



# PIPELINES AND PATHOGENESIS: NEW HORIZONS

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## Abstract

Inspired by a brave couple whose lives have been marred by Huntington's disease (HD), participants at the "Pipelines and Pathogenesis: New Horizons" workshop identified key areas of research and strategies to help translate basic findings into treatment possibilities for HD. Although HD has presented a formidable challenge for scientists trying to understand its pathology and develop therapies, new results and approaches suggest effective treatments may emerge in the near future. In addition, the lessons learned from HD promise to help find treatments for other neurological disorders.

Directly attacking HD's root cause emerged as a particularly promising approach. Specifically, participants discussed how to speed the development of RNA interference (RNAi) strategies to knock down mutant huntingtin mRNA specifically, or both mutant and wildtype huntingtin mRNAs. They also described the use of intracellular antibodies, or intrabodies, to block mutant huntingtin's harmful actions or facilitate its destruction.

In addition, participants noted the potential benefits of correcting various downstream disruptions associated with HD. For example, they discussed mutant huntingtin's ability to reduce brain-derived neurotrophic factor (BDNF), and how reversing this reduction may help eliminate, or at least attenuate, several of the pathologies associated with HD.

Communications within the network of neurons that is most affected by HD were also identified as potential sources for therapeutic interventions. Participants emphasized the need to understand basal ganglia circuitry more precisely. They also noted that, although the most visibly damaged area of the brain in HD is the striatum, the dysfunction of neurons in the cortex seems to be a crucial source of HD pathology.

Modulating proteins involved in the cellular stress response was also discussed as a promising therapeutic avenue. Recent findings indicate that the status of heat shock proteins plays an important role in defining mutant huntingtin's toxicity, and conversely, mutant huntingtin affects protein folding and clearance. Compounds that

regulate heat shock proteins and appear to ameliorate HD pathology have been identified and searches for additional compounds are underway.

A collection of global, unbiased approaches to search for key players in HD pathology holds particular promise for further expanding these therapeutic options. In addition to the heat shock proteins, early findings implicate synaptic proteins, as well as proteins involved in metabolism and aging. Important advances in the identification of genetic factors that affect the age of onset of HD were also discussed.

To make the most of these findings, participants agreed it will be important to prioritize the growing portfolio of therapeutic candidates. In addition, guidelines for translating the results of animal studies into clinical procedures will be needed, as well as new biomarkers to follow HD progression. Participants also emphasized the opportunity of applying lessons learned from HD to the study of other neurodegenerative disorders, and vice versa. In sum, participants were optimistic about the future and hopeful they will soon be able to answer the plea for help put forward by the couple who generously shared their experiences at the meeting.

## The far-reaching consequences of HD

The participation of a 59-year-old woman suffering from HD and her ex-husband greatly helped workshop attendees grasp the far-reaching and complex consequences of HD. By sharing their poignant story, the couple highlighted the many ways in which HD disrupts lives, even before symptoms emerge.

Because the woman was unable to speak intelligibly, her ex-husband (and primary caregiver) spoke to the group. He described how his ex-wife had spent most of her life in fear of developing HD because her father seemed to have the disease. When the woman began experiencing early symptoms of HD, she tried hiding them. Then, when she was diagnosed in 1993, she asked her husband for a divorce, revealing that she had married him primarily for security, to have someone to care for her if she developed HD. Despite this admission, her steadfast husband stayed by her side and when the

woman's friends declined to move in with her to help out, he became her dedicated caregiver. The woman now lives in a nursing home where her ex-husband visits her daily for several hours and keeps abreast of every detail of her condition.

Most HD patients, of course, are not so fortunate. Many divorces triggered by HD are the result of spouses who are unable to cope with the burden of caregiving. In addition, as noted by Nancy Wexler, for many HD patients, divorce is the only means to become eligible for adequate healthcare benefits.

When Alice Wexler asked the couple what was the most disabling aspect of the woman's disease, the woman nodded vigorously to every point her ex-husband mentioned, including the recent HD diagnosis of her son, memory loss, disrupted movements, cognitive impairments and an inability to communicate. In the caregiver's opinion, this last deficit is the most difficult to deal with. Furthermore, HD seems to have also affected the woman's relationship with her children. Two of her three children are estranged and, as noted by Anne Young, such broken family bonds occur frequently in HD families.

At the end of the couple's visit, the brave caregiver, who until then had been calm and composed, put forth an emotional and desperate plea for help. Prefacing his words with an apology for the burden he felt he was placing on the participants, he stressed the need for HD research to bear fruits that will lead to an urgently needed cure.

## Current status of the clinical pipeline

Shaken and inspired by the account, participants began by discussing the status of the HD clinical pipeline. Summarizing the scope of current clinical studies, Young noted they include pre-symptomatic and symptomatic individuals in various locations, including the U.S., Europe and Australia. Both disease-modifying and symptomatic drugs are being tested. A major driving force behind these studies is the Huntington Study Group (HSG). As described by Ira Shoulson, the HSG is a growing consortium which currently includes approximately 80 international sites at which controlled clinical trials, as well as observational studies, are being conducted. Shoulson recommended visiting [www.huntington-study-group.org](http://www.huntington-study-group.org) and [www.huntington-project.org](http://www.huntington-project.org) for more information.

HSG clinical trials include several compounds that are candidates for promoting cellular health, such as coenzyme Q10 (CoQ10), creatine, minocycline, and cysteamine. At the moment, high doses of coenzyme Q10 (2400 mg/day) are being tested in a manifest population. A pre-manifest study led by Chris Ross is also underway. An interesting point, noted by Kurt Fischbeck, is that CoQ10, or its synthetic analog idebenone, is in trials for Friedreich's ataxia and, consistent with other findings, higher doses appear to be more effective.

Another antioxidant in clinical trials is creatine. Shoulson noted that, despite the similarity in function, creatine and CoQ10 have distinct mechanisms of action, as well as different intracellular distributions. CoQ10 is an essential cofactor of the mitochondrial electron transport chain, while creatine regulates ATP levels through its conversion to phosphocreatine. In addition, creatine can reduce glutamate release, and potentially stabilize the mitochondrial permeability transition (MPT). A recent pilot study by Steven Hersch and colleagues suggests high doses of creatine can ameliorate several HD-associated alterations in patients. To extend these observations, Hersch is now planning a full-scale clinical trial.

Minocycline and cysteamine are in earlier stages of the clinical

pipeline. Shoulson noted that the safety and tolerability of minocycline have been established, and now a "futility" study, designed to determine if minocycline followed up by larger and longer studies, is underway. In the case of cysteamine, safety and tolerability studies are expected to begin soon.

Another cellular health-promoting compound discussed at the workshop was Vorinostat (suberoylanilide hydroxamic acid, SAHA). SAHA is the only histone deacetylase inhibitor that had proved efficacious in stringent tests conducted by Gill Bates to assess these compounds' effects on HD mice. N. Wexler noted that it was recently approved for cancer treatment, and Leslie Thompson said Merck is currently discussing clinically testing such inhibitors for HD.

A few compounds are also being tested for their ability to ameliorate HD symptoms. As noted by Shoulson, this class of drugs has received less attention than those aimed at preventing or reversing HD pathology, yet they are potentially very valuable. Many palliative treatments for other neurodegenerative disorders have been approved, and are now helping patients. The only such drug approved for HD (in Europe) is tetrabenazine, an anti-choreic medication whose approval by the FDA is still pending. Participants hoped the approval will be finalized soon and other compounds that target HD symptoms will be developed.

In this regard, a new candidate is Dimebon, a drug which has been approved as an antihistamine in Russia since 1983. As noted by Shoulson, in a recent Alzheimer's disease trial in Russia, Dimebon enhanced cognition as assessed by five independent measures, with minimal side-effects. Working with the company Medivation, Karl Kiebertz and colleagues are now trying to establish dosage and tolerability for HD.

Other compounds that might ameliorate HD symptoms may be on the horizon. For example, Shoulson noted that some HD symptoms—such as difficulty speaking, inappropriate laughter, rapid emotional transitions, and difficulty swallowing—match those of pseudobulbar palsy, a common condition in neurodegenerative and de-myelinating disorders such as amyotrophic lateral sclerosis and multiple sclerosis. Medications for pseudobulbar palsy are currently under development and may be useful for managing HD.

