

World Congress on Huntington's Disease 2007

by Lisa J. Bain

HD in Perspective: Looking Back and Looking Forward

Speaking at the opening session of the 2007 World Congress on Huntington's Disease in Dresden, Germany, **Alice Wexler** described the village of East Hampton where George Huntington grew up and observed the disease that local people called St. Vitus' dance, but would come to be known as Huntington's chorea and eventually Huntington disease. Although known today for its wealth and celebrities, East Hampton in the nineteenth century was a modest farming and fishing village, where several families affected with "St. Vitus's dance" had lived for several generations. The families known to George Huntington were descendents of a woman named Phebe Hedges, who took her own life in 1806 at age 40, evidently because of her dread of the disease that affected her mother at the time, and that she too was starting to develop. Phebe herself was held in great esteem, as were many of her descendents, who were viewed not only as neighbors, but often as leaders in the community.

George Huntington's position as a member of that community, where his father and grandfather had practiced medicine for nearly seventy five years, helped make possible his description of a hereditary pattern that the English biologist William Bateson later recognized as consistent with Mendelian dominant inheritance. Likewise, in 2007, progress continues to be made in the search for treatments through a community wide effort with affected people, family members, scientists, and clinicians working together. Thus, it was fitting that George Huntington's spirit was invoked at the World Congress meeting.

HD-affected families have played a critical role in advancing research and shaping the clinical management of the disease. As the mother of two adult children with HD, **Helga von Wilucki** joined other family members and people affected with HD to start a self-help movement, which in 1979 led to the founding of the International Huntington Association (IHA). In the early days, said von Wilucki, researchers were reluctant to meet with families about their work, resulting in large gaps of knowledge and information on both sides: families not knowing about the latest discoveries and researchers not understanding the patient and family perspective. Since that time, however, the organization has grown from 6 member countries to 47 member countries today, with another 16 countries working to establish membership. More importantly, they have established a collaborative relationship with the research community, resulting in joint meetings with the World Federation of Neurology's Research Group on Huntington Disease, which evolved into the World Congress on Huntington Disease.



The spirit of Milton Wexler, another key figure in HD history, was also invoked at the Dresden meeting. Milton Wexler died on March 16, 2007 at the age of 98, leaving behind a legacy of breaking down divisions between families and scientists, as well as an organization to carry on his work searching for a cure for HD. Wexler, along with daughters Alice and Nancy, had founded the Hereditary Disease Foundation (HDF) in 1968 after his ex-wife and their mother, Leonore, developed HD. Milton Wexler took the concept of free association, which he used in his work as a psychoanalyst, to the HD research community. The HDF convened scientific workshops where small groups of scientists with different perspectives were encouraged to think freely and creatively for solutions to the vexing problems they encountered.

In fact, it was at one of these early HDF workshops that a plan to search for the HD gene was first hatched. **Nancy Wexler** recalled how the idea of looking at DNA variations to find the gene was first suggested at the workshop. "A lot of people thought we had lost our marbles" and that it was a waste of time and money to pursue this line of research. David Housman, however, who had organized the workshop, said that with a large enough family, they might be able to look for variants that would signal where the gene was located. Nancy Wexler found such a family in Lake Maracaibo, Venezuela, and has continued to follow them ever since, making frequent return trips to Venezuela with groups of neurologists who conduct examinations and collect clinical data and blood. It was this work, these families, and their participation in the research that culminated in identification of the gene in 1993. These studies showed that the mutation consists of a large number of trinucleotide (CAG) repeats in one part of the huntingtin gene. While everyone has the huntingtin gene, people with more than 40 repeats get the disease while those with fewer than 35 repeats do not.

The pedigree now numbers more than 18,000 people, of which at least 5,000 have HD or are at risk, and they continue to provide data that clarifies the consequences of the HD mutation. For example, it was these data that documented the relationship between the size of the mutation and the age of onset. Yet repeat length alone does not account for all of the variability in age of onset. Thus, researchers are now studying these same families for clues about genetic modifiers that might contribute to this variability, as well as to other aspects of the disease that vary among people with the gene, such as rate of progression and symptomatology. The hope, said Wexler, is that finding other genes that affect age of onset or progression may lead to new therapeutic strategies.

James Gusella, in providing a geneticist's perspective on "What the Future Holds," affirmed that "it starts and ends with patients and families." HD is a rare disease in more ways than one, noted Gusella. With most diseases, treatments focus on the late stages of the disease process but not before. However, because there is a completely predictive molecular test for HD that will identify who will develop clinical symptoms, researchers and clinicians have the unusual ability to study and try to intervene or ameliorate the disease process from the beginning to the end.



Drugs can be targeted at any stage of the disease process, said Gusella, requiring a different strategy and different approaches depending on the stage. Clinical trials to this point have all targeted the symptomatic phase rather than the beginning of the disease process. Similarly, discovery research has largely been focused on this late stage. Knowing that neurons die in HD, researchers have typically searched for drugs that would save those neurons, rather than those that would halt the disease process long before severe neuronal dysfunction occurs. Refocusing drug discovery efforts on this earlier stage requires a greater emphasis on basic research that relies on what we can learn from genetics and patients, said Gusella. Scientists do not have to be limited by the traditional approach, because they have the opportunity to take advantage of the homogeneous genetic nature of HD, he said.

“HD families still have an enormous amount to teach us, and ultimately the gold standard is what is happening in people,” he said. Everyone with HD starts at the same point, with the mutated gene. Although symptoms are usually not evident for decades, drug discovery efforts have not yet taken advantage of this long window of an ongoing disease process that this purely genetic disorder provides. There is still much to learn about the consequences of the mutation and how it triggers the disorder, the importance of the CAG repeat length, the dominance of the mutated over the normal protein, and the contribution of the remainder of the protein (i.e., the areas other than the polyglutamine repeats). Recognition of these other important questions is needed to change the HD drug development pipeline to reflect a more integrated model that considers the interdependence of events in the disease process at all stages. The drug development process may also be transformed by cooperative projects such as COHORT (HSG) and REGISTRY (Euro HD) that are capturing and sharing biological and clinical data from as many HD patients and family members as possible, which will enable more informed laboratory studies.

Gusella pointed to one other important project that demonstrates the importance of patient, family, clinician and researcher partnerships: the HD-MAPS (HD Modifiers of Age of Onset in Pairs of Siblings) project, which is collecting DNA from pairs of siblings collected by over 20 research groups worldwide. Genotyping of the DNA from 1200 individuals using the latest technology is set to begin soon and will provide a genome-wide assessment of the role of other genes in modifying age of onset in HD. This study, in combination with COHORT, REGISTRY and other cooperative studies, should provide the power that can only come from worldwide collaborations. The modifiers that it identifies act prior to the symptomatic phase of the disease and will therefore provide valid therapeutic targets earlier in the disease process.

