcriptional alterations associated with HD is yielding information of potential clinical value was presented by Christian Neri. His group is using an "omics" approach to integrate data from a wide variety of sources, ranging from yeast to humans, and including HD transcriptional profiles, proteomics experiments, interactome analyses and genetic modifier studies. (A working group of the European HD Network led by Neri and Gill Bates is setting up the database so that it is readily accessible to the community over the internet.)

To mine this wealth of data, Neri's team has developed a bioinformatics data analysis package that helps prioritize genes that may be relevant to HD drug discovery, emphasizing conserved components of longevity and cell maintenance pathways. The group is focusing on these pathways based on their previous work indicating that activators of sirtuin proteins, NAD-dependent histone deacetylases implicated in the regulation of lifespan, protect neurons from HD cytotoxicity via the *daf-16*/FoxO transcription factor family. FoxO lies at the center of a large network of genes with numerous modulators and transcriptional targets. More recent data from their lab indicate that FoxO targets are enriched in modifiers of neuronal dysfunction produced by polyglutamine expansion. Thus, the FoxO network is an appealing source of candidate targets, not only for HD, but potentially for a wide variety of neurodegenerative disorders.

Providing an update of their findings, Neri described the identification of several genes in the FoxO network that appear to be promising neuroprotective targets. Previously, Neri's team had reported that resveratrol, a strong modulator of lifespan that activates the Sir2 sirtuin, ameliorated HD pathology. However, resveratrol has limitations as a therapeutic compound: it doesn't cross the blood-brain barrier and it has effects on several proteins in addition to Sir2. Working with the CHDI, Neri has now identified resveratrol-like compounds that are able to cross the blood-brain barrier. However, he is still searching for new ways to stimulate the neuroprotective capabilities of the FoxO network and develop more specific interventions and combination therapies. One of his approaches is to use glycogen synthase kinase 3 $\beta$  (*GSK-3 $\beta$* ) inhibitors, such as lithium chloride, to activate neuroprotection by increasing beta-catenin levels. Beta-catenin binds directly to the FOXO protein and enhances FOXO transcriptional activity. Thus, combining Sir2-FoxO modulators with *GSK-3 $\beta$*  inhibitors may provide more robust neuroprotection. Neri also noted that the mitochondrial uncoupling protein UCP-4 is of interest

because it is a FOXO/DAF-16 target which is required for Sir2-mediated neuroprotection and modulates the survival of striatal HD cells. Furthermore, the gene encoding the transcriptional regulator CA150 has emerged as a modifier of age of HD onset, and experiments from Neri's lab indicate that CA150 expression rescues striatal cell death in HD models. Interestingly, preliminary data suggest a link between CA150 and the FoxO network.

Additional therapeutic targets may soon emerge from an unbiased screen for compounds that rescue Sp1-regulated transcription. As previously noted, huntingtin interacts with Sp1 and the polyglutamine expansion appears to enhance this interaction and interfere with Sp1's activity as a transcription factor. In search of compounds that may restore Sp1-mediated transcription in HD, Krainc and Paganetti are using a luciferase reporter assay to monitor Sp1 activity in high throughput screens of immortalized striatal cells expressing mutant huntingtin. So far, they have screened 1.2 million compounds resulting in a few thousand hits which are now being evaluated in secondary assays using primary neurons. Promising hits will then be tested in animals. A major challenge, however, will be validating the various candidate targets, which may include proteins or modifications involved in: transcription factor binding to DNA, huntingtin's interactions with the transcription machinery, chromatin modification, etc.

In sum, the path ahead for the development of transcription-based treatments looks promising, but challenging. It will be important to consider that transcriptional regulators may have complex effects on HD pathology beyond their direct regulation of transcription, noted Krainc. For example, Saudou reported that HDAC6 inhibition results in increased tubulin acetylation which ameliorates HD by improving BDNF transport. Also, the acetylation state of huntingtin itself can be affected by histone modulators in complex ways. For example, the CREB-binding protein (CBP) can promote huntingtin acetylation, causing huntingtin to localize to autophagosomes and, in turn, huntingtin can affect histone acetylation through its interaction with CBP's histone acetyl transferase domain. Similarly, HDAC inhibitors may affect huntingtin's acetylation state and, consequently, its clearance. Indeed, Steffan reported that inhibitors of the nicotinamide-dependent class 3 HDACs induce huntingtin fragmentation. Another factor to consider is that HD transcriptional pathology is not restricted to neurons. Work from Muchowski's lab suggests that mutant huntingtin can perturb transcriptional programs in microglia, resulting in toxic activation of the kynurenine pathway (see *Synaptic Alterations*). This activation can be blocked by treatment with a neuroprotective HDAC inhibitor. It will be important to keep this complexity in mind when developing therapeutic targets based on transcriptional modulation.

## **Looking Ahead**

As illustrated in the previous sections, the list of candidate pathological mechanisms underlying HD and the corresponding list of potential therapeutic interventions is large and still growing. Although the continuing emergence of such a wealth of candidate targets and compounds is encouraging, it also poses a major challenge. The time and cost of clinical trials is such that it will be impossible to test them all in the clinic. In a discussion led by Bob Hughes, participants described strategies to address this issue, and identified tools and approaches to improve target and compound validation and prioritization, as well as new ways to streamline clinical trials.