## Targets

Participants also identified key areas of research and strategies to help translate basic findings into treatment possibilities for HD. They discussed the benefits and challenges of targeting mutant huntingtin, the primary source of HD pathology, as well as advantages and disadvantages of downstream interventions.

### Targeting mutant huntingtin mRNA: RNAi

The use of RNA interference (RNAi) to knock down mutant huntingtin mRNA emerged as one of the most conceptually appealing options for treating HD because it targets the root cause of the disease. The therapeutic potential of this strategy has been demonstrated by several recent studies, such as Beverly Davidson's report indicating that reducing mutant huntingtin mRNA with RNAi prevents behavioral and neuropathological alterations in HD mice.

However, several issues regarding its use as a therapeutic agent have yet to be resolved. Importantly, the safety of this procedure needs to be firmly established. Because it is difficult to target mutant huntingtin mRNA without affecting its wildtype counterpart, a major question in the field is whether partially reducing wildtype huntingtin is safe. The question is particularly complicated

because, as noted by Bill Kaemmerer, multiple functions have been attributed to normal huntingtin.

So far, however, studies in mice suggest that reducing wildtype huntingtin in the adult brain is tolerable. For example, Davidson's group delivered an anti-huntingtin small hairpin RNA (shRNA) to mice striata using adeno-associated viruses (AAV) which down-regulated huntingtin expression by 40–60% and caused no apparent toxicity or behavioral defects, at least for 4 months. The researchers are now analyzing animals 9 months after the injection. In addition, as noted by Marina Chicurel, using species-specific RNAi in rodent models expressing human mutant huntingtin genes, Nicole Déglon's group found that reducing mutant huntingtin mRNA specifically reduces HD-like pathology, and the effects are very similar to those achieved by reducing both wildtype and mutant huntingtin. Survival rates also appear to be similar.

Future studies that promise to shed more light on this key issue include safety tests in rhesus monkeys currently being carried out by Kaemmerer's group and by Krys Bankiewicz at UCSF. In addition, Daniel Goldowitz is generating mouse lines in which huntingtin is selectively knocked out in the adult brain. Because of huntingtin's developmental role, particularly in the cortex, Goldowitz noted that inducible knockouts will probably be the most useful for addressing RNAi safety. Kaemmerer cautioned, however, that whereas RNAi knocks down huntingtin levels, Goldowitz's genetic manipulations knock it out. Thus, any toxicity observed in Goldowitz's mice does not necessarily imply RNAi toxicity.

A strategy that circumvents the problem of reducing wildtype huntingtin is to design allele-specific molecules using single nucleotide polymorphisms (SNPs) to exclusively knock down mutant huntingtin mRNA. Both Neil Aronin and Davidson are working on this approach and Kaemmerer said his group has conducted proof-of-principle experiments demonstrating the strategy's feasibility in cell culture. To determine the extent to which this therapy could work in humans, however, it will be important to assess the degree of heterozygosity in the patient population. Another challenge will be obtaining FDA approval given that the cost and time required for conducting individual clinical trials for each molecule is prohibitive. One possible solution, suggested by Jang-Ho Cha, is to use a cocktail of allelic-specific molecules for safety trials.

Whether using allele-specific or non-allele specific RNAi, Davidson emphasized that trial-and-error is a necessary component of testing the safety of RNAi molecules. Indeed, in her lab, two of three shRNAs carefully designed following well-established rules of sequence selection were toxic. Davidson suspects that inappropriate strand loading onto the RNA-induced silencing complex (RISC) may be causing toxicity via the silencing of unintended mRNAs. Based on these observations, she urged Kaemmerer to run toxicity tests *in vivo* and check strand loading using Northern blots before testing his anti-huntingtin shRNA in monkeys. In addition, she suggested testing more than one shRNA.

Participants also discussed issues associated with delivery. Synthetic small interfering RNAs (siRNAs) can be delivered as pharmaceuticals, using pumps for example, or viruses can be used to induce the expression of shRNAs in infected cells. As noted by Kaemmerer, each strategy has its advantages and limitations. Viral delivery, usually via AAV, requires a single dose and there is little need for formulation optimization. On the other hand, viral infections are irreversible, dosages are harder to titrate, and viral vectors present less of a financial incentive for pharmaceutical companies.

Regarding toxicity, Davidson noted that at least 4 clinical safety trials using AAV in the central nervous system are underway. So far,

the virus seems to be well tolerated inducing only low levels of immune response. However, optimizing dosage is important because overexpression of shRNA can be toxic. Besides off-target effects, one possible source of toxicity is that the shRNA might compete with endogenous microRNAs (miRNAs) for the same RNA processing machinery. Because siRNAs do not require the same processing, in theory at least, they should not suffer from this problem. However, more work is needed to fully assess their safety risks. Davidson noted that her group's attempts at using non-viral delivery of siRNAs in mice have resulted in robust knockdown of huntingtin, but have induced toxicity.

An alternative to siRNA and standard shRNA is to embed the anti-huntingtin sequence in a known miRNA. As noted by Davidson, miRNAs are processed much more efficiently than the other two RNA molecules, such that lower doses are required for mRNA knockdown. So far, her team has had success transferring anti-huntingtin sequences to the miRNA format and they are now conducting safety studies in animals.

Deciding where to deliver RNAi molecules for HD treatment is yet another issue that needs to be resolved. As noted by Carl Johnson, most efforts to date have focused on striatal delivery based on the visible pathology observed in this region, particularly in the later stages of disease. However, increasing evidence indicates that the cortex is a prime site of HD dysfunction (see *Targeting cell-cell interactions: basal ganglia circuitry in HD*). Kaemmerer noted that his group will work with William Yang to decide which brain area(s) to target. He also noted that it will be important to determine how much of the target area needs to be covered—striatal volume scales approximately 2000-fold from mouse to human. Davidson noted that Parkinson's disease studies in rhesus monkeys and humans suggest it is feasible to cover the caudate-putamen area. However, further work will be needed to determine cortical coverage requirements and options. Davidson and Johnson pointed out that the Bankiewicz group has shown that injections into the fiber tracts of the corona radiata appear to be a route for effective delivery to the cortex. Alternatively, hoping to harness retrograde transport, Kaemmerer's group has searched for cortical labeling after delivering RNAi molecules to the striatum. Unfortunately, so far, they have observed transport only towards the substantia nigra. Furthermore, as noted by Marie-Françoise Chesselet, these delivery methods may be ineffectual if cortical interneurons are the primary source of HD dysfunction, as suggested by some recent studies (see *Targeting cell-cell interactions: basal ganglia circuitry in HD*).

To broaden RNAi coverage throughout the brain, Paul Patterson suggested using peripheral injections of mannitol to weaken the blood-brain barrier. His group has observed that administering mannitol at the same time that viral vectors are delivered to the brain, enhances viral distribution. Davidson acknowledged the possible usefulness of this technique, but noted that usually convection-enhanced delivery is sufficient to obtain fairly widespread viral distribution of RNAi. Patterson suggested organizing a workshop to discuss delivery issues, not only related to RNAi, but to other compounds.

Improving delivery could also facilitate the development of potentially powerful combination therapies. For example, Kaemmerer noted his interest in co-delivering brain-derived neurotrophic factor (BDNF) with RNAi agents (see *Targeting the reduction of BDNF*).

Another important issue is determining at what stage of HD RNAi treatment is most effective. Kaemmerer suggested using longitudinal data from HD observational studies such as PREDICT-HD

and PHAROS to guide this decision. Young noted, however, that these data may take several years to collect.

Participants agreed that identifying sensitive and reliable biomarkers to follow disease progression will be critical for the evaluation and optimization of RNAi, as well as other candidate therapies (see *Improving measures of HD in humans*). For RNAi in particular, Kaemmerer considered that intracranial markers would be most useful because peripheral changes may be difficult to detect. As noted by N. Wexler, promising brain biomarkers are emerging from the work of Yvette Bordelon and John Mazziota based on contrast imaging. Davidson emphasized the need for more imaging markers and noted the use of laser-capture-microdissection analyses in animals to follow RNAi effects. Stephen Tapscott wondered if, at least for initial efficacy studies, peripheral administration and assessment of RNAi effects might be useful. Davidson responded that her group is using peripheral markers for safety studies.