Understanding the Biology of HD: Basic Mechanisms

Returning to the importance of understanding the basic biology of HD, **Leslie Thompson** provided an overview of the biological mechanisms that lead “from a faulty gene to a bad

disease.” The complexity of these mechanisms has made the search for treatments more difficult than had been anticipated when the gene was first discovered; however at the same time, multiple mechanisms provide multiple potential therapeutic targets.

Many cellular processes have been shown to be disrupted in HD, among them gene transcription, protein modification, cleavage and degradation of aberrant proteins, neurotransmission, processes regulated by the cytoskeleton such as trafficking of proteins to where they are needed, and energy production. These disruptions are due in some cases to gains of function that result from the expanded polyglutamine repeat and in some cases to a loss of normal huntingtin protein function. Scientists have developed multiple models in which to study these cellular processes ranging from cell lines to a host of animal models. *Drosophila* (fruit fly) models have been engineered to recapitulate many aspects of the disease, and thus provide an efficient model with which to monitor the effects of various compounds. *C. elegans* (worm) models allow visualization of the transition from a protein that is soluble to one that aggregates when the number of repeats exceeds the critical threshold. Mouse models have helped scientists understand the effects of the mutation on behavioral and cognitive function, survival, gene expression, and many other pathogenic pathways that are affected in HD. Moreover, these mouse models have been used extensively as pre-clinical models to test the effectiveness of various compounds.

Thompson discussed how these models have been used to clarify the biological mechanisms underlying HD. For example, Erich Wanker has demonstrated in yeast that the huntingtin protein interacts with many cellular proteins, and Robert Hughes has shown that many of these interactions appear to be functionally relevant, possibly explaining why there are so many cellular processes affected. Other researchers have studied the aggregation of the huntingtin protein and the resulting intra-nuclear inclusions that characterize the diseased brain. Still others have shown that impaired mitochondrial activity is an early hallmark of HD and continue to investigate whether mitochondrial damage is a primary event or secondary to some other malfunction such as misregulation of transcription. Transcriptional dysregulation itself has been the focus of much research, with many possible pathways emerging as potentially relevant, including pathways involved in cholesterol metabolism, signal transduction, BDNF (brain-derived neurotrophic factor, a protein that is involved in the growth and health of neurons), neuroinflammation, and mitochondrial function. An emerging area of research focuses on post-translational modifications to the huntingtin protein, which may confer either protective or toxic properties to the protein.

The tendency of the mutant huntingtin protein to form aggregates long been thought be a central mechanism underlying the disease process. As a result, inhibiting the formation of aggregates or increasing their disposal have been investigated as important therapeutic strategies. **Ron Wetzel** has been studying the biophysical properties of the huntingtin protein and the proteins involved in other neurodegenerative diseases that misfold and aggregate in cells.



Proteins are synthesized as long strings of amino acids, but their natural tendency is to fold into more compact, lower energy states. According to Wetzel, protein folding follows a set of biophysical rules. Understanding what those rules are, and what might lead to a protein misfolding, may lead to a better understanding of how the mutant protein disrupts normal cellular processes. Cells have elaborate mechanisms to deal with the aggregates that inevitably form in side reactions during protein folding. These mechanisms include molecular chaperones that can salvage misfolded proteins, the ubiquitin-proteasome system that recognizes aggregates as unwanted and digests them, and aggresomes and a process called autophagy that collects and disposes of aggregates too large for the proteasome. Normally these pathways are able to deal with aggregates and misfolded proteins that form, but Wetzel and others believe that in HD and other neurodegenerative diseases, these systems are overwhelmed by the large number and many different species of aggregates created. Which of these species (e.g., protofibrils, oligomers, amorphous aggregates, etc.) are responsible for toxicity is another important question under investigation.

Aggregates might lead to neuronal death and dysfunction through a number of possible mechanisms: A breakdown in the ubiquitin/proteasome system could upset homeostasis in the cells. Large aggregates may also encroach on cellular process such as trafficking of proteins around the cell or at the synapses between neurons. They may also recruit and sequester other proteins that are needed for other cellular functions. The body might launch an uncontrolled inflammatory response against aggregates and this could lead to a host of downstream effects. Aggregates may also lead to mitochondrial dysfunction or may alter specific enzyme activities. Each of these mechanisms suggests one or more possible therapeutic strategies. This include inhibiting the proteolytic events that lead to the toxic species; stimulating the formation of, or stabilizing non-toxic species; stimulating removal or breakdown of the aggregates; or blocking the downstream effects of the cytotoxic mechanism that is activated by aggregates.

While dysfunction and death of neurons in the striatum have long been looked upon as the key events in HD, recent studies have focused attention on the cortex and the corticostriatal pathways as equally important in the neurodegenerative process, since this pathway sends excitatory signals from the cortex to the striatum. **Michael Levine** and colleagues have been examining changes in brain circuitry in mouse models of HD using an electrophysiological approach. Their studies have confirmed that the corticostriatal circuits are influential in HD, and that in HD mice, a loss of excitatory input in the striatum as the phenotype progresses is accompanied by opposite effects in the cortex. This makes sense, said Levine, when you think about synapses. Synapses are the points where neurons meet and communicate with adjacent neurons, sending their impulses from one neuron to the next. In the HD mouse, there is a loss of synaptic markers and spines in the striatum, such that even when the cortex becomes hyperexcitable, the striatum is disconnected. Levine and colleagues also have shown that there are properties intrinsic to striatal neurons that are disrupted in the presence of the HD mutation. As a

result of all these changes, the firing patterns of striatal neurons are disorganized in HD mice *in vivo* as shown by George Rebec's laboratory and striatal outputs become abnormal. Moreover, it appears that these dysfunctions precede neurodegeneration and may underlie the development of symptoms.

Experiments by Levine and colleagues using mice generated by Michelle Gray in William Yang's laboratory have further shown that if you take the mutant gene out of the cortex, you obtain recovery of some of the electrophysiological properties in the striatum, supporting the idea that when looking for therapeutic targets, it will be important to look beyond the striatum.

Helping HD: Clinical Studies and Trials in HD

All these efforts to better understand the biology of HD have as their goal the discovery of means to treat and ultimately cure the disease. In preparation for clinical trials of potential treatments, HD researchers have been attempting to identify early, pre-diagnostic markers of the disease that would allow them to determine when a treatment should be started and whether it is having its desired effect. Potential biomarkers include compounds that could be measured in blood, urine, or other body fluids; results from imaging studies; and measures of specific motor and cognitive abilities.