Giving priority to therapeutic approaches that target the root cause of HD such as silencing the huntingtin gene or eliminating the mutant protein is one strategy to help focus

translational efforts. The strategy is appealing because it circumvents HD's complexity and, furthermore, it is beginning to yield encouraging results. However, as suggested by several participants, it may also be valuable, and perhaps even necessary, to tackle HD as a complex problem. Indeed, as noted by Don Cleveland, some neurological disorders must be understood at this level to help develop effective treatments. In amyotrophic lateral sclerosis, for example, different cell types drive different stages of the disease. Reducing the mutant protein in motor neurons slows disease onset, but to alter disease progression, it is necessary to reduce the mutant protein in astrocytes, at one stage, and in microglia, at another. Tools and model systems that offer the possibility of collecting global datasets may help sort out HD's complexity. As noted by Hughes and others, high throughput screening approaches and bioinformatic analysis tools are making it possible to obtain global perspectives of HD dysfunction.

Another consideration emphasized at the meeting was the value of testing targets and compounds across several model systems, using various experimental approaches. As noted by Andy Strand, a validated target for HD does not exist (there is no FDA-approved drug that cures HD in humans), therefore, there is no yardstick to quantify the relative value of different model systems. Each model system offers distinct strengths and limitations as it strikes a unique balance between accurately reflecting the human HD phenotype and providing a practical experimental tool. The goal is to inflict the least possible insult that generates an HD-like phenotype which is experimentally tractable. Furthermore, Juan Botas, who favored continued research into all of HD's apparently relevant mechanisms, pointed out the value of using multiple models and approaches to distinguish between mechanisms that are specific to HD and those that are peculiarities of individual experimental systems. Moreover, William Yang considered that large pharmaceutical companies will be more willing to invest in HD drug development if there's an integrated pipeline in which candidates are validated at many levels, by several different approaches.

## **HD models**

### *Cells, slices, and invertebrates*

Offering an overview of the wide range of models and tools available to researchers in the HD field, participants described the strengths and limitations of a few representative approaches. Given the high degree of conservation in fundamental cell mechanisms across cell types, Ai Yamamoto highlighted the value of using HeLa cells which are robust and easy to manipulate. On the other hand, she pointed out that these cells are limited in their ability to model neuronal behaviors because of their high rate of cell division, low cytoplasmic volume and lack of neuron-specific signaling mechanisms. Providing complementary models to non-neuronal cell systems, participants also discussed the use and development of neuronal cell models. For example, Paganetti described his team's development of a highly homogenous population of stem cell-derived glutamatergic neurons, and a fusion cell line of primary hippocampal neurons and neuroblastoma cells which can be easily differentiated into neuron-like cells with neuritic processes. To help establish the relevance of cell-based readouts, Paganetti urged participants to develop conditional models in which the reversibility of different effects can be readily assessed.

Co-culture models were also discussed at the meeting. Although harder to set up and manipulate than homogenous cultures, co-cultures provide a system that allows the study of the non-cell autonomous features of HD. As noted by Linda Kaltenbach, striatal neurons require

cortical neurons to develop dendritic spines and several studies have implicated multiple cells—including striatal, cortical, interneurons and glial cells—in HD pathology. Based on these considerations, Kaltenbach and colleagues have developed a cortico-striatal culture system to screen compounds for neuroprotective activities. The cortical and striatal cells are isolated separately, labeled with different fluorescent protein markers, and plated on a bed of glia. Using various synaptic markers and electrophysiological measurements, Kaltenbach has established that synapses are formed and become functionally active within a few days. In addition, differences in the survival of cells expressing wildtype and mutant huntingtin can be detected using an object-detection algorithm to count fluorescent cells.

Several hits have emerged from compound screens using this system, including an adenosine 2A receptor antagonist, a phosphodiesterase type 4 inhibitor and a non-selective kinase inhibitor. In the future, Kaltenbach and team plan to set up co-cultures with transgenic mouse cells and optimize conditions to extend the lifespan of the cultures. She also plans to monitor other outcomes in addition to survival, such as dendrite length and synaptic integrity.

To test the effects of compounds on cortical and striatal cells separately, Austen Milnerwood and colleagues have developed another co-culture system in which cortical cells are separated from striatal medium spiny cells by a barrier which allows the passage of neurites, but not liquids. In this manner, the cortical cells can form synapses with the striatal cells, but bath application of compounds can be limited to a single cell type. In addition, Milnerwood noted that this co-culture system allows medium spiny cells to survive in culture for several weeks.

Another model system that models HD pathogenesis in medium spiny cells is a rat cortico-striatal brain slice model described by Don Lo. This system preserves the local tissue environment of striatal cells in the mammalian brain, retaining many of their neuronal connections intact. To produce an HD phenotype, the slices are biolistically transfected with huntingtin constructs using gold particles coated with DNA expression constructs. Co-transfection of a mutant huntingtin exon 1 construct and a fluorescent protein allows the visualization of transfected cells. Labeled medium spiny neurons expressing mutant huntingtin exon 1 degenerate prematurely, and exhibit intracellular inclusions, dysmorphic dendrites, and altered electrical properties prior to death.