### Targeting mutant huntingtin protein

Interfering with mutant huntingtin at the post-translational level was also discussed as a potential therapeutic option. Patterson, for example, described his group's work with anti-huntingtin intrabodies, antibodies that are expressed intracellularly. Whereas intrabodies that recognize polyglutamine actually exacerbate toxicity (perhaps by stabilizing mutant huntingtin's conformation), intrabodies against huntingtin's proline-enriched domain, polyproline region or N-terminus are capable of reducing toxicity and aggregation in cell culture, flies, and slice models of HD. Tests in mice are currently underway.

As explained by Patterson, intrabodies' efficacy, measured as the ratio of intrabody to antigen required for optimal function, as well as their mechanisms of action, vary greatly. For example, the VL12.3 intrabody developed by David Colby in Dane Wittrup's laboratory, which recognizes huntingtin's first 17 amino acids, obliterates aggregates but does not affect soluble oligomer levels, whereas the Happ intrabodies, that target the proline-rich domain, reduce both aggregates and soluble oligomers, by affecting the turnover of aggregate precursors. The Happs also increase the turnover of mutant huntingtin much more than that of wildtype huntingtin. Current experiments in Patterson's lab testing these virally-delivered intrabodies in mice promise to shed light on the therapeutic possibilities of these reagents. So far, they have achieved good viral distribution, but have yet to optimize expression levels.

Participants also discussed current knowledge of mutant huntingtin aggregation and its potential as a therapeutic target. Noting the diversity and complexity of aggregate forms, Paul Muchowski emphasized the need for additional and better tools to study aggregates, especially *in vivo*. Although he considered that anti-aggregation compounds *per se* are unlikely to emerge as promising therapeutic candidates given that most drugs act on receptors or catalytic sites, he thought that increased knowledge of aggregate conformations is likely to be helpful for developing new therapies.

Current approaches to study aggregate conformation include conformation-specific antibodies which Muchowski, Thompson, and Steven Finkbeiner are using to correlate different aggregate forms with toxicity. Johnson noted that an antibody generated by Charlie Glabe appears to specifically recognize toxic soluble oligomers of various disease proteins. Although this antibody has proven useful for various studies, Muchowski noted the recent discovery that heat shock protein 70 is also a ligand of this antibody

which complicates its use (see *Targeting the cellular stress response*).

Finkbeiner noted his group has generated several antibodies, one of which seems to be a particularly good predictor of toxicity. Working with Muchowski, they have observed that the antibody is capable of dissolving fibrils and can pull down mutant human huntingtin in YAC128 brain tissue. In addition, Finkbeiner's team has determined the structure of the antigen-recognizing region and developed a screen to identify compounds that disrupt its binding to polyglutamine. So far, they have found 7 druggable compounds. Noting that Aurora Biosciences performed related screens, Bob Hughes suggested examining their data.

Additional tools for studying aggregates were described by several participants. Muchowski noted the value of using yeast expressing polyglutamine expanded proteins to help correlate specific oligomeric structures with toxicity. In addition, Morimoto pointed out his group's use of fluorescence correlation spectroscopy to probe the relationships between aggregate forms, toxicity, and molecular chaperone function. To quantify insoluble amyloid, Bates recommended a commercial ligand that binds to fibrillar structures as revealed by electron microscopy, and has allowed her to perform highly reliable and quantitative ELISAs.

### Targeting the cellular stress response

In addition to studying mutant huntingtin's conformation and behavior, participants emphasized the value of understanding and harnessing cells' endogenous mechanisms for handling aberrant proteins. Attendees considered that regulating heat shock proteins (Hsp), which act as molecular chaperones involved in protein folding and oligomerization, may lead to effective treatments for HD.

Several studies suggest a role for chaperone function in various forms of neurodegeneration. For example, participants noted that mutations of certain chaperones can cause neurodegenerative disorders such as Charcot-Marie-Tooth disease. Of particular interest, Morimoto's group has found that overexpressing heat shock transcription factor 1 (HSF1) in *C. elegans* suppresses polyglutamine protein aggregation, whereas reduction of HSF1 by RNAi potentiates aggregation.

Morimoto's group has also identified specific chaperones that can alter the course of mutant huntingtin aggregation and toxicity in mammalian cells. A recent article by Kimura and colleagues, for example, describes how depletion of the CCT chaperonin results in the appearance of soluble mutant huntingtin aggregates, while CCT overexpression suppresses aggregation and cell death. Experiments from Bates's lab crossing HD R6/2 mice with mice carrying transgenic heat shock response (HSR) genes promise to complement these studies. Recent results reveal very weak effects using Hsp70 transgenic mice and no effects using Hsp27 transgenic mice. Because Hsp27 acts acutely, however, Bates is interested in repeating this last experiment using an inducible transgene. She is also planning to cross mice expressing an inducible copy of HSF1.

Morimoto predicted that understanding the changes in chaperone function associated with the early stages of HD could yield valuable targets, as well as biomarkers of disease. Tapscott added that many of the alterations associated with HD might be secondary to stress responses. Consequently, understanding the stress response and how it evolves over time might be even more fruitful than focusing on individual HD pathologies.

Enlisting chaperones to fight disease effectively will probably require the ability to modulate them specifically in time and space, however. As noted by Morimoto, the chaperone network is large

and complex, including 330 chaperones belonging to 15 different classes in humans. It is also ancient and highly conserved such that gross manipulations of core chaperone function may result in serious side-effects.

A more promising approach, suggested Morimoto, is to target specific chaperone regulators. Using worms as model systems facilitates the initial task of differentiating core chaperones and regulators from more specialized ones. It will then be important to map the differential expression of chaperones and their regulators in the brain, and define their specificities and subcellular locations. Morimoto's group is focusing on J-domain proteins which, in humans, include 35 co-chaperones. These proteins are critical for protein translocation and may provide specific handles for fine-tuning the system without causing widespread alterations.

David Housman noted that his lab have data indicating it is possible to obtain regulators that are client specific. The data come from work by Ruth Bodner who is conducting screens to identify compounds that selectively decrease levels of a mutant, but not a wildtype, huntingtin fragment. To then test whether the compounds affect chaperone function, the group is using a cell-free assay that monitors the re-folding of heat-denatured luciferase. Working with these tools, Bodner and colleagues have identified an inhibitor of Hsp90 that appears to be client specific.

Another approach that may reveal HD-specific targets was described by William Yang. His group is characterizing mutant huntingtin's interactome by immunoprecipitating the full-length protein from various regions of the mouse brain, including cortex, striatum, and cerebellum, at different stages of disease. Yang considered that analyses of chaperone co-immunoprecipitation will likely provide insights into the links between chaperone function and HD.

Examining inter-individual variations in Hsp levels and their association with HD phenotypes might also be informative. Morimoto noted that a close link appears to exist between HSF expression levels and longevity. It is possible that similar relationships exist between HS genes and neurodegenerative disorders. When Morimoto's group informally measured Hsp levels in 17 lab members, they observed 5–10% variability in the levels of various Hsp proteins. Interestingly, Hsp70 levels varied 1000-fold within the population, yet the variability almost disappeared when cells were examined in the induced state. Housman noted that his team could extend these observations and search for potential correlations with HD phenotypes, particularly if the variability of Hsp levels is heritable in cell lines.

Participants identified several other paths to follow up on previous studies and accelerate the identification of therapeutic candidates. Working with The Scripps Research Institute Molecular Screening Center, Morimoto is setting up a massive screen to identify heat shock regulators using HeLa cells carrying a luciferase gene driven by an Hsp promoter. The goal is to screen one million molecules. The Scripps center will perform primary screenings and vetting. The results will be confirmed by Morimoto's group and candidate compounds will be ranked according to their chemical characteristics. Mechanistic studies on the most druggable compounds will then be performed, including proteome analyses. In addition, compounds will be tested in HD cell models, as well as fed to worms. Testing in higher animals will be performed by collaborating labs. Additional searches for compounds that regulate Hsp function are being performed by Bates and Rainer Kuhn, who is using Hsp reporter transgenic lines.