The Predict-HD study was designed to identify these early markers of HD. **Jane Paulsen**, principal investigator of this large, multi-center, multi-disciplinary, longitudinal project said that the design of the study came, in part, from families coming to her with ideas about subtle signs that might signal development of the disease. Pragmatic issues also played a central role in designing the study. "These are individuals in the prime of their lives," said Paulsen. So although the investigators wanted to gather as much data as possible, their desires were tempered by concerns about how much testing the participants would be willing to tolerate. In fact, one of the main goals of the study was to recruit and maintain the involvement of an adequate number of participants, and this goal has been accomplished. "It really speaks to what Jim Gusella was saying this morning about collaborative research," said Paulsen. "The days of scientists isolated from families is gone. These families have been remarkable."

Other goals have also been achieved, among the most important of them a reduction of up to 40% in the needed sample size for a clinical trial of a treatment for pre-symptomatic HD subjects. This reduction is possible because when investigators can limit a clinical study only to those individuals who will develop HD over the next couple of years, fewer subjects are needed. The study has also identified and validated markers of disease progression, validated the formula used to predict at what age a person will develop symptoms of HD based on their CAG repeat size, and developed a wide database of MRI scans, biological specimens, and clinical assessments.



Paulsen presented data on cognitive predictors of HD. Eighteen different neurocognitive tests were evaluated in 543 gene-positive, pre-HD participants. During the 4-year study period, 68 of these individuals converted to a definite diagnosis of HD. This allowed the investigators to assess which of the 18 tests had the best predictive value. What they learned was that even after the participant's CAG repeat length, current age, results of their motor exam, and results of their MRI scan were considered, results from 5 neurocognitive tests increased the probability of receiving a diagnosis of HD. By further refining the ability to accurately predict how close a person is to diagnosis, these results will improve the efficiency of preventive clinical trials and reduce the number of subjects needed.

Even better tools are still needed, said **Julie Stout**, another investigator involved in the Predict-HD study. Stout described the HD Toolkit project, which applies the principles of evidence-based medicine to the identification of assessment strategies. After conducting a systematic review and meta-analysis of the published literature on HD studies that used cognitive, motor, neuropsychiatric, behavioral, functional, and quality-of-life assessments, Stout and colleagues rated all these assessments according to their sensitivity. A test was rated as having "proven sensitivity" if it detected decline in pre-HD subjects over 2-4 years and if this decline was demonstrated in more than 30 samples. Lesser ratings were "probably sensitivity," "promising," "not recommended," and "proven insensitivity." They identified 9 measures, all in the cognitive and motor domains, that show proven sensitivity in detecting a decline in pre-HD subjects over 2-4 years. Few tests were categorized as having proven sensitivity in the other categories.

The team is still evaluating the redundancy of these measures, trying to build a cognitive battery that will achieve the maximum sensitivity with the fewest tests. Stout said that more tests of executive function are needed in addition to functional and behavioral measures. "There is still a lot of work to be done, and lots of participants are needed," she said.

Brain imaging tools not only provide information about disease progression, but also can help explain the neurological basis of the clinical abnormalities seen in HD, said **Diana Rosas**. Atrophy in the striatum has been proposed as a useful biomarker for assessing pre-symptomatic disease status. But atrophy actually occurs in all brain structures, including the cortex, white matter, and subcortical structures. Moreover, cortical changes during the early symptomatic stages may help explain why, despite more than 50 percent striatal loss at the time of diagnosis, clinical symptoms continue to progress as further loss occurs and spreads. Rosas analyzes magnetic resonance images (MRI) using segmentation and surface reconstruction techniques to create 3-D images that show thinning across different regions of the cortex in pre-manifest HD subjects. These images show the most extensive thinning in the motor, sensorimotor, and occipital areas of the cortex, with preservation of the frontal cortices.



Rosas also uses another MRI technique called diffusion tensor imaging (DTI) to assess the microstructural anatomy in the white matter of HD brains. The white matter is where neurons connect with other neurons to create the complex circuitry necessary for normal cognitive and neuropsychological function. DTI assesses the fractional anisotropy, or connectivity, in the brain. This technique shows that there is some disorganization even in pre-manifest HD subjects, and that it progresses to become much more severe in people who have been diagnosed with HD. This may help explain some of the clinical features of HD that have previously been unexplainable, and also may lead to treatments that exert their effects specifically in vulnerable brain areas.

“We need to understand the ‘where’ in order understand the ‘how’, ‘why’, and ‘when’” said Rosas. In addition to increasing overall understanding of HD pathogenesis, both DTI and cortical thickness measures may prove useful as biomarkers of HD.

At the same time that investigators such as Paulsen, Stout, and Rosas are developing better tools to assess disease progression, others are searching for compounds to treat the disease, and some compounds have already entered clinical trials. **Robert Pacifici** presented an update of the work CHDI, Inc. is doing in the area of drug discovery and development. Since putting together a strategy document in 2005, CHDI has progressed to the point where they have begun to demonstrate statistically significant robust effects of some compounds in animal models.

“We have a pipeline, but as we all know, the failure rate for drug discovery is incredibly high,” said Pacifici. “We need to have multiple shots on goal.”

CHDI’s strategy encompasses multiple parallel approaches that target the entire range of mechanisms that were discussed earlier by Leslie Thompson. They begin with target identification and validation, then proceed to develop screens based on those targets; use those screens to identify “hits” and then lead compounds; optimize lead compounds and select the best candidates from those optimization studies; conduct long term toxicology studies; and finally conduct clinical trials. Targets must be both biologically validated and chemically tractable, or “drugable.” For example, the huntingtin protein is the most well validated target, in that it clearly leads to the disease, but is not easily modified through chemical means. Other targets, such as caspase 6, an enzyme that cleaves the huntingtin protein into toxic fragments; and transglutaminase, an enzyme involved in the aggregation of huntingtin protein, are more drugable targets.

In order to find a pharmacological agent that will hit a target, CHDI assembles a team of internal personnel and external partners in academia and industry that bring to the table the best practices to address all aspects of a particular target/drug interaction. For example, in searching for a caspase 6 inhibitor, questions that must be addressed include whether it is actually caspase 6 and not another member of the caspase family that is responsible for generating toxic fragments; whether an inhibitor selective for caspase 6 can be found, and if such a molecule is found, whether it penetrates into the brain; and



whether caspase 6 might have a necessary function such that inhibition of the enzyme could have deleterious consequences.

In selecting targets to pursue, Pacifici said there is nothing more valuable than observations in humans. So, for example, after Coenzyme Q10 showed some signs of efficacy in the human trials, CHDI began to explore whether modifications could be made at the molecular level that would result in properties that might improve efficacy by altering the subcellular distribution of the drug, its penetrance into the brain, or its potency.

Meanwhile, interventional trials continue around the world. **Ira Shoulson** outlined the recently completed and upcoming interventional trials conducted by the HSG, and **Bernhard Landwehrmeyer** summarized those trials conducted by EHDN. Most of these trials have enrolled subjects with manifest HD and are aimed at improving symptoms. However, there are now efforts to begin conducting neuroprotection trials in pre-manifest subjects. The results of studies like PREDICT-HD, described earlier by Jane Paulsen, have provided the information necessary to design trials in these subjects, which take significantly longer to complete.