Lo explained that the system is proving particularly useful as a secondary screen for testing hits emerging from primary, higher throughput screens. So far, Lo's team has screened approximately 500 compounds, helping rank-order candidates emerging from a wide range of primary drug screens. Although many different readouts can be monitored in the slices, including electrophysiological behaviors, Lo is primarily assaying morphological endpoints associated with neurodegeneration which enables him to perform moderately high throughput screens. Supporting the value of applying many different types of assays and model systems to the study of HD, Lo noted that the hits he has obtained so far come from a wide variety of primary screens with no single screen appearing to be comprehensive in terms of representing all possible HD-associated pathogenic mechanisms.

Invertebrates offer yet another bridge between cell assays and mammalian whole animal models. As explained by Juan Botas, invertebrates such as *Drosophila* and *C. elegans* have well-studied genetics which enable the performance of whole genomic screens, as well as many other sophisticated genetic interventions, using high throughput systems. Of course, the evolutionary distance between these animals and humans limits their use to studying only evolutionarily conserved cellular pathways.

### Mouse models

New mouse models and new insights into current mouse models were also discussed. As noted by Gill Bates, mice offer an *in vivo* mammalian system that is cheaper and easier to manipulate than larger animals, such as monkeys and sheep. Of particular importance, the genetic tools available to study mice—such as a large collection of knockout animals and the availability of methods to regulate gene expression in space and time—are very powerful. Mice also have the advantage of developing HD pathology and symptoms relatively quickly. However, compared to cell cultures and invertebrates, mice are more costly and offer a much lower screening throughput. Because of this, Bates, who has conducted 20-25 screens and genetic crosses over several years, only tests compounds or targets that have been shown to have robust effects in several model systems and/or whose mechanisms of action are supported by particularly solid lines of evidence.

Additional mouse model systems, however, promise to expand the possibilities for drug screening in mice. Liliana Menalled and colleagues, for example, are conducting a detailed characterization of many lines of mice, four of which were presented at the meeting—two YAC and two BAC models in different genetic backgrounds—to try to identify robust phenotypes that will allow the detection of small (10-20%) drug effects reliably in full-length models of HD. As described by Daniela Brunner, Menalled and co-workers are performing longitudinal studies (4-100 weeks) of rotarod performance, grip strength, open field activity during both dark and light phases, rearing and climbing, pre-pulse inhibition of the startle response, anxiety behaviors, and cognitive skills. So far, the team has observed reproducible hypoactivity in all the lines tested, as well as rotarod deficits, increased anxiety behaviors and robust cognitive impairments, as assessed by tests based on those developed by Jenny Morton. Motor deficits are particularly robust and progressive in BAC mice, while cognitive deficits are more robust in YAC mice.

Moreover, Mary Heng described a new mouse model, the heterozygous HdhQ200 mouse, which is a knock-in model, and thus expresses full-length huntingtin in its natural genomic and protein context, but which has a more robust and rapid phenotype than other knock-in mice. Heng and colleagues had previously characterized the HdhQ150 mouse model of HD which displays a late onset behavioral phenotype with motor abnormalities appearing at 100 weeks of age and striatal pathology at 70 weeks of age in heterozygotes. Hoping to accelerate and exacerbate this phenotype, the team performed a series of backcrosses to increase the repeat length. Their new model, with approximately 200 CAG repeats, develops its behavioral phenotype approximately twice as fast, with motor deficits appearing as early as 50 weeks. The mice also show regional-selective pathology in the striatum and cortex, including nuclear inclusions and gliosis, and display age-dependent weight loss beginning at approximately 70 weeks.

Increasing CAG repeat length to exacerbate the HD phenotype may have drawbacks, however. As noted by Bates, in transgenic animals, a large number of CAG repeats, which is necessary to obtain a robust phenotype, results in a disease that is more like juvenile than adult-onset HD, with pathologies that are more widespread than those observed in humans. In addition, the effects of CAG repeat length are not always straightforward. For example, Damian Cummings and colleagues compared R6/2 lines with 110, 150, 210, and 280 CAG repeats, and found that the most severe and earlier phenotypes—including weight loss, clasping abnormalities, onset of motor dysfunction, and altered electrophysiological behavior of striatal medium spiny neurons—are seen with 110 and 150 repeats. Repeats over 200 produce milder phenotypes with a later onset.

However, several considerations should be taken into account when analyzing these seemingly paradoxical findings. For example, Tony Reiner has observed lower expression levels of huntingtin mRNA and protein in R6/2 mice carrying very large CAG repeats. He also noted that mice with 150 repeats have mostly nuclear inclusions, whereas those with repeats over 200 have a greater proportion of cytoplasmic inclusions. Jenny Morton, who has also observed improved phenotypes with increasing repeat length, including enhanced survival, added that above approximately 288 CAG repeats, her team observes a qualitative difference in the expression of the disease, with differences in both pathology and behavior. Yet another consideration, noted by Morton, is that repeat length in individual neurons can vary widely as demonstrated by Peggy Shelbourne. Somatic instability is a well-established feature of HD, which complicates results collected from whole animals whose genotypes across individual cells are not uniform. It is possible, for example, that the repeat length identified in a blood sample is, in fact, different from the repeat length found in the same animal's neuronal cells. Genomic context, including effects from the anti-sense strand, should also be considered, noted Margolis. Anne Messer agreed and added that somatic expansion depends greatly on strain background. Heng added that transgenic fragment models of HD and knock-in models may behave differently—as described above, her team has observed an exacerbation of the HD phenotype by increasing repeat length to 200.