One recently identified regulator of the HSR, celastrol, is currently being examined by Morimoto's group. The team is analyzing

several modified versions of the compound which was identified by the NINDS Neurodegeneration Drug Screening Consortium. Celastrol is a triterpenoid derived from the *Celastraceae* plant family and was initially described as a potent activator of HSF1. However, it is now thought to have more widespread effects through its modulation of the oxidation of SH groups by a hit-and-run mechanism. As explained by Morimoto, Hsp90 is a major target of celastrol because it is a very abundant cellular protein, but other proteins are affected as well. In particular, celastrol affects the nuclear factor erythroid 2-related factor 2 (Nrf2) pathway involved in antioxidation and detoxification. Morimoto's group has generated celastrol derivatives with promising pharmacological features for drug development—increased solubility and activities in the high nanomolar range. However, at this stage, the group is focusing on understanding the compounds' mechanism of action before initiating preclinical tests. Kuhn is also working with identified HSR regulators. His team is preparing to examine the effects on HD of Hsp90 inhibitors that penetrate the blood-brain barrier *in vivo*.

Participants concluded their discussion of chaperones on an optimistic note. Morimoto considered that the amelioration of HD pathology achieved with such gross manipulations as overexpression of a major chaperone (without knowledge of its concentration or that of its substrates), is a heartening indication of what might be possible with more subtle and targeted interventions. Bates added that it may be unnecessary, and perhaps even desirable, to alter chaperone function without much tissue specificity, given that HD is a multi-system disease. Participants also noted that it is possible that cells are fairly competent at dealing with mutant huntingtin, as suggested by HD's late onset, but eventually become overwhelmed and protein homeostasis breaks down. If so, a transient tweaking of chaperone function may be sufficient to re-equilibrate the system, noted Morimoto, with minimal secondary effects. Hughes wondered if a selective upregulation of the stress response, perhaps early in life, could be protective.

### **Targeting mutant huntingtin processing**

Another potential target for therapeutic intervention is the processing of mutant huntingtin. Numerous reports have suggested that mutant huntingtin's toxicity arises from its cleavage and the accumulation of amino-terminal fragments, particularly within the nuclei of vulnerable cells. Working with scientists at Novartis, Marian DiFiglia noted she is interested in identifying the proteases involved in this fragmentation, as well as identifying potential regulators, particularly because protease cleavage sites are a favorable target for drug development. One of the approaches she is pursuing is to search for inhibitors of the production of Cp-A and Cp-B, small N-terminal huntingtin fragments identified by Yvon Trottier which appear to be key to the formation of nuclear inclusions. The protease(s) that generate Cp-A and Cp-B have yet to be conclusively identified. To identify them and find corresponding inhibitors, DiFiglia has set up an ELISA-based assay that monitors the effects of protease inhibitors on the production of CpA and CpB. The assay is done in the presence of a proteasome inhibitor to minimize non-specific fragmentation. With a focused library of compounds to test, DiFiglia says the assay works well—in 3 weeks her team was able to test 100 compounds in triplicate.

DiFiglia is also working with Michael Hayden to examine the role of caspase-6 in HD. Hayden's team has shown that the caspase-6 site on huntingtin, but not the sites of other caspases, plays a key role in huntingtin toxicity in the YAC128 model of HD. To extend these studies, DiFiglia and colleagues are now generating a neo-epitope antibody that will recognize huntingtin fragments generated by

caspase-6 cleavage to more easily assess this fragment's generation in the brain. Caspase-6 fragments are known to exist in the brain, but now the researchers want to compare wildtype and mutant huntingtin fragments, as well as examine the processing of huntingtin carrying a mutation in the caspase-6 site. So far, no other proteins are known to bind to the caspase-6 site, however, when the site is mutated, huntingtin is cleaved at another site. One possible explanation for this observation, suggested Morimoto, is that the mutation causes a conformational change.

Some participants suggested confirming this process's relevance to HD pathology before investing too much effort on its characterization. Although DiFiglia emphasized that the effect reported by Hayden is robust and highly reproducible, Johnson was concerned that YAC128 mice have a very mild phenotype such that the magnitude of the protection conferred by mutating huntingtin's caspase-6 site is small. To amplify the signal-to-noise ratio, he proposed testing the caspase-6 mutation in another mouse model with a stronger phenotype, such as Yang's BAC mice.

Although discussed only briefly, the potential value of targeting cellular clearance mechanisms was also recognized. DiFiglia noted that autophagosomes appear to be importantly involved in huntingtin clearance and Morimoto pointed out that inhibiting the proteasome enhances HD toxicity. Morimoto's group has identified a compound that increases proteasome activity in a screen for compounds that ameliorate mutant superoxide dismutase toxicity, and they now plan to test its effects on HD toxicity.

### **Targeting mutant huntingtin's disruption of transcription**

Moving a step downstream, participants discussed some of mutant huntingtin's effects on cellular functions and their possible value as therapeutic targets. It has long been recognized that transcription is significantly disrupted in HD, but the underlying mechanisms of this alteration and its consequences have yet to be fully understood. Cha noted that disruptions in the regulation of chromatin structure by histones may contribute to HD transcriptional pathology. Indeed, histone deacetylase (HDAC) inhibitors have been reported to ameliorate HD pathology and several groups, including Kuhn's and Bates's, are now searching for more effective and specific HDAC inhibitors.

The underlying mechanisms by which HDAC inhibitors benefit HD, however, remain unclear. To address this question, Cha is examining the acetylation, ubiquitination, and phosphorylation of histones in HD. His approach involves analyzing histones associated with individual genes to correlate the histones' post-translational modifications with gene expression levels. Using chromatin immunoprecipitation (ChIP), Cha has observed that genes that are downregulated in HD are associated with histones modified in ways that are known to suppress transcription.

Working with Leslie Thompson, Cha has now begun to examine how HD may induce the formation of facultative heterochromatin—localized areas of chromatin that may spread in a manner correlated with disease progression. Thompson noted that the post-translational status of huntingtin, in particular its SUMOylation state, appears to affect the recruitment of HDACs and other transcriptional regulators. To determine which HDAC types are relevant to HD, Thompson is conducting genetic tests in collaboration with Larry Marsh. So far, it appears that reducing specific proteins within the HDAC classes I and III, but not II, in *Drosophila* alleviates HD toxicity.

At least part of huntingtin's effects on transcription, suggested Cha, may be due to its direct association with DNA. Cha's team has observed co-immunoprecipitation of DNA with both mutant and

wildtype huntingtin. In addition, they observe DNA binding to huntingtin under *in vitro* conditions used to study DNA-binding proteins. Furthermore, both wildtype and mutant huntingtin can be found associated with the promoters of genes whose expression is altered in HD. To identify the DNA sequences huntingtin binds to, Cha sequenced DNA fragments derived from ChIP subclones and found mostly repetitive sequences. Confirming these findings, Cha and colleagues have observed, so far, a lack of sequence specificity in "ChIP on chip" experiments using high resolution genomic DNA microarrays. Thus, the results suggest that huntingtin may act as a structural protein in the nucleus, binding DNA in a sequence-independent manner. Mutant huntingtin, which binds DNA more readily, may disrupt this normal function and affect transcription.

Participants also discussed huntingtin's role in sequestering REST, a transcriptional repressor which binds to NRSE, a neuronal silencer present in approximately 1300 neuronal genes. Cattaneo noted that ChIP experiments using cells and mouse tissue to monitor REST binding, consistently show that an increase in wildtype huntingtin results in a decrease in NRSE occupancy. Conversely, mice with low levels of wildtype huntingtin have increased NRSE occupancy, as do HD mice. Transient transfections of a REST dominant-negative mutant in cultured cells rescue the phenotype, restoring endogenous levels of NRSE-containing genes, including genes coding for BDNF, synapsin 1, and several neurotransmitter receptors. Cattaneo is now setting up to deliver the REST dominant-negative mutant to HD mice using AAV.

To identify compounds that may correct NRSE regulation in HD, Cattaneo is conducting screens using transfected ST14A cells with a reporter construct consisting of 300 base pairs of the NRSE coupled to the luciferase gene. As a complement to the assay, Cattaneo can measure the expression of BDNF and other endogenous genes. So far, the researchers have identified several compounds, including one that is effective at 20 nM and toxic only at a 25-fold higher concentration.

### **Targeting the reduction of BDNF**

Although the expression of many genes is affected in HD, the reduction of cortical BDNF expression, in particular, seems to be strongly associated with HD pathology. Crossing HD mice with BDNF mutants accelerates striatal degeneration, whereas infusing BDNF can ameliorate at least some aspects of HD pathology. Furthermore, Cattaneo pointed out Kevin Jones's recent findings indicating that mice lacking BDNF expression in the cortex share striking phenotypic similarities with HD mice. She also noted that, as observed by Andy Strand's group, these mice have striatal gene expression profiles that mirror those of human HD patients very closely—more so than R6/2 mice or mice treated with 3-nitropropionic acid.