Name of Study	Drug	Status	Results	Notes
PHEND-HD	Phenylbutyrate	Phase II complete		HSG
TREND-HD	Ethyl-EPA	Phase III complete	No significant benefit	HSG & EHDN
DIMOND-A	Dimebon	Phase I-IIA complete	Safety & tolerability confirmed	HSG
TETRA-HD	Tetrabenazine	Phase III complete	Reduction of chorea	HSG
EHDI	Riluzole	Phase III complete	No significant difference in TFC, CGI, or motor score	EHDN
DOMINO	Minocycline	Phase II active		HSG
DIMOND-B	Dimebon	Phase III active		HSG
2-CARE	Coenzyme Q10	Phase III upcoming		HSG
CREST-E	Creatine	Phase III Upcoming		HSG
PREQUEL	CoQ + upiquinone in	Upcoming		HSG



	pre-manifest HD			
ACR-16	ACR-16, a dopaminergic stabilizer	Phase III upcoming		NeuroSearch

Intervention trials need not necessarily involve drugs. **Monica Busse** discussed the use of physical therapy (PT) as a means of improving movement and function in people with HD. There is anecdotal support for PT as a potential intervention, however a systematic review of PT intervention trials showed insufficient evidence to support the routine implementation of PT in HD patients. Confounders to such studies include the difficulty of quantifying the “dose” of the intervention, the lack of reliable and valid outcome measures, lack of clarity about what would constitute a clinically significant outcome, and the lack of a meaningful control intervention. Busse and colleagues at Cardiff University in Wales have been working to change this by focusing on developing task-specific interventions and understanding disease-specific impairments. In longitudinal studies, they are evaluating PT assessment tools to determine which will provide robust outcome measures, and they are also examining the content of physiotherapy according to the stage of the patient’s condition and the level of impairment. This work aims to provide concrete evidence to support the use of PT, and to design future clinical trials to assess its overall effectiveness.

Patients with HD may also be helped by day-care rehabilitative approaches that combine occupational therapy, physiotherapy, speech and swallowing therapy, medication, neuropsychological interventions, and social work, according to **Herwig W. Lange**. Assessment of this type of intervention, however, faces similar obstacles as discussed above for PT. In one small study, no significant benefit was shown, although there were some interesting trends toward a decrease in motor score, an increase in cognitive score, and a lessening of depression, said Lange.

Pathogenesis and Pathophysiology of HD – The HD gene

At the first scientific session, investigators presented more detailed information about the various mechanisms that may underlie HD pathogenesis. **Beverly Davidson** started out by discussing how RNA interference (RNAi) might be used to target the huntingtin gene itself as a means of silencing the gene and removing the downstream toxic effects. RNAi was only discovered in 1998 but has rapidly become one of the most exciting areas of scientific investigation, both for understanding the biological function of genes and as a possible therapeutic strategy. Davidson’s lab designed short hairpin RNAs (shRNAs) against segments of the huntingtin gene, which when delivered using a viral vector to HD mice, resulted in reduced expression of the huntingtin protein and an improvement in the motor behavior of the mice, although many of these shRNAs resulted in significant toxicity in the brain. The observed toxicity prompted the scientists to investigate micro RNA (miRNAs), which are naturally occurring, small non-coding RNA sequences that

have recently emerged as key players in gene regulation. They produced artificial miRNAs and showed that, in comparison to shRNAs, miRNAs reduced the production of huntingtin protein to a similar extent but showed less toxicity.

These studies raise the question of whether miRNAs, which may be embedded within the huntingtin gene, contribute to neurodegeneration in HD and other diseases. Davidson has shown that at least one miRNA (mir128b) is reduced by as much as 30% in HD tissue. She hypothesized that normal brain signals in the cortex may lead to the expression of this miRNA, which suppresses the expression of certain proteins and promotes neuronal survival; but that in HD, there is a reduction in expression of mir128b, resulting in neuronal death. If this indeed is the case, mir128b and other inhibitory RNAs may represent novel drug targets for HD.

Another aspect of the huntingtin mutation that may hold clues about pathogenic mechanisms is somatic instability of the CAG repeat. Somatic instability, in which the CAG repeat expands, can result in longer CAG repeats in certain tissues, which may affect the toxicity of mutant protein and thus pathogenicity. According to **Gillian Bates**, CAG repeat mosaicism (different CAG repeat lengths in different tissues) has been observed in mouse models and in human tissue, with characteristic regional and temporal patterns. Her lab has been studying the phenomenon in three mouse models that have different CAG repeat lengths and that develop disease at different time points. In both mice and humans, larger repeats are seen predominantly in neuronal cells; and in Bates' mice, more instability is seen in mice who live longer, indicating that this instability is related to age rather than to the stage of the disease. Bates has also shown that the expansion of CAG repeats occurs synchronously in terminally-differentiated neurons and that more instability is seen in the striatum in comparison to other brain regions. These observations dissociate instability from cell division. Regional differences in the rate of instability may be due to specialization of DNA repair mechanisms, said Bates, noting that the mismatch repair enzyme MSH3 is expressed predominantly in neuronal cells in the striatum. With advancing age, increased oxidative damage may put more strain on these DNA repair mechanisms. Taken together, these observations suggest that somatic instability may act as a modifier of disease onset and progression.

Pathogenesis and Pathophysiology of HD – the Protein

Moving beyond the mutant gene, a series of presentations were delivered on properties and interactions of the mutant protein that influence pathogenesis, including proteolysis, post-translational modifications, aggregation, interactions with other proteins, and the consequences of all of these events.

Ron Wetzel has been studying the mechanism of polyglutamine aggregation both in vitro and in cell models. Misfolding and aggregation of exon 1 of the huntingtin protein (the segment of the protein that contains the polyglutamine repeats) in neuronal cells has long been suspected of playing a central role in HD pathogenesis, although the complexity of



the aggregation process has made it difficult to prove or disprove. Wetzel's earlier work demonstrated that aggregation of simple polyglutamine sequences begins with the formation of a nucleus, followed by elongation to form a growing amyloid-like fibrillar aggregate. In contrast, work in other labs with huntingtin exon1 revealed more varied aggregated products, including, besides fibrils, spherical oligomers and protofibrils. Recently Wetzel's lab has been studying the aggregation of chemically synthesized models of huntingtin exon1 in vitro, as well as monitoring the aggregation of expressed exon1 in cells using a novel staining procedure. In these studies they observed a diverse collection of aggregated species, rather than the homogeneous fibrils obtained previously with simple polyglutamine. This work implicates flanking sequences next to the polyglutamine segment of exon1 as adding complexity to the aggregation mechanism and aggregation products both in vitro and in cells. It thus points the way to further research that should clarify the mechanism of aggregate formation and its role in the disease process.