Participants also discussed other characteristics of mouse models that should be taken into account to avoid experimental distortions. For example, Brunner noted that BAC and YAC mice, especially BAC females, are heavier than wildtype mice and this can affect behavioral evaluations. BAC males, however, display rotarod deficits even when groups are matched for body weight. William Yang, who developed the BAC mouse model, added that his team has several lines of evidence suggesting that the weight gain in BAC males does not appear to account for the motor deficits in BAC mice, nor with the psychiatric deficits. Brunner also pointed out that strain differences are very important. For example, C57BL/6 mice don't perform well on climbing tests because the wildtype animals don't engage much in this activity naturally. On the other hand, *FVB/N* mice are not well-suited for tests that require visual skills due to their very poor eyesight. Brunner also noted that mouse lines obtained from different vendors can vary widely in their motor and cognitive abilities. The team ordered the same wildtype lines from several US and European vendors and discovered that the mice behaved very differently depending on their source.

### Large animal models

The development of new, large mammalian models of HD was also discussed at the meeting. Particularly exciting was the description of the first non-human primate model of HD, with its high degree of genetic, physiological, neurological, behavioral and cognitive similarities to humans, its large body and brain size, and the possibility of conducting lengthy longitudinal studies.

As described by Tony Chan, five full-term rhesus macaques with mutant huntingtin exon 1 integrated in one or more genomic sites have been born. Monkeys with 83 or 84 repeats have developed dystonia and chorea very similar to human HD and their brain pathology, including nuclear inclusions and neuropil aggregates, is also similar to that seen in humans. Chan speculated that monkeys with lower repeat numbers may show a later onset of the disease—one monkey with 29 repeats (10-11 repeats is the normal range) has not yet shown any unusual phenotype at 4 months of age, whereas those with more than 80 repeats began showing

symptoms by 1 month of age. Chan and colleagues have developed a modified version of the UHDRS to rate HD progression which should help extend these studies. Current work includes analyzing the expression of the huntingtin constructs, as well as of other genes related to HD, and assessing possible effects due to insertion localization. The researchers are also examining the monkeys' metabolic profiles, at least some of which appear to have an HD-distinctive pattern. Chan is particularly interested in examining monkeys at the time they cease to gain weight, which seems to be a turning point marked by an acceleration of the animals' phenotypic decline.

For the second generation of transgenic HD monkeys, Chan's team will use the human huntingtin promoter (instead of a ubiquitin promoter), and a longer fragment of huntingtin (500 amino acids with 73 glutamine repeats). They hope to conduct longitudinal studies, develop better cognitive tests to take advantage of the model's cognitive capabilities, continue generating cell lines from various tissues, perform monthly blood tests to examine gene expression and metabolic profiles, and conduct MRI scans every 6 months. They also hope to characterize the monkeys' motor alterations in greater detail.

Making the model widely available to the HD community will be a challenge, however, Chan asked participants to be patient noting that monkeys take 3 to 4 years to reach puberty. Luckily, all HD monkey founders are all male, which will accelerate the breeding process once they reached puberty. Furthermore, the use of assisted reproductive technologies, which have been in nonhuman primates, will help accelerate the process. For example, Chan noted that it should be possible to collect semen from transgenic HD monkeys for artificial inseminations and in vitro fertilizations followed by embryo transfer.

Another new, large-animal model of HD described at the meeting was the sheep transgenic model. As noted by Russell Snell, sheep have the advantage of being cheap, easily maintained and having a reproductive system that can be readily manipulated following well-established farm techniques. In addition, as noted by Richard Faull, sheep have well differentiated forebrains and striata that are very similar to humans' in structure and neurochemical composition (e.g., distribution patterns of neurotransmitter receptors). So far, Snell and colleagues have obtained six transgenic sheep carrying a full-length copy of human huntingtin with 73 CAG repeats under the regulation of the human huntingtin promoter. In some sheep, they have observed decreases in DARPP-32 and the cannabinoid receptor type 1 in the putamen and globus pallidus, consistent with HD pathology. However, the researchers have not yet observed inclusions, at least by 7 months of age, nor any obvious HD symptoms. Future plans include conducting behavioral tests and monitoring pre-symptomatic markers. Snell also noted that his team hopes to breed more animals and investigate why some of the transgenic animals have failed to express the transgene. Taking advantage of the sheep genome project that is currently underway, the team may also conduct gene expression studies using microarrays. Ultimately, Snell envisions the sheep being particularly useful for conducting pre-symptomatic drug trials.

## **Clinical Trials**

As summarized by Steven Hersch, at present, two major organizations, the Huntington Study Group (HSG) and the Euro-HD Group, are carrying out clinical HD trials. The HSG is involved in coordinating several multi-center therapeutic trials, while Euro-HD is focusing on observational studies to improve outcome measures for therapeutic trials and to help identify

clinical biomarkers. Additional organizations involved in these types of studies include the NIH, the CHDI, and the Venezuela Project.