Although, as noted by Fischbeck, several lines of evidence point to a gain-of-function mutation underlying HD pathology, BDNF reduction may still be a major contributor to disease. As noted by Bates, a gain-of-function mutation can express as a functional loss in certain situations—for example, if the mutant protein leads to sequestration of the wildtype protein. In addition, Cattaneo noted that mutant huntingtin appears to affect the expression of BDNF in more than one way, including both loss- and gain-of-function disruptions.

Participants discussed the possibilities and challenges of developing treatments to increase BDNF signaling in HD. As noted by Tapscott and Shoulson, clinical trials to test the effects of neurotrophins on Parkinson's disease (PD) have been performed.

Unfortunately, the results were negative—GDNF’s effects did not differ significantly from those of a placebo, and GDNF caused some toxicity. In addition, Yang pointed out that work by Susumu Tonegawa’s group indicates that overexpressing BDNF can affect brain development.

How relevant these problems are to HD treatment is uncertain. As noted by Cattaneo, Ron Mandel and colleagues tried using a viral system to deliver BDNF to HD mouse brains, and observed high levels of toxicity, which persisted to some degree even when they reduced the dosage by a factor of 100. However, as noted by N. Wexler, these experiments were performed by a single group, many years ago, without the knowledge and tools that are available today. Thompson added that HD may be better suited to BDNF therapy than PD given that BDNF seems to play a central role in HD pathology which may not be shared by PD.

One difficulty encountered in the PD trials that is likely to carry over to HD trials, however, is delivery. As noted by Shoulson, achieving good delivery levels of neurotrophins has been a major challenge. An additional challenge is monitoring BDNF levels. For example, Michael Levine said his team was unable to obtain consistent measurements of BDNF in R6/2 mice which showed electrophysiological improvements associated with exercise.

Participants proposed approaches to circumvent some of these problems. Cattaneo noted that, although difficult, it is possible to obtain reliable measures of BDNF as demonstrated by Yves Barde’s group. Addressing delivery and toxicity, Yang and Johnson suggested localized delivery of BDNF coupled with moderate expression to minimize toxicity. Davidson added that protein delivery may be more effective than viral systems. Furthermore, Cattaneo noted that BDNF mimetics, such as those being developed by Frank Longo, may provide enhanced specificity and efficacy. Yet another option, suggested by Tapscott, is the use of small molecules to target signaling molecules downstream of BDNF. Cattaneo agreed that such targeting might be effective, especially once the pathways disrupted in HD are better understood—Trk receptors appear to be altered in HD and adaptor molecules involved in BDNF signaling, such as the Shc proteins, can change as a consequence of brain damage. One compound identified at Cephalon, noted Thompson, appears to be extremely neurotrophic, inducing the upregulation of TrkA and TrkB receptors. Preliminary data suggest it rescues R6/2 neurons.

In addition to correcting the HD-associated reduction in BDNF, procedures that enhance BDNF levels or signaling, may help counteract striatal cell loss. As noted by Chicurel, Steven Goldman’s group recently succeeded in inducing striatal neuron recruitment in R6/2 mice using viral delivery of BDNF and Noggin, an inhibitor of the signaling pathway involved in astrocyte genesis. The recruitment resulted in delayed motor impairment, as well as extended survival.

### **Targeting cell-cell interactions: basal ganglia circuitry**

The role of cortical neurons as suppliers of BDNF to the striatum is not the only cell-cell interaction that appears to be disrupted in HD. Increasing evidence indicates that alterations in the synaptic circuitry of the basal ganglia are also key to HD pathology. Setting the stage for discussing this issue, Young and Charlie Wilson provided an overview of basal ganglia circuitry. Young sketched a diagram showing the striatum as the main input structure of the basal ganglia, receiving excitatory afferents from the cerebral cortex and thalamus. The main output, through

the direct and indirect pathways, is the internal globus pallidus, and ultimately, the thalamus.

Although several functions have been ascribed to the basal ganglia, a major one is comparing movement templates to actual movements, a process key to learning and executing repetitive motor tasks. As described by Young, the importance of this function is illustrated by experiments in songbirds. Male songbirds learn to sing by first listening to their father, and then practicing the song, a task that involves the basal ganglia. After a period of practice, the song is said to “crystallize”—birds don’t require hearing the template song anymore to reproduce it faithfully. However, birds still need the feedback information from their own voice to keep the song from deteriorating. If a bird is deafened after crystallization, its song will slowly degrade over time. But if deafening is paired with a lesion to the basal ganglia circuitry, the song will persist without deterioration. Thus, the function of the basal ganglia is key to both learning and updating movement information. The results also suggest that a damaged basal ganglia circuit that cannot compare properly may have more serious consequences than a completely non-functional circuit which at least leaves previously learned information intact.

Consistent with the basal ganglia’s role in learning new movements, Young noted, HD patients have trouble executing new motions, even simple ones, on command. However, before reaching the advanced stages of disease, patients are often able to perform fairly complicated patterns of movement that have already been learned (e.g., tying a shoelace). It is possible that the updating of previously learned information is affected only later in the disease process, or that basal ganglia function is so compromised early on that it has no effect on updating.

To understand how basal ganglia accomplish these functions it is necessary to understand their electrophysiology, a task that has proven more complicated than was expected. As explained by Wilson, striatal neurons were originally thought to have long, slow responses. In addition, their firing rates were thought to be steady and definable by scalar numbers. Yet recent studies indicate that the behavior of striatal neurons, and the basal ganglia as a whole, is more complex. For example, researchers have found that, in addition to slow activities, responses in the 1–5 millisecond range are essential for proper basal ganglia function. The precise timing of signals is critical and tightly regulated. Indeed, in the indirect pathway, the diameter of the axons innervating the globus pallidus is strongly correlated with the length of the axons innervating the substantia nigra. Because the globus pallidus is farther away from the subthalamic nucleus than the substantia nigra, axons innervating the former are thicker. This relationship is tightly conserved across species and results in the signals from the thalamus emerging in a precisely timed sequence. DiFiglia wondered if the shrinking observed in HD brains and the high degree of white matter loss could affect this timing, especially if different caliber axons are affected differently.

### **Targeting cell-cell interactions: basal ganglia circuitry in HD**

Resolving questions about how basal ganglia circuitry is altered in HD is key for defining the mechanistic underpinnings of the disease, as well as for identifying therapeutic opportunities and relevant readouts for animal studies, noted Bates. George Rebec’s data, which include extracellular recordings from the striatum, cortex and substantia nigra of live HD mice, provide a valuable starting point for addressing this issue. Rebec reported that the

striatum is the most affected of the regions he analyzed. Firing rates are 5–10 times faster in R6/2 striatal neurons than in wildtype striatal cells. Cortical rates are also faster and, in CAG140 knock-in mice, there is a higher degree of bursting activity. On the other hand, Rebec observed hyper-slow firing rates in the substantia nigra. In addition, using multi-wire recordings, Rebec found that R6/2 striatal firing was noisier than wildtype firing. In wildtype animals, one fourth to one third of neurons show correlated firing, whereas fewer than 5% of these neurons were correlated in R6/2 striata. Increased noisiness was also observed in the substantia nigra.

Levine's findings in brain slices from various HD mouse models—including YAC128, R6/2, and CAG140 mice—are consistent with these observations. At very early stages of the disease, Levine observes spontaneous depolarizations in striatal spiny cells that emanate from the cortex (a similar effect can be seen in normal animals when the cortex is made hyperexcitable using picrotoxin to block GABA receptors). However, this hyperresponsiveness is transient and followed by progressive reductions in spontaneous excitatory currents, as the cortex and striatum appear to become functionally disconnected. The lack of correlated firing seen by Rebec could be an indication of the initial phases of this disconnection, given that individual cortical axons connect with several striatal cells through en-passant boutons.