Yvon Trottier, another proponent of aggregation as an important pathogenic event, has been looking specifically at the role of expanded polyglutamine in the aggregation process. In HD and other CAG-repeat disorders, neurotoxicity occurs when the polyglutamine length exceeds a certain threshold, suggesting that polyglutamine becomes toxic when it reaches the minimal size to form a stable pathogenic structure, which promotes aggregation. Trottier, however, showed that expanded and non-expanded polyglutamines share similar structural properties before and during aggregation; his data suggest that the polyglutamine length influences the kinetics of aggregation and the stability of aggregates but not their structure. Trottier proposed an alternative model in which toxicity gradually increases as polyglutamine length increases and is modulated by the protein context; and that the toxicity threshold is reached when the cellular processes designed to protect cells against the effect of aggregates are overwhelmed.

The intracellular protein aggregates seen in HD include, in addition to the huntingtin protein itself, a number of other proteins including molecular chaperones, which appear to also play important roles in pathogenesis. **Harm Kampinga**, calling chaperones the "guardians of the proteome," said that emerging evidence suggests that chaperones differ both in their intracellular localization and in functionality. Using a reverse genetic overexpression screen, Kampinga and colleagues identified a new set of non-canonical Hsp40 family members that are potent suppressors of polyglutamine aggregation and toxicity in both cell and in vivo models, suggesting that these chaperones may represent a novel therapeutic target.

Apoptosis, one type of programmed cell death, is another cellular process that is influenced by the mutant huntingtin protein. According to **Joel Ybe**, dissociation of the huntingtin protein from its binding partner HIP1 (huntingtin-interacting protein 1) initiates a pathway that leads first to the binding of HIP1 to another protein called HIPPI, and eventually to cell death by apoptosis. The expanded polyglutamine stretch in the huntingtin protein interferes with its binding to HIP1. Ybe and colleagues have been

studying the crystal structure of the binding region of HIP1 to HIPPI and recently solved the structure to 2.8Å resolution, revealing structural features that may help explain, at a molecular level, how the mutant huntingtin protein causes cell death.

In humans and mouse models, the intranuclear inclusions seen in HD brains consist of N-terminal fragments of the huntingtin protein, which are generated when proteolytic enzymes such as the caspases cleave the huntingtin protein at specific cleavage sites. The resulting fragments are thought to have different propensity for forming inclusion bodies as well as different levels of toxicity, so there has been much effort directed at understanding how the huntingtin protein is cleaved and what fragments are created. Five different caspase cleavage sites have been identified in the huntingtin protein, said **Michael Hayden**: 3 cleavage sites (513, 552 and 586aa) have been shown to be cleaved *in vitro* and *in vivo*, while 2 sites are silent (530, 589aa). Cleavage at each of these sites yields fragments of different sizes. Working in a yeast artificial chromosome (YAC) mouse model of HD that includes the full-length huntingtin gene with 120 CAG repeats and recapitulates the phenotype of HD, his lab has shown that in mice with a mutation that prevents cleavage by caspase 6 at amino acid 586, the pathological features of HD are ameliorated. Further characterization of these caspase-6 resistant (C6R) mice has shown that they are also insensitive to NMDA-induced excitotoxicity, demonstrating that the 586aa fragment plays a critical role early in the disease process. Of note, his lab has shown that excitotoxic stress triggers caspase-6 amplification. C6R mice are also protected against abnormalities seen in the forced swim test, which is a measure of depression and anxiety in mice. Interestingly, C6R mice have a significant increase in neuronal inclusions, suggesting that while different fragments may all lead to inclusions, only specific fragments, such as the one generated by caspase-6, are toxic.

Although definitive experiments have not been conducted that would prove caspase 6 is the enzyme responsible for cleavage at amino acid 586, experimental evidence strongly suggests that it is. In patients, an increase in caspase 6 activity is seen in the striatum, and caspase-6 activation is an early marker of neuronal dysfunction in YAC128 mice and aged individuals. Hayden's lab is now working to identify inhibitors of caspase-6 that might be useful therapeutically.

Cleavage is not the only process that alters the newly synthesized huntingtin protein. A number of chemical modifications can be made to the protein, and these modifications can alter the protein's function, localization, and interaction with other proteins. Thus, post-translational modifications can influence pathogenesis and introduce new potential therapeutic targets. **Leslie Thompson** summarized the post-translational modifications to huntingtin that appear to influence the accumulation of the protein and contribute to cellular dysfunction. For example, phosphorylation of the amino acid serine at two different positions within the protein increases nuclear localization, and thus could mediate movement of the protein into the nucleus. Other post-translational modifications that affect various functions of the protein include SUMOylation, ubiquitination, palmitoylation, and acetylation. Each of these modifications involves adding a chemical



group or even linking another peptide to the huntingtin protein, thus changing its properties. SUMO modification, for example, affects protein stability, interactions with other proteins, subcellular localization, and transcriptional regulation. Further, oxidative stress has been shown to increase SUMOylation, which may help explain how oxidative stress leads to cellular dysfunction.

Pathogenesis and Pathophysiology of HD – Beyond the Huntingtin Gene and Protein

Downstream consequences of HD gene and protein expression that are thought to be important in HD pathogenesis and that were discussed at the Congress include transcriptional dysregulation, excitotoxicity and mitochondrial dysfunction.

Although altered gene expression has been demonstrated in both human HD brain tissue and in various model systems and could help explain a number of the phenotypic features of the disease, the mechanism of transcriptional dysregulation is unclear. **Jang-Ho Cha** and colleagues have shown that the neuronal intranuclear inclusions (NII) do not themselves cause transcriptional dysregulation of genes that are known to be downregulated in HD, such as the dopamine D2 receptor gene. Rather, their studies suggest that downregulated genes, but not normally expressed genes, have decreased association with the transcription factor Sp1. They have also explored the role of histone modifications in gene regulation. Histones are proteins that combine with DNA to form the chromatin complexes that make up chromosomes. Chemical modifications of histones (such as acetylation and ubiquitination, discussed above) are known to change the chromatin structure and regulate the transcription of genes. Cha's studies show that in the brains of HD mice, downregulated genes have decreased acetylation of histone H3, increased monoubiquitylation of histone H2A, and decreased monoubiquitylation of histone H2B. These modifications may explain the repression or activation of different genes in HD and also provide therapeutic targets. Histone deacetylase (HDAC) inhibitors have already been shown to improve the phenotype in HD mouse models. These studies may help explain how these drugs work and suggest how their effectiveness could be improved.

Cha also offered evidence for another possible mechanism of transcriptional dysregulation in HD. His studies show that the huntingtin protein itself binds to DNA, and that there are different binding sites for wild-type and mutant huntingtin protein. He suggested that direct binding of huntingtin to DNA might regulate transcription of various genes.