Hersch noted that current interventional studies include trials to test the effects of omega-3 fatty acid ethyl eicosapentanoic acid (EPA), the dopaminergic modulator ACR16, and atomoxetine, a drug currently used to treat attention-deficit/hyperactivity disorder (ADHD). Tetrabenazine, which has been shown to ameliorate HD's hyperkinetic movements, is in the process of being approved by the FDA. Disease-modifying studies include a phase II trial for the HDAC inhibitor phenyl butyrate, a dosage study of Dimebon, a drug used in Russia as an antihistamine which appears to enhance cognition in Alzheimer's disease, and a futility study to assess the therapeutic potential of the antibiotic minocycline which affects multiple targets.

Two therapeutic candidates, coenzyme Q10 and creatine, are already in phase III trials. Trials involving pre-manifest individuals are also underway. Chris Ross and colleagues have confirmed the safety and tolerability of coenzyme Q10 in such trials, and Hersch and colleagues are currently enrolling individuals in a pre-manifest trial to study the effects of high doses of creatine. The results from these studies are expected to be very informative. As noted by Hersch, they will provide the first solid feedback on the process of translating HD data from animal models to the clinic. Experience from other diseases, such as stroke and amyotrophic lateral sclerosis, indicate that there are many ways in which the translation process can fail. Hopefully, the particularly good animal models available for studying HD will help minimize the setbacks (see *HD models*).

Still other challenges lie ahead. As noted by Hersch, the drug development process is costly and time-consuming. It requires treatment standardizations, pharmacokinetic analyses, safety studies, and the development of a well understood manufacturing process in which impurities and compound stability are well characterized. Clinical exposures to new treatments must be very gradual, and because of HD's slow, progressive nature, a large number of individuals treated over lengthy periods is required to detect statistically significant effects. In addition, as noted by Diana Rosas, classic HD outcome measures are fairly insensitive, the disease phenotype is very variable, and the pathology starts decades before symptoms develop. As described below, several strategies are being pursued to address these challenges.

## **HD Biomarkers**

Several participants emphasized the critical importance of obtaining reproducible, sensitive and specific biomarkers of HD to streamline clinical trials. Offering the ability to monitor the disease process and predict phenoconversion, biomarkers can greatly increase the power of clinical trials, reducing the number of subjects and time to detect therapeutic effects. In addition, presymptomatic biomarkers can help test early therapeutic interventions, as well as reveal mechanisms of pathology and new drug targets.

### Neuroimaging

Imaging of the brain is emerging as a particularly valuable source of pre- and post-diagnostic HD biomarkers. In previous meetings, changes in striatal volume, cortical thickness, and white matter appearance were presented as candidate biomarkers for tracking HD progression. This year, more in-depth analyses of imaging data provided more specific indicators of disease, as well as new insights into HD pathology and phenotypic variability. For example, Rosas noted that her team has generated a composite magnetic resonance imaging (MRI) map of

33 patients to assess the changes associated with HD at three different stages of progression. The researchers confirmed their earlier observations of cortical thinning associated with HD, and found that thinning is first seen in primary cortex, followed by unimodal cortical areas, and subsequently heteromodal areas (in some cortical areas, such as the anterior cingulate, a transient thickening is observed perhaps due to gliosis). A very strong correlation is observed between patients' Total Functional Capacity (TFC) scores and the thinning of certain cortical areas. The team has also found very clear correlations between specific symptoms and particular cortical alterations (see *HD phenotypic heterogeneity*). Using a fine grain approach, noted Rosas, neuroimaging can be an extremely sensitive indicator of HD progression, allowing the identification of 3% changes per year in the most affected brain regions (while total brain volume changes less than 1% per year).

In addition to cortical changes, Rosas described regional- and temporal-specific changes in the amygdala, hippocampus, thalamus and in white matter areas, particularly the corpus callosum. Previous findings suggested the corpus callosum may be affected in HD, but the extent, timing, and specific location of the damage were largely unknown. Using new tools, including 3-dimensional reconstruction of diffusion coherence, Rosas's team has now discovered that fiber density is reduced throughout the corpus callosum of HD patients and the alteration of its different regions matches corresponding cortical maps. In the posterior regions, which are affected earlier, fiber drop-off can be detected 11 years or more before symptom onset. These data underscore the fact that HD is a systems network disease with complex spatial and temporal dynamics. They also reinforce the value of developing presymptomatic treatments, indicating that significant physical damage occurs before symptoms manifest.

Illustrating the potential of using changes detected by MRI as biomarkers for evaluating drug efficacy in clinical trials, Rosas described the results of a creatine dosage-escalation study initiated 30 months ago. Mirroring creatine's beneficial effects as assessed by cognitive tests and reduced 8-OH2'dG serum concentrations, thinning of the cortex and white matter alterations were significantly reduced in response to creatine treatment (approximately 30%). A statistical model revealed a continued slowing down of disease progression. As noted by Rosas, these studies show how biomarkers can help identify which brain areas are most relevant for therapy evaluation and increase the detection power of clinical trials. Theoretically, these biomarkers could allow the detection of a 25% effect in a 3-year phase III clinical trial, using only 140 subjects (650 subjects would be needed to detect the same effect using currently available indicators).