Together with data from other groups that also implicate early cortical dysfunction, these findings are fueling a shift in focus from the striatum to the cortex as a primary site of HD pathology. For example, studies by Goldowitz's group using chimeric mice with mixtures of wildtype and R6/2 cells, show gliosis, measured as GFAP reactivity, associated with the striatal projections of cortical R6/2 neurons, but not with the area containing the cell bodies of R6/2 striatal neurons. Yang's work with the BACHD model, in which full-length mutant huntingtin can be selectively switched off in specific cell types, also points to the cortex. At 6 months of age—concurrent with the onset of motor deficits—BACHD mice have a myriad of cortical and striatal electrophysiological alterations (as revealed by collaborative work with Mike Levine's and Istvan Mody's groups at UCLA). Genetic analyses are currently underway to assess whether switching off full length mutant huntingtin expression in the cortex may have an impact on the striatal pathogenesis in BACHD mice.

Participants had several suggestions to extend these findings. For example, Wilson proposed examining the histology and electrophysiology of R6/2 cortical innervation of wildtype striatal neurons in Goldowitz's chimeras. In addition, Fischbeck suggested assessing the roles of glia with different genotypes in these animals. Goldowitz noted that because R6/2 glial cells don't have visible aggregates, they are difficult to identify unequivocally, but preliminary data suggest that both wildtype and R6/2 glia contribute to HD-associated gliosis. Yang added that when mutant huntingtin is expressed exclusively in neurons, his team still observes gliosis. To integrate and analyze in greater depth these and other relevant findings, Levine suggested organizing a workshop dedicated to the involvement of the cortex in HD.

In addition to redirecting attention from the striatum to the cortex, Levine urged participants to focus more on interneurons. Although interneuron loss is minimal in HD, altered interneuron function seems to emerge very early on, as indicated by Yang's and Levine's findings discussed above. To examine interneuron changes more precisely, Levine is crossing R6/2 mice with mice that have labeled interneurons. Levine considered that the study of both GABAergic and cholinergic interneurons should be pursued. In addition, he emphasized the importance of performing careful time courses to

clarify which disruptions precede others. Rebec added that it will be important to determine which changes are compensatory.

### **Targeting cell-cell interactions: synaptic disruptions in HD**

Given that the glutamatergic corticostriatal pathway is a key site of HD pathology, compounds that regulate glutamate levels in the striatum may be therapeutically useful. Indeed, Rebec reported that an antibiotic that increases the expression of a glial glutamate transporter can ameliorate HD symptoms. Rebec explained that his team observed low levels of ascorbate in striatal extracellular fluid of R6/2 mice, and wondered if glutamate transport, which is known to be linked to ascorbate, was affected. To test the idea, the researchers treated R6/2 mice with ceftriaxone, a beta-lactam antibiotic known to increase the expression of the glutamate transporter subtype 1 (GLT1). After 5 days of treatment, striatal GLT1 was increased and extracellular glutamate levels were correspondingly decreased. In addition, clasping, rotarod and open field performances improved. Treatment of wildtype mice also increased GLT1 levels, but had no obvious behavioral effects. Rebec is now examining the electrophysiological consequences of the treatment and plans to use capillary electrophoresis to measure extracellular glutamate levels.

Participants discussed the implications of Rebec's results in the context of data from other groups. Levine emphasized the importance of considering timing when comparing data. He noted that changes in glutamate responsiveness in HD are complex and dynamic. In both YAC and R6/2 mice, for example, NMDA and AMPA receptor responses are increased early on, but the hypersensitivity decreases with disease progression and may even turn into hyposensitivity. Young added that Patrick Brundin's findings on excitotoxic vulnerability in HD mice are developmentally-dependent, as are findings from Lynn Raymond's group regarding glutamate responses in different YAC mouse models, noted Levine. Observations on the role of glia in HD were also discussed in relation to Rebec's findings. As noted by Chicurel, Xiao-Jiang Li's proposal that mutant huntingtin expressed in glial cells results in decreased glutamate uptake which contributes to neuronal excitotoxicity, appears to be consistent with Rebec's observations. The proposal is based on data from co-culture experiments indicating that wild-type glial cells protect neurons against mutant huntingtin-mediated neurotoxicity, whereas glial cells expressing mutant huntingtin increase neuronal vulnerability.

Participants also discussed the therapeutic implications of Rebec's observations. Drawing an analogy to many currently used psychiatric drugs which were originally identified in the 1880's but applied to psychiatry only fairly recently, Rebec noted that the full potential of antibiotics is only beginning to be appreciated. He also emphasized that the GLT1 transporter is a good therapeutic target because of its specificity. Fischbeck added that ceftriaxone is currently in clinical trials for amyotrophic lateral sclerosis.

Additional targets involved in synaptic function were described by Hughes. His team identified SNARE complex proteins in screens for huntingtin-associated proteins. When tested in flies by Juan Botas's group, the genes emerged as loss-of-function suppressors of HD neurodegeneration (see *Targeting modifiers of HD: Genetic screens*). The SNARE modifiers seem to work by restoring the normal probability of synaptic vesicle fusion—transmission at the neuromuscular junction of these HD flies, unlike that of wildtype flies, appears to be hyperfunctional, essentially never failing. Although proving huntingtin's specific association with the SNARE complex is difficult because of the complex's intrinsic stickiness, the

fly data strongly suggest SNARE proteins are importantly linked to HD. Moreover, Yang noted his group has also found SNARE proteins in independent studies of huntingtin protein interactions.

A particularly potent and apparently druggable suppressor associated with the SNARE complex, noted Hughes, is the delta subunit of the voltage-sensitive calcium channel (VSCC), the putative target of Gabapentin. To follow up on this finding, Hughes is now interested in testing the effects of gabapentin on HD pathology. Fischbeck added that conotoxin, a more specific drug, may also be useful, at least as a tool for future studies. Hughes agreed, but noted that, as a therapeutic agent, conotoxin is limited because it can cause hallucinations.

Several participants considered that a link between VSCCs and HD is consistent, or at least not in opposition, with available electrophysiological data. Wilson noted that Yang's observations of electrophysiological changes in BACHD mice could be related to the altered function of VSCCs. Furthermore, he noted that gabapentin is used for treating epilepsy and Levine's results indicate HD shares some similarities with epilepsy—at early stages of HD, striatal responses mimic those of normal animals treated with cortical picrotoxin. Rebec and Levine agreed, noting gabapentin may indeed be beneficial for HD. Levine also noted his group performed some experiments using tetrodotoxin in R6/2 slices to dissect synaptic alterations. Although the studies didn't reveal any obvious presynaptic anomalies, they were not comprehensive and didn't include recordings in calcium-free medium.

### **Targeting modifiers of HD: Genetic screens**

Participants agreed that unbiased searches for genetic modifiers of HD, such as the *Drosophila* screen mentioned above, constitute a particularly efficient approach for identifying downstream targets. Hughes emphasized the value of finding non-essential, loss-of-function suppressors of HD as therapeutic targets and noted that ongoing global RNAi screens promise to provide additional targets, as well as confirm ones implicated by other studies.

Genetic modifier studies in humans are also underway. Michael Andresen summarized recent results from a genome-wide single nucleotide polymorphism (SNP) linkage analysis in large Venezuelan HD kindreds to find modifiers of age of onset. Working with over 800 samples and 5000 markers, the team has identified 11 loci with LOD scores greater than 1.5, encompassing approximately 800 genes. One locus, on chromosome 2, has a strikingly high LOD score of over 4 (the odds are 10,000 to 1 against the data appearing as they did if the locus were unlinked). The data replicate some findings from a study with a US population, such as identification of the GRIK2 gene coding for a kainate GluR6 receptor subunit, but important differences exist.

Participants discussed several possibilities for future investigations. Chesselet offered to connect Andresen with a researcher who has access to a family with extremely late onset HD. In addition, Young wondered what fraction of the heritability of age of onset was accounted for by the locus with the very high LOD score. Andresen said this was yet to be established, as well as the prevalence of the different modifier alleles within the population. To determine the effects of different polymorphisms, Hughes and Morimoto suggested using *C. elegans*. Another issue discussed was the extent of the effects of the modifier loci. For example, Finkbeiner wondered if age of onset was the only characteristic affected by the identified loci. Housman noted that only age of onset has been studied, but it will be interesting to analyze other

phenotypes, such as longevity and, as suggested by Thompson, psychiatric symptoms. Andresen noted that, as long as a phenotype can be quantified, it can be analyzed with the collected genotype data.