Excitotoxicity has long been thought to play a role in neuronal death. **Michael Levine** and colleagues have been studying what excitotoxicity does to cells before they die; that is, what kind of functional alterations occur that may explain the phenotypic progression of the disease. As these mechanisms become clarified, they may suggest new therapeutic targets. Excitotoxicity results from increased release or decreased reuptake of the



excitatory neurotransmitter, glutamate; or from alterations in glutamate receptors, particularly the NMDA receptor; or alterations in calcium homeostasis as a consequence of activation of glutamate receptors. Levine has been looking at the change in glutamate receptor-mediated electrophysiological responses in HD mouse brains. He has shown that when exposed to NMDA, subpopulations of striatal cells have heightened responses and that this appears to be related to a change in sensitivity to Mg^{2+} . Further, he has shown that this increased response is lost as the phenotype progresses, suggesting that excitotoxicity occurs early during development of the phenotype and that therapies aimed at the glutamate receptor or glutamate release will have to be given very early in the disease process, before the glutamate receptors have lost their responsiveness.

Mitochondrial toxicity has also been proposed as a mechanism of neurodegeneration. **Frank Gellerich** and colleagues have developed a rat model of HD with only 51 CAG repeats to more accurately model the adult form of the disease. They isolated and examined mitochondria in these mice before and after 21 months of age, and showed that in the older rats, the rate of Ca^{2+} accumulation was decreased along with a decreased threshold for the mitochondrial permeability transition (MPT), which has been implicated in neurodegeneration. These changes in mitochondrial properties were normalized in vitro with cyclosporin A. Gellerich and colleagues concluded that the mutated huntingtin protein interacts with Ca^{2+} binding sites in the mitochondrial outer compartment, causing an impairment in Ca^{2+} regulation and oxidative phosphorylation, which leads to energetic depression, cell death, and tissue atrophy; and that these impairments should be suppressed with cyclosporin-like drugs.

In the final presentation of this session, **Eduardo Gonzalez-Couto** discussed an in silico modeling technique used to develop protein-protein interaction networks into pathways integrating the various pathogenic pathways demonstrated in HD. He has used this technique to combine experimental proteomics data acquired in house with information gleaned from the literature. The pathway diagrams developed with this technique not only promote and support interdisciplinary research but also can be used as tools for target identification in drug development.

Inflammatory and Metabolic Alterations in HD

Immune dysfunction, both in the CNS and the periphery, has recently begun to gain attention as a pathogenic mechanism in HD. In the process of searching for plasma biomarkers of HD through proteomic profiling, **Edward Wild** and colleagues identified a group of 18 proteins that were differentially expressed as the disease progressed, most notably alpha-2 macroglobulin, clusterin, and components of the complement cascade. What struck the investigators was that so many of the proteins they identified are involved in the innate immune response. So rather than look at these proteins individually, they examined levels of the related molecule interleukin-6 (IL-6), which drives the acute phase inflammatory response, driving up levels of the proteins they had identified. Using an enzyme-linked immunosorbent assay (ELISA), they showed that IL-



6 levels increase as the disease advances in both humans and mouse models of HD, suggesting that innate immune activation is a characteristic and quantifiable feature of HD. In addition to immune activation, IL-6 is also involved in the regulation of energy balance and in the release of corticosteroids, through its action on the hypothalamic/pituitary/adrenal axis, suggesting that IL-6 activation may be responsible for phenotypic features of HD such as unexplained weight loss. What causes the increase in IL-6 has yet to be identified.

One of Wild's collaborators, **Maria Björkqvist**, extended this study to look at other factors that are involved in the peripheral immune response in HD patients and mouse models. Using a technique called electrochemoluminescence, Björkqvist and colleagues showed that not only IL-6, but also IL-8 and tumor necrosis factor alpha (TNF- α) track with disease progression. Other cytokines were also altered, but IL-6 and IL-8 were the most significantly correlated. Björkqvist said this could reflect either central nervous system pathology, with spillover of these factors into the periphery; or there may be an inflammation in the periphery that plays a role in HD pathogenesis. Measurement of IL-6, IL-8, and IL-10 may also be useful as biomarkers.

Inflammation within the CNS, or neuroinflammation, may also contribute to HD pathogenesis. Neuroinflammation, an innate immune response in the brain, results in increased vascular permeability, invasion of immune cells, and release of inflammatory mediators. Chronic neuroinflammation, more typically associated with diseases such as multiple sclerosis, has also been linked to neurodegenerative diseases including HD. Microglia, the resident immune cells of the CNS, have emerged as the principal mediators of the neuroinflammatory response. They are the smallest type of glial (non-neuronal) cells in the brain, with phagocytic as well as other immunological properties. Positron emission tomography (PET) scanning using a ligand that binds to activated microglia suggests that activation of microglia is an early sign of HD, according to **Thomas Möller**. His lab has studied 20 human HD post-mortem brain samples to determine regionally specific levels of inflammatory markers and growth factors such as TNF- α , IL-6, IL-8, and BDNF. These studies show a very distinct inflammatory pattern in the HD brain, with elevated expression of certain markers correlating with disease progression, and with dysregulation much more pronounced in the striatum as compared to the cortex or cerebellum. Möller said his lab is now set to confirm these results in animal studies and will also conduct future studies to examine pre-manifest tissue. Diana Rosas suggested that he also look further into other parts of the cortex.

Meanwhile, **Paul Muchowski** has been exploring microglia signaling pathways as a potential therapeutic target. Muchowski focuses on the kynurenine pathway, which is localized in glial cells and is the main route of tryptophan degradation; and in particular on one enzyme of the kynurenine pathway, the 3-HK biosynthetic enzyme kynurenine 3-monooxygenase (KMO). In humans, KMO is expressed exclusively in microglia, but it catalyzes the formation of two toxic metabolites (3-HK and quinolinic acid) that can act on neurons by causing excitotoxicity and mitochondrial stress. Robert Schwarcz



previously showed that injection of low quantities of quinolinic acid into the striatum of rats can produce lesions that are remarkably similar to those found in HD patients. A small molecule inhibitor of KMO called Ro 61-8048, which was shown to be orally active and neuroprotective in animal models of brain ischemia, had been identified by Roche Pharmaceuticals. In a small pilot study led by David Howland (HighQ Foundation) and Liliana Menalled (Psychogenics) Ro 61-8048 showed promising results in the R6/2 mouse model of HD. However, it was later shown that this KMO inhibitor did not penetrate the blood-brain-barrier efficiently. Therefore, Paul Muchowski collaborated with his father, Joseph Muchowski, an organic chemist with expertise in drug development, to synthesize novel brain-penetrating KMO inhibitors. One of these inhibitors, which they call JM6, had a dramatic, significant, and dose-dependent effect on survival and stabilization of body weight in R6/2 mice. Indeed, in this study 40% of mice treated with JM6 survived nearly 50% longer than R6/2 mice fed a vehicle control. They are now evaluating JM6 in other mouse models.