Positron emission tomography (PET) may also yield powerful biomarkers for HD. As described by Yvette Bordelon, a new PET ligand that binds protein aggregates which assume an amyloid or amyloid-like structure, known as [F18]-FDDNP, may prove useful. Based on work by Barrio and colleagues showing that [F18]-FDDNP, a positively charged tertiary amine, can be used to detect amyloid plaques and neurofibrillary tangles in the brains of Alzheimer's disease patients, Bordelon is now testing the ligand in HD patients. Encouraging results have emerged from a pilot study, including two presymptomatic individuals, two individuals with mild HD and three individuals with moderate HD, in addition to age-matched controls. Bordelon observed [F18]-FDDNP uptake in the striatum of all HD subjects and cortical uptake in individuals with mild or moderate HD. Longitudinal data for one patient showed an increase in [F18]-FDDNP uptake that correlated with a declining Unified HD Rating Scale (UHDRS) score. Bordelon's future plans include conducting larger scale studies with more subjects, correlating ligand uptake

with post-mortem pathology, and determining the earliest time points at which uptake can be detected in presymptomatic individuals.

### Biochemical biomarkers

Several biochemical biomarker candidates were also discussed. Of particular interest, Marta Valenza described a compound that is easily measured in plasma and appears to correlate well with disease stage. As previously mentioned, the expression of genes involved in cholesterol synthesis is decreased in HD (see *Transcriptional Dysregulation*). To monitor this alteration, Valenza and colleagues measured 24S-hydroxycholesterol, a cholesterol derivative produced mostly by striatal and cortical neurons that cross the blood brain barrier. The derivative was found to be decreased in the brain and plasma of YAC mice, mirroring brain cholesterol levels, and was also decreased in plasma from HD patients. Valenza also found a significant correlation between caudate volume, an indicator of neurodegeneration, and 24S-hydroxycholesterol plasma levels. A small number of pre-manifest individuals were also tested. Some had levels similar to the controls, whereas others had levels similar to symptomatic patients. Interestingly, the probability of onset of disease within the next 5 years correlated well with these individuals' 24S-hydroxycholesterol levels. Valenza plans to confirm and extend these studies by analyzing the plasma of a larger sample of pre-manifest individuals.

Alterations in immune signaling molecules also emerged as potential candidates for tracking HD progression. Analyzing HD plasma using proteomic profiling, Edward Wild and colleagues found evidence for immune activation in HD which they investigated in greater depth using multiplex ELISA. Their data indicate that innate immune activation occurs early in HD and increases with disease progression as reflected in the levels of several immune signaling molecules, in particular interleukins -6 and -8 (IL-6 and IL-8). IL-8 levels correlate well with UHDRS scores, and an increase in IL-6, the primary cytokine of the innate immune system, was detected in a group of gene carriers an average of 16 years before the predicted onset of symptoms. Testing the activities of monocytes from HD patients, as well as macrophages and microglia from several mouse models, Wild and colleagues found that expression of mutant huntingtin in these cells induces pathological hyperactivity in response to stimulation. Also, immune hyperactivity was detected in the cerebrospinal fluid and striatum of HD patients. Thus, HD appears to involve both central and peripheral immune alterations triggered by cell-autonomous mechanisms. The data shed new light on HD pathology, as well as suggest potential new biomarkers to track disease.

Another candidate biomarker was described by Ray Truant. As previously mentioned (see *Transcriptional Dysregulation*), Truant and colleagues reported that mutant huntingtin stabilizes actin rods in cells' nuclei by affecting the exchange of profilin and cofilin on actin filaments. Analyzing cofilin and actin in human lymphocytes, Truant observed disulfide-linked higher-order complexes present in HD, but not control, lymphocytes. And in a preliminary longitudinal study, Truant observed that the ratio of free cofilin to complexed cofilin correlates well with disease severity and is detectable in pre-symptomatic patients. To further evaluate this ratio as a potential biomarker of HD, Truant is now performing studies in animal models, including R6/2 mice and rhesus monkeys. As noted by Truant, the findings also suggest potential new drug targets. For example, several kinases are likely to regulate the process, including LIM kinase (which activates profilin), and protein kinase C and CAM kinase II which phosphorylate huntingtin domains involved in actin rod formation.

Mutant huntingtin itself was also suggested as a biomarker of disease. Using an antibody specific for mutant huntingtin, the MW1 antibody developed by Paul Patterson, Andreas Weiss and Paolo Paganetti described a TR-FRET assay (see *Protein Clearance*) that generates a fluorescent signal proportional to the soluble pool of mutant huntingtin, which is inversely correlated with mutant huntingtin aggregation and disease progression. (Although huntingtin is a cytoplasmic protein, it is detectable in bodily fluids, such as blood and cerebrospinal fluid, probably due to its release from ruptured cells.). Weiss has tested the assay in transgenic cells, knock-in cells, and different animal tissues, including plasma and cerebrospinal fluids of R6/2 and knock-in mice. In addition, he has tested blood samples from five HD patients and four controls. A clear difference is seen between the two groups and the signals appear to mirror progression.