Much of the discussion of genetic modifiers of HD, however, centered around the process of narrowing down the identified loci to specific genes or regulators. Andresen noted that none of the genes in the highly linked loci are obvious candidates for modifying HD. As next steps, the team plans to compare their data with those emerging from other approaches, and conduct association analyses of candidate genes. Tapscott suggested avoiding an overly gene-centric examination of the data which could overlook non-coding DNA modifiers. Goldowitz proposed using informatics tools and the Gene Ontology resource to help prioritize identified genes. In addition, he noted that talking with Sue Kingsley, leader of the Canadian Pleiades Promoter Project, may be useful. The Pleiades project is composed of a multi-disciplinary group—including informaticists, molecular biologists, geneticists—whose goal is to develop technologies for gene therapy. Of particular interest, they have a procedure to insert genes in cassettes and deliver them to specific tissues. The technology is similar to that used to generate the GENSAT mice, but a wide variety of genes, not only GFP, can be inserted.

Another tool that promises to accelerate the identification of HD modifiers is a knowledge base developed by Christian Neri's group. The database integrates information associated with HD modifiers, spanning studies from yeast to humans, and including data derived from a wide range of approaches, including genetics, proteomics, and interactome analyses. The resulting integration, said Neri, should help researchers conduct hypothesis-driven research from a systems biology perspective. Illustrating the tool's potential, Neri noted that by applying it to his own data (see **Targeting modifiers of HD: Aging and metabolism**), his team has identified 200 candidate HD modifier genes, several of which are located in the loci described by Andresen and colleagues. Neri is examining some of these genes in greater depth, such as the FOXO3A transcription factor for which SNP data are available. To extend the knowledge base's reach, Neri plans to incorporate additional information, including data associated with stress response modulators (see **Targeting the cellular stress response**).

### **Targeting modifiers of HD: Aging and metabolism**

One of the advantages of targeting modifiers of HD, rather than mutant huntingtin itself, is the possibility of finding treatment options with broad applicability to neurodegenerative disorders. For example, targeting proteins that slow aging may have therapeutic value for HD, as well as other neurodegenerative diseases. Indeed, Neri has found that activating the Sir2 sirtuins, a group of NAD-dependent protein deacetylases reported to increase longevity in some animal models, ameliorates HD toxicity. In mammals, the Sir2 ortholog Sirt1 regulates, among other things, the FOXO and PGC-1<sub>α</sub> transcription factors. FOXO proteins act as stress sensors, and control the expression of genes that regulate apoptosis, as well as pathways involved in resistance to oxidative and chemical stress. Approximately 600 genes have been identified as downstream effectors of FOXO and Neri's group has found that 200 of them can act as modifiers of HD, half of which are highly conserved in humans. PGC-1<sub>α</sub> is a master regulator of mitochondrial biogenesis.

Thompson cautioned, however, that Sir2's effects on longevity and neurodegeneration are not entirely understood. Data from her

group and others, for example, indicate that Sir2 pathways can produce opposite effects on longevity and HD neurodegeneration. In *Drosophila*, Thompson observes an increase in lifespan with Sir2 upregulation. However, she observes a decrease in huntingtin-mediated eye neurodegeneration in response to Sir2 downregulation. In addition, as reported by Frederic Saudou and colleagues, activation of insulin growth factor 1 (IGF-1) and its consequent phosphorylation of huntingtin by the serine/threonine kinase Akt/PKB inhibits mutant huntingtin-mediated neuronal death. Yet activation of the insulin pathway and Akt also results in the cytoplasmic sequestration of FOXO which is expected to decrease longevity.

Several factors may contribute to these seemingly incongruous results. Neri pointed out that the IGF pathway has many branches characterized by a high degree of cross-talk. He also noted that Thompson's results could be affected by high baseline levels of Sir2. Inter-species differences may also come into play, as noted by Morimoto. In addition, Sir2 is known to have differential effects on chronological versus replicative aging in yeast, said Thompson. And as noted by Yang, different brain regions expressing distinct sirtuins and downstream effectors may behave differently. Genetic studies in mice may help unravel these uncertainties, he proposed.

Another facet of sirtuin biology that may need to be investigated more fully for the development of HD therapies is its relationship to metabolism. As noted by Yang, resveratrol, a compound that appears to activate sirtuins, has been shown in two recent publications to have striking effects on the longevity of animals eating high fat, but not normal, diets. HD patients are often instructed to eat high fat diets and gain weight because, as noted by Young, clinical observations indicate that increased body weight correlates with milder symptoms. On the other hand, noted Hughes, at least one study reported that caloric restriction ameliorated HD symptoms.

Participants suggested several alternatives to help resolve these questions. For example, Hughes wondered if testing the effects of restricting calorie intake every other day may be worthwhile. Animals put on this regimen are heavier than those that eat *ad libitum*, he noted, but enjoy the longevity benefits of caloric restriction. Thompson added that it will be important to establish whether the effects of caloric restriction change with disease stage. To confirm the correlation between greater body weight and milder symptoms, Young suggested examining the Venezuelan database which includes weight measurements.

Although participants did not spend much time discussing other regulators of metabolism, Kuhn mentioned his group is interested in compounds that regulate PGC-1. PGC-1<sub>α</sub> is an appealing target because, as shown by Dmitri Krainc, it links transcriptional dysregulation and mitochondrial pathology in HD.

### **Targeting environmental modifiers of HD**

Participants agreed that, in addition to genetic modifiers, environmental factors play a key role in determining several aspects of HD. For example, John Mazziotta noted that the quality of caregiving makes a huge difference in HD patients' lifespans after onset. Indeed, people with HD onset in their 30's can live into their 70's or 80's if they receive high quality care. Andresen noted that whereas age of onset is very heritable, life expectancy after onset is not, perhaps because of the overriding effects of quality of care. Cha added that environmental enrichment in mice has dramatic effects on the disease phenotype, ameliorating symptoms, as well as neuropathology. Both Cha and Mazziotta considered that examining caregiving practices more systematically may yield new

insights for improving disease management. However, extending life *per se* should not be the ultimate goal, opined A. Wexler. Slowing progression at the late stages of disease may result in simply extending a person's suffering. Carl Leventhal agreed, noting it will be important to factor in quality of life in such studies.

Participants also discussed the ways in which environmental and genetic factors may interact to influence HD progression and age of onset. For example, Hughes suggested examining longevity in non-affected members of HD families to assess how lifespan modifiers affect HD progression. Shoulson added that although CAG repeat length correlates most strongly with age of onset, some influence of repeat length on progression is observed in populations adjusted for age. Regarding disease onset, Cattaneo asked about the effects of the normal huntingtin allele's repeat length. Andresen noted his group hasn't identified any, but work by Rick Myers suggests they may exist. In addition, A. Wexler wondered if other factors, possibly environmental, affect age of onset. Perhaps new lessons can be learned from individuals with particularly late onset HD.

## **Accelerating flow through the pipeline**

### **Streamlining the transition from animals to humans**

Participants agreed that translating this wealth of information into clinical trials and, ultimately, useful therapies, is key. But several challenges lie ahead. As noted by Johnson, current HD clinical trials involve compounds that have been previously tested, at least to some degree, in humans. Clinically testing new compounds that have never been used in humans, however, will require more work. As described by Kuhn, reliable data, dose-response curves, confirmation of effects in several animal models, pharmacokinetic data and toxicology are key requirements for moving animal studies to humans. In particular, Kuhn emphasized obtaining robust pharmacokinetic and toxicology profiles. It is important to determine that a sufficiently high concentration of the compound can be delivered to the brain and to have guidelines for administration regimens and dosing. Shoulson noted that dosage decisions are usually difficult and often require integrating various sources of data that are neither comprehensive nor totally standardized. Kuhn added that having a reliable pharmacodynamic readout greatly facilitates the process of dosage determination. The optimization of creatine and CoQ10 dosages have benefited from iterated trials, and Johnson wondered if this strategy could be used for other compounds. Shoulson said dosage testing in humans is very constrained by regulatory laws, such that the majority of testing must be done in animals. Yet, as noted by Zaven Khachaturian and Kuhn, even data from monkeys is often not easily extrapolated to humans.

Kuhn also emphasized that toxicology tests should be run early on. His team usually performs a few *in vitro* toxicity tests followed by animal tests, including selectivity profiles, and cytochrome P450 (Cyt P450) assessments. The latter are particularly valuable because Cyt P450s play a central role in the processing, and pharmacological and toxicological effects of drugs. If after conducting these tests a compound is still considered a promising candidate, then more sophisticated assays can be run by specialized companies.

However, Kuhn and Shoulson cautioned that even with extensive safety testing, getting a drug approved by the Federal Drug Administration (FDA) can be very difficult. Shoulson added that the FDA usually requires time-consuming LD50 studies in large

animals such as dogs, even when safety and tolerability have been established in humans. Moreover, although initial toxicology tests are not very expensive, a full-blown toxicology assessment can cost 1–2 million dollars, noted Fischbeck. Khachaturian said the NIH institutes offer grants for toxicology studies, and Fischbeck added that the FDA’s exploratory Investigational New Drug (IND) program can help fast-track drug approval, especially for rare diseases. However, as noted by Kuhn, this program is limited to the micro-dose studies.

Markers to monitor drugs’ engagement of molecular mechanisms, as well as biomarkers of disease (see below) emerged as key tools for moving drugs into the clinic. Although several participants noted that understanding a drug’s mechanism of action is not a prerequisite for clinical testing, the value of being able to assess whether a drug is affecting its predicted target was emphasized. As noted by Johnson and Kuhn, if a drug fails to induce its expected effects, it is important to know whether the failure is due to the drug’s inability to engage its targets (due to delivery issues, e.g.) or because its mechanism of action is fundamentally ineffective at combating the disease. If the former is true, then there may be ways of optimizing drug administration to obtain positive results. If the latter is true, then efforts and resources can be re-directed to ensure that candidates with similar mechanisms of action are not pursued.

Participants agreed that an important step towards optimizing the community’s efforts to move promising compounds into the clinic will be to set up guidelines for the process. As noted by Khachaturian, many promising compounds in the laboratory fail in clinical trials, so it will be important to minimize this attrition as much as possible. Finkbeiner proposed establishing a rational approach for prioritizing compounds, as well as standards for comparing data across studies. The HDF’s contacts at big pharmaceutical companies, such as Novartis, could help shape these guidelines, as well as provide other valuable input and collaborative opportunities, noted Cattaneo and Yang. One possible forum for the publication of the guidelines, noted Khachaturian, is the peer-reviewed journal *Alzheimer’s and Dementia*, a new publication of which Khachaturian is editor-in-chief.

Bates suggested also drawing up guidelines for publishing animal studies. She noted that minimum requirements for publishing pharmacologic data, for example, should be established. Kaemmerer agreed, adding that these guidelines could help prevent situations in which an interesting finding is published without sufficient characterization data important for therapeutic development. There is often little incentive for other scientists to obtain these missing data, which may not even be publishable as a separate article.

Participants also discussed a few ways in which animal studies can be improved for accelerating drug development. Bates suggested storing samples which may be useful for future screens. For example, her team is storing urine samples of mice treated with different compounds which may prove useful for metabolic profiling studies (see below). Yang pointed out the need for better behavioral readouts (particularly cognitive readouts) and predicted that work such as that of Jenny Morton’s group will likely provide important new tools. To facilitate comparisons between studies, Levine recommended characterization of mouse strains that can vary between labs. In particular, he noted that R6/2 mice vary significantly in their repeat lengths, resulting in different electrophysiological and survival time courses.

### **Improving measures of HD in humans**

Participants also noted that improving measures of HD onset and progression in humans should help accelerate flow through the therapeutic pipeline. Because age of motor onset is such a key feature

of HD and has long been used to characterize the disease, it is of interest to examine the strengths and limitations of the methodology used for its determination. Young explained that age of onset is defined by motor, not psychiatric, symptoms because the latter are associated with other common illnesses and are more variable in their expression. For the genetic modifier studies, Housman noted that each subject was evaluated by two neurologists, at least one of them a senior one, and all data were prospective.

Despite efforts to optimize and standardize evaluations, however, participants pointed out some persistent challenges. As noted by N. Wexler, there is a broad range of movement deficiencies in HD and, as noted by Young, several of these deficiencies, in the early stages of disease, are similar to movements of unaffected individuals who are simply fidgety or under stress. If an individual has a drinking problem, the difficulty of diagnosing HD onset is even greater. In addition, Cha noted that he’s observed altered body movements in unaffected spouses of HD patients, even before the patients themselves are diagnosed with HD. Body language and movements are very strong social cues, he stressed. Cha also noted that evaluations across datasets are not identical—for example, the HD-MAPS (Huntington’s Disease Modifiers of Age at Onset in Pairs of Siblings) dataset derives from more neurologists seeing fewer patients than the Venezuela dataset.

Although these diagnostic problems are small compared to those associated with many psychiatric diseases, noted Cha, their effects can be significant. As emphasized by N. Wexler and Young, even a few diagnostic miscalls, plus a few rare genotyping errors, can seriously distort a study’s conclusions. Tapscott wondered if measuring saccades might be a more sensitive and reliable diagnostic tool. Unfortunately, the predictive value of this measure has not yet been systematically assessed, noted Young, and its correlation with HD onset seems to vary with age. In addition, saccade data are not included in the Venezuela dataset.

Hughes noted that another discrete measurement that could be used to characterize HD is lifespan after disease onset. N. Wexler said survival data will be examined in the Venezuelan modifier study, but Housman pointed out several limitations associated with this parameter. Most importantly, lifespan seems to be very dependent on care, even in Venezuela (see *Targeting environmental modifiers of HD*). In addition, because individuals die of causes other than HD, the population available for survival analyses is smaller than that available for motor onset evaluation, thus reducing the study’s power.

Participants also discussed the importance of identifying early, quantitative, and reproducible biomarkers that track disease progression. These biomarkers promise to be particularly valuable for reducing the duration and number of subjects required for evaluating drugs in clinical trials. Shoulson noted that several biochemical markers—including DNA, RNA, and metabolites—as well as neuroimaging markers are currently being evaluated. One of the simplest and most stable markers, noted Young, is 8-OH2’dG, a marker of DNA oxidation that is increased up to four-fold in HD plasma. Although it is not very specific (it also increases in other diseases), it has already proved useful in creatine studies led by Steve Hersch. A more specific biomarker identified by Diana Rosas’s neuroimaging studies is cortical thinning, which correlates strikingly well with HD progression. RNA markers identified by Dmitri Krainc in peripheral blood and Andy Strand in muscle, noted Tapscott, may also prove useful. Such peripheral markers are desirable because of their accessibility, but Patterson cautioned that

they may be limited in their ability to reflect changes mediated by drugs that are delivered intracerebrally. Patterson also noted that identifying biomarkers that track HD progression in mice, as well as humans, will help accelerate the transition from animal to human studies (see *Streamlining the transition from animals to humans*).

As described by Shoulson, the HSG is conducting several observational studies that promise to reveal yet additional markers of HD, particularly presymptomatic markers. For example, Jane Paulsen is leading the PREDICT-HD study, with approximately 800 presymptomatic gene carriers who have never been diagnosed with the disease. Based on the study's findings so far, cognitive measures appear to be particularly useful for monitoring presymptomatic HD and estimating the number of years before clinical onset. Shoulson added that PHAROS, with approximately 1000 individuals at risk of developing HD, but who have never been genetically tested for HD, is also expected to contribute useful biomarker information. The European Huntington Disease Network (EHDN) consortium, a sister organization of the Huntington Study Group in Europe, is involved in biomarker identification as well.

## Concluding thoughts

In sum, participants were optimistic about the status of HD research. The development of strategies to combat HD at its primary source, including RNAi and intrabodies, is now well underway. In addition, multiple downstream pathways and molecules are emerging as feasible therapeutic targets. Modulating the cellular stress response, correcting the BDNF deficiency associated with HD, and targeting synaptic proteins such as glutamate transporters or calcium channels are some of the many encouraging candidates that may yield effective treatments. Furthermore, systematic, unbiased approaches to identify HD modifiers promise to further enrich the opportunities for therapeutic interventions.

A clear indicator of the recent progress made in the field was the priority participants gave to identifying concrete strategies to streamline the translation of lab results into clinical trials. A genuine interest in identifying key requirements and setting up guidelines for scientists to begin moving their data into the therapeutic pipeline was evident. The optimization of this translational process, together with the recent strides in HD research, suggest it may not be very long before the plea for help put forth at the beginning of the workshop is answered.