Metabolic derangements have long been noted in HD patients, manifesting primarily as weight loss. HD mice also exhibit metabolic symptoms, including “adaptive thermogenesis,” wherein cold temperatures bring out the HD phenotype while warm temperatures extend survival. These metabolic derangements have been attributed to mitochondrial dysfunction, although the molecular basis of this has been unclear. **Patrick Weydt** presented data showing that PGC-1 α , a transcriptional coactivator that is expressed in brown adipose tissue, is not only a key mediator of adaptive thermogenesis via its role in mitochondrial function, but also regulates transcription, thus linking these two key features of HD to a common pathway. For example, in mice subjected to a cold challenge, many PGC-1 α -dependent genes are down regulated in the striatum, suggesting that the striatum is vulnerable to loss of PGC-1 α function. Whether PGC-1 α is expressed at lower levels in HD mice or its function is in some other way compromised has yet to be determined. In either case, the PGC-1 α pathway may reveal novel therapeutic targets as well as potential biomarkers. Some studies suggest that it may also function as a genetic modifier of disease onset or progression.

Hypothalamic dysfunction is another non-motor feature of HD that has not been well studied, although data suggest that it could be responsible for psychiatric symptoms as well as weight loss, sleep changes, and circadian rhythm changes that are seen in HD. **Åsa Petersén, Ahmad Aziz, and Marios Politis** all presented research that supports a role for hypothalamic dysfunction in HD. Petersén said that hypothalamic atrophy and cell loss have been observed in MR images from HD patients; and both HD mice and patients also show a loss of orexin-and somatostatin-containing neurons in the hypothalamus. Orexin (also known as hypocretin) is a neuropeptide thought to play an important role in sleep regulation. Aziz also looked at orexin neurons, as well as melanin-concentrating hormone (MCH) neurons. These two populations of neurons are both found in the hypothalamus and both play a role in regulating body weight, sleep, and autonomic function. Aziz showed that the number of orexin neurons was reduced by 30% in HD brains, while the MCH neurons were not significantly altered. Both investigators also



reported that CSF levels of orexin remain unchanged in HD patients and mice, although Petersén said that levels of other hypothalamic peptides, cocaine- and amphetamine-regulated transcript (CART) and neuropeptide Y, are increased.

Politis presented data from PET studies of HD patients and asymptomatic gene carriers compared to normal controls after image analysis with both region-of-interest and voxel-by-voxel approaches. Using PET with [¹¹C]raclopride, a selective D2 receptor ligand and a marker of the availability of these receptors, Politis and colleagues demonstrated decreased binding in the hypothalamus of both patients and gene carriers, suggesting that hypothalamic dysfunction is an early event in the disease process. Taken together, these studies all suggest an important role for the hypothalamus in HD pathogenesis and also point to possible biomarkers and therapeutic targets. Petersén, however, stressed the need for more research to understand the role of the hypothalamus in HD, particularly comparative analyses between human and mouse models.

Cell Replacement in HD

Even as investigators work to better understand the pathogenic mechanisms underlying HD, efforts to treat the disease are also proceeding apace. Regardless of the mechanism of neurodegeneration, neural replacement, either through transplantation or neurogenesis offers the possibility of replacing the medium spiny neurons lost in HD patients. Although neural transplantation has been attempted in HD patients since 1990, there is still much to be learned from preclinical animal models, according to **Claire Kelly**. Transplanted neurons must not only survive in the host brain, they must also make the appropriate connections with other nerve cells in order to reconstruct the circuits that have been disrupted. Preclinical studies in animal models allow progress in neural transplantation to continue while the ethical, technical, safety, and trial design issues for human studies are worked out.

One of the major issues to be addressed involves safe sourcing of tissue for transplantation. Primary fetal (PF) tissue, adult CNS stem cells, and embryonic stem cells have all been proposed as sources of transplantable neurons. While transplantation with PF tissue has already been shown to be effective in improving function in HD patients, the scarcity of this tissue makes the search for other sources especially important. Kelly showed data from her studies, which compared fetal neural precursors (FNPs) expanded in culture to yield a larger number of transplantable cells, to PF neurons. Both cell types were transplanted into a lesion model of HD. By harvesting both mouse and human cells, Kelly and colleagues were able to test these cell types in both an allo- and xenograft environments. The data demonstrate that fiber outgrowth was consistently higher in transplants from FNPs than from PF striatal graft, irrespective of donor source and host environment. While these data support the superiority of FNPs as a source of transplantable cells, Kelly said that more work is needed before taking FNPs to the clinic.

Clinical experience with fetal neural transplantation in humans was reported by **Anne-Catherine Bachoud-Lévi** (France) and **Guido Nikkhah** (Germany). Bachoud-Lévi and colleagues transplanted fetal whole ganglionic eminences into the brains of five HD patients and have followed these patients for 6 years. Although three of the patients showed clinical improvements two years after surgery, these improvements plateaued and then faded.

Another option for cell replacement, increased neurogenesis, was presented by **Tomas Deierborg**. Neural stem cells line the ventricular wall of the subventricular zone in the brain, said Deierborg, raising the possibility that these cells could be induced to differentiate and restore cells that are lost during neurodegeneration. Indeed, neurogenesis has been reported to be increased in HD patients, indicating that the brain had already begun a process of self-repair. Augmenting the repair mechanism offers a novel approach to treating HD, suggested Deierborg.

Experimental Models and HD Therapies in Experimental Models

A wide range of experimental models have been developed for studying the pathogenic process in HD and for testing potential therapies. **Gillian Bates** began this session by comparing two mouse models: R6/2 and CHL2^{Q150/Q150}; the former a transgenic fragment model and the latter a knock-in model with full-length huntingtin. Although the knock-in model develops disease manifestations later than the R6/2, the disease phenotypes at end stage are similar, said Bates. Surprisingly, this included the presence of aggregates in the brain, which were expected to be greater in the fragment model.

In collaboration with Ruth Luthi-Carter's lab, Bates compared striatal gene expression profiles of these mice with human striatal gene expression (all at end stage), demonstrating a high degree of similarity. These data support the notion that the mouse models recapitulate the human disease and are thus relevant for studying the disease. So far, said Bates, the R6/2 model has been used to complete 13 efficacy trials of potential drug treatments in her own lab. Only the HDAC inhibitor suberoylanilide hydroxamic acid (SAHA) was shown to be efficacious; however it was also shown to be toxic in mice. Because the knock-in models develop disease more slowly, they are less suitable for preclinical studies, said Bates.

Blair Leavitt continued the session with a discussion of the yeast artificial chromosome (YAC) models developed in Michael Hayden's laboratory. These models express full-length huntingtin with different CAG repeat sizes. The YAC128 recapitulates the motor dysfunction, cognitive deficits, and regionally selective neuropathology of human HD, with rotarod deficits apparent at 4 months and a decrease in striatal volume at 12 months. These mice have been used in 15 preclinical trials, showing efficacy only in one trial of cystamine, which decreased striatal neurodegeneration but did not reverse some of the functional endpoints. Although these trials require longer studies than those that use R6/2 mice, Leavitt said the phenotype is robust and reliable.



Rats are also used as animal models of HD. According to **Huu Phuc Nguyen**, rats offer numerous advantages over mice. Their neurophysiology has been studied in greater detail and their larger size makes them better models for neurotransplantation, in vivo imaging, and biomarker studies that require analysis of biofluids. Their size also makes them the preferred model for studies of therapies such as deep brain stimulation. Nguyen and colleagues have shown that transgenic HD rats begin to show behavioral abnormalities of HD even before neuropathological markers appear, suggesting that pathological processes are occurring which have yet to be identified. At one month of age, these rats exhibit reduced anxiety and improved rotarod performance, but by 7-8 months motor performance is significantly decreased. They begin to show neuropathology at 6 months, cognitive decline at 9 months, aggregate formation at about 9 months, and striatal atrophy at 12 months.

Rodents are by no means the only animal models used in HD preclinical research. **Larry Marsh** and colleagues have developed a *Drosophila* (fruit fly) model that mimics neurodegeneration in humans and that can be engineered, using tissue specific promoters, to express foreign genes anywhere at anytime. Flies that express the huntingtin gene in photoreceptor cells of the eyes show a characteristic degeneration pattern that is easy to quantify visually; and these flies have shown particular value for studying two major potential targets for HD therapy: protein aggregation and transcriptional dysregulation. As mentioned earlier, different species of aggregates have different levels of toxicity, with oligomers thought to be more toxic than fibrils. In vitro studies have identified molecules that selectively target oligomers rather than fibrils. Marsh and colleagues have used the fly model to evaluate the effects of these small molecule inhibitors of aggregation on the pathogenic process. They have shown, for example, that methylene blue, which promotes fibril and inhibits oligomer formation, reduces the formation of aggregates and rescues photoreceptor neurons in HD transgenic flies.

They have also used the transgenic flies to identify selective HDAC targets that contribute to neurodegeneration, reasoning that selective inhibition of these targets might offer a better therapeutic approach. HDAC inhibitors have been shown to reverse the transcriptional dysregulation that contributes to HD pathogenesis; however those currently available are non-specific and potentially toxic. Marsh's studies suggest that inhibition of two particular HDAC targets, Rpd3 and/or Sir2, effectively suppress pathogenesis in the HD flies.

Alex Kazantsev has taken this work further, focusing on selective inhibitors of sirtuin 2 (SIRT2) alpha-tubulin deacetylase, which regulates microtubule stability in cells. His lab has shown that these SIRT2 inhibitors rescue cytotoxicity in both cell-based and fly models of Parkinson disease. Now he is extending this work to evaluate these inhibitors in HD models.



The development of new therapeutic strategies for HD involves a constant interplay between investigations in various *in vitro* and *in vivo* models, as observations made in one model suggest new questions to be explored in others. For example, insoluble protein aggregates were first noted as a feature of HD and other neurodegenerative diseases in human brain tissue, and have gone on to be studied biochemically and physiologically in many different models. Subsequent studies suggested that inclusions once thought to be toxic may in fact be protective, but as noted earlier, the aggregation pathway and its various intermediates remain obscure. **Erich Wanker** brings together some of the earlier mentioned studies of aggregate formation with drug discovery studies by looking at the effects of small molecules on protein misfolding, oligomerization, and fibrillogenesis in both cell-free and cell-based model systems. His lab identified a compound found in green tea, epigallocatechin-3-gallate (EGCG), which inhibits huntingtin fibril formation and reduces toxicity. Further, he has shown that this compound stimulates the formation of a new type of non-toxic soluble oligomer, suggesting that the conversion of misfolded proteins into an off-pathway non-toxic species might slow neurodegeneration in HD.

Sandrine Humbert and colleagues have taken yet another approach to identify therapeutic targets in HD. Using a technique called 3D fast video microscopy, Humbert has shown that the intracellular dynamics are impaired in HD, and has demonstrated that huntingtin alters the microtubule-based transport of neurotrophic factors such as BDNF (brain derived neurotrophic factor). Further, they demonstrated that HDAC inhibitors, specifically inhibitors of HDAC 6, increase vesicular transport of BDNF by preventing acetylation at lysine 40 of alpha-tubulin, identifying this as a potential therapeutic target in HD. Moreover, they have shown that cystamine, a drug that has been shown to be neuroprotective in several models, regulates BDNF secretion by restoring intracellular dynamics.

Understanding the Clinical Features of HD

In parallel with studies about the molecular characteristics of HD, another group of clinician/scientists are investigating the clinical features of this complex and multi-dimensional disease. While motor disability is commonly used to diagnose disease, evidence increasingly points to psychological symptoms as some of the earliest, and equally disabling, signs of the disease. **Erik van Dujin** and colleagues reported that both presymptomatic and symptomatic HD gene carriers have higher levels of psychopathology than first-degree non-gene carriers or the general population. Major depressive disorder was the most common psychiatric symptom seen in gene carriers. **Kevin Duff** and colleagues studied how scores on the Beck Depression Inventory II changed over time in presymptomatic HD gene carriers being followed in the Predict-HD study. They showed that depression increased over one year in presymptomatic gene carriers compared to non-carriers, particularly in those with motor signs of the disease.

Pre-symptomatic and symptomatic HD subjects also have been reported to have an impaired ability to recognize emotions, particularly disgust, either in facial or vocal

expressions. Moreover, studies indicate that this impairment results from dysfunction in the basal ganglia, suggesting that a person's emotion recognition profile is an early reflection of neuropathology. **Julie Snowden** and colleagues tested the ability to recognize emotions in two groups of patients -- one with HD and one with fronto-temporal dementia (FTD) – to determine if the emotion recognition profile could distinguish between the two disorders. Contrary to other studies, impaired recognition of disgust was not more prominent than recognition of other negative emotions in the HD patients, with the greatest impairments being present for the emotion of anger. While HD patients showed less pervasive impairments in emotion recognition than FTD patients, the two groups showed no consistent difference in the pattern of emotion recognition.

Obsessive compulsive symptoms (OCS), irritability, and aggression are among other aspects of psychopathology that are prevalent in people with HD. **Leigh J. Beglinger** evaluated OCS in pre-symptomatic HD subjects enrolled in the Predict-HD study and demonstrated that sub-clinical OCS are common in pre-symptomatic subjects and tracked with disease progression, contributing to functional impairments. **Stefan Klöppel** and colleagues studied irritability in pre-symptomatic subjects not only with questionnaires, but also with functional imaging studies. Using a simple task that induces irritability in subjects by providing negative feedback, he showed that while both gene-