As noted by Weiss, the assay may be particularly useful for monitoring treatments that seek to reduce mutant huntingtin or modulate its aggregation, such as siRNA or chaperone-modifying therapies. In addition, the assay can be multiplexed to monitor several proteins simultaneously—for example, huntingtin cleavage fragments and/or different forms of post-translationally modified huntingtin could be concurrently tracked. Furthermore, the assay is very sensitive: it can detect less than 4 ng of huntingtin protein requiring only three microliters of blood per sample. The assay is also fast—Weiss and colleagues recently measured mutant huntingtin in 200 samples from 100 subjects in two hours.

### **HD phenotypic heterogeneity**

As noted by Rosas, it is unlikely that a single biomarker will become the standard for tracking HD progression. Different biomarkers provide complementary information about disease status, which is particularly valuable in a disease that displays such heterogeneity of expression as HD. Despite the simplicity of HD's root cause—a single, dominant mutation—its clinical phenotype is surprisingly variable. As noted by Richard Faull, some individuals suffer mostly from motor symptoms, whereas others have more pronounced mood alterations, or neuropsychological symptoms. Still others have mixtures of symptoms that are not easily classified. Even within the motor category of symptoms, some individuals have dystonia as the predominant symptom, while others are more affected by chorea. New findings by three groups shed light on the neuropathological underpinnings of these variations and suggested ways to help monitor different facets of HD and perhaps, in the future, allow the tailoring of treatments to match individuals' specific needs.

Faull and colleagues, for example, reported post-mortem cortical alterations that correlated with various HD symptoms. Last year, the team published a report describing a correlation between striatal pathology and symptoms—the loss of staining for GABA<sub>A</sub> receptors in striosomes correlated with mood symptoms, whereas matrix receptor loss correlated with motor and mixed (motor/mood) symptom profiles. Now the researchers have studied cell loss in the cortices of 16 HD patients and 15 controls using stereological techniques, and compared these data to the patients' family and medical records, made possible by the establishment of close partnerships with the HD families. Strikingly, patients in which motor symptoms predominate showed massive cell loss in primary motor cortex (approximately 30%), while patients with mainly mood symptomatology showed major losses in the anterior cingulate cortex (54%). In contrast, the primary motor cortex appeared unaffected in mood patients and, conversely, the anterior cingulate cortex was seemingly intact in motor patients. Patients with

mixed symptoms had approximately 22% cell loss in the primary motor cortex and 44% loss in the anterior cingulate cortex.

Although cell loss in these two cortical areas showed the most striking distinction between patient groups, losses in other areas also correlated with symptomatology. Overall, patients with mainly motor symptoms suffered reductions in cell number in primary motor, primary sensory, secondary motor and superior parietal cortices; whereas patients with mainly mood symptoms suffered losses in the cingulate, secondary visual, temporal association, secondary motor, and superior parietal cortices. Patients with mixed symptoms showed widespread cell loss, except for primary visual cortex, in which only neuropil shrinkage was observed, apparently due to reductions in the size and numbers of dendritic branches. Faull added that cortical cell losses correlate with striatal pathology, although more work is needed to understand precisely how the two are linked. He also noted that some losses occur earlier than others. For example, cell number reduction in the cingulate and secondary motor cortices occurs when striatal pathology is still in its early stages, while other cortical areas are affected later. So far, Faull has not found a direct correlation between patterns of cell loss and CAG repeat length, but he noted that this may be due to the study's small sample size.

Another study in which HD clinical heterogeneity was mapped to variations in neuropathology was presented by Tony Reiner. Reiner explained that some HD patients suffer primarily from chorea, while others exhibit mostly rigidity or dystonia, a condition characterized by sustained contractions of opposing muscle groups which are painful and can cause twisting movements or abnormal postures. Based on the circuitry of the basal ganglia and on previous studies implicating the loss of parvalbuminergic striatal interneurons in the development of dystonia in a line of mutant hamsters, Reiner and colleagues evaluated the loss of parvalbuminergic interneurons in HD striata. Using immunolabeling to detect these cells in fixed brain sections, Reiner found that striata from patients with chorea as the predominant motor symptom were indistinguishable from controls. In contrast, parvalbuminergic striatal interneurons were reduced by 50% in cases exhibiting both chorea and dystonia, and by 85% in cases with dystonia or rigidity as the predominant symptom.

Reiner plans to increase the study's current small sample size, and urged participants to inform him of the availability of high quality tissue specimens associated with good clinical records. Reiner also noted his plans to compare striata from different stages of HD. To more clearly dissociate disease stage from motor symptom effects, Reiner is particularly interested in analyzing early-stage cases of HD with dystonia and late-stage cases of HD without dystonia (often dystonia emerges in the later stages of disease). It will also be interesting to determine whether the striatal changes Reiner has observed are reflected in cortical alterations. The mutant hamsters that develop dystonia have both striatal and cortical alterations. Furthermore, William Yang's team has observed changes in cortical interneurons associated with HD. In addition, data from Rosas' lab indicate that patients with the same degree of disease severity but with different motor symptoms—hyperkinetic versus hypokinetic—have a very different distribution of cortical alterations, as assessed by MRI. For example, bradykinesia, a slowness of movement execution, appears to correlate with premotor and supplementary motor cortex involvement.

In addition to providing new insights into HD clinical heterogeneity, Reiner's study suggests potential new therapeutic approaches. For example, boosting GABAergic inhibition, or increasing BDNF levels (parvalbuminergic striatal neurons express high levels of the TrkB receptor), might help ameliorate HD-associated dystonia. Achieving such amelioration would be particularly valuable, noted Reiner, because dystonia is one of HD's most disabling symptoms.

## Predictions

As participants considered the future of HD research and the long-sought goal of obtaining a cure for the devastating disorder, they put forth several ideas. Many predicted a combination therapy was most likely to emerge as the best treatment for HD. They also noted the importance of timing in developing treatments, given HD's changing phenotype. The value of learning from research in other neurological disorders and, conversely, using HD findings to help advance the understanding of other neuropathologies, was also emphasized. Although everyone was cautious about predicting when there might be a cure for HD, the fact that the question was even raised reflected the prevailing optimism stemming from the significant progress that has been recently made in the field.

Sharing results from an informal survey conducted at the meeting, Michael Levine noted that several participants predicted that a cure will most likely come from multi-pronged approaches, and that the cure will probably be a combination of treatments. Even the HD phenotype expressed by *Drosophila* which, as noted by Alex Kazantsev, can be ostensibly reversed by some current experimental treatments, cannot be completely cured by a single "magic bullet," noted Emily Thompson. A potentially fruitful strategy, suggested by Dmitri Krainc, will be to focus on targets of pathways that are found to converge or have some degree of cross-talk. And, as has proven useful in cancer research, drugs that have small, but reproducible effects in animal models may prove valuable in the clinic as part of combination therapies, noted Andy Strand.

The temporal complexity of HD was also discussed, noting that the development of future treatments will likely need to be targeted to specific stages of the disease process. For example, the use of modifiers of synaptic activity should take into account that changes in activity associated with HD are biphasic, as described by Carlos Cepeda. The effects of huntingtin phosphorylation by IKK also illustrate the importance of timing—as proposed by Joan Steffan, IKK phosphorylation may help clear mutant huntingtin in the early stages of disease, but enhance its toxicity later. Providing a clinical example of the importance of timing, Diana Rosas noted that the stage at which creatine is administered to patients appears to be critical—patients receiving the treatment early in the disease process appear to benefit the most. Another important aspect of the temporal progression of HD is that, because the disease develops slowly over decades, therapies will likely be long-term and, thus, their safety and tolerability must be very carefully evaluated, noted Paolo Paganetti.

Several participants noted the likely benefits of applying lessons learned from other diseases to HD research and vice versa. For example, as noted by David Rubinsztein, autophagy seems to be involved in the clearance of intracytoplasmic aggregate-prone proteins associated with several neurodegenerative disorders in addition to HD. By studying autophagy and autophagy modulation in models of several of these disorders, his team is succeeding in translating information learned from one disease to another. A similar situation applies to the study of longevity and cell maintenance pathways that confer neuroprotection, as described by Christian Neri. In particular, Neri has identified the FoxO gene network as a rich source of modifiers of neuronal dysfunction that may prove useful for the development of therapies for various neurodegenerative disorders. Moreover, Krainc noted that many CAG disorders in addition to HD, such as several of the spinocerebellar ataxias, involve transcriptional deficiencies that may stem from similar mechanisms of pathology. Also, Ray Truant emphasized the similarity between the cofilin rods he has observed in HD and those reported in Alzheimer's

disease. A concrete example of using knowledge and tools developed for other diseases was provided by Yvette Bordelon, who is testing [F18]-FDDNP, a compound used to detect amyloid plaques and neurofibrillary tangles in the brains of Alzheimer's disease patients, as a biomarker for HD.

The importance of context was also emphasized. In his closing remarks, Michael Levine noted that abnormalities induced by HD are not isolated, but occur in the context of networks of cellular proteins, interconnected neuronal circuits, and interacting physiological processes in the whole animal and, thus, future efforts will likely benefit from focusing on systems, rather than on isolated processes or components. Nancy Wexler agreed and urged participants to also apply this perspective to the task of research itself—progress in HD is the result of the scientific community's efforts as a whole, so individuals should consider the context of what their colleagues have done and are doing when designing their own research agendas.

Recounting the long and arduous path to developing the first therapy for lysosomal storage diseases, Henri Termeer, the CEO of Genzyme, emphasized the importance of persistence, flexibility, and drive, as well as luck, to take a scientific idea from its inception to its approval and ultimate application in the clinic. Although Termeer joked that he had been successful because as a non-scientist he wasn't "burdened by too much knowledge," he credited scientists, particularly in academia, as being the root sources of ideas and innovations. He also noted the value of obtaining positive clinical results, even if not extensive, to encourage investors and others to support additional research and development efforts. Referring to the HD field in particular, Termeer opined that a priority should be to narrow down the list of candidate targets and compounds which, as previously described, is very large and still growing.

Although Levine noted that in his informal survey, most participants were unwilling to speculate about when a cure for HD will be available, David Housman ventured to predict it could be as close as ten years away. The potential for streamlining clinical trials with improved biomarkers and the encouraging advances presented at the meeting, said Housman, gave him reason to expect more accurate clinical data and a dramatic shortening of clinical trial length. Nancy Wexler agreed with Housman's perspective, and said that, through the years, her father had repeatedly asked Housman about the time it would take to develop a cure. Although the answer is not in yet, Milton Wexler would have undoubtedly appreciated the new level of optimism permeating the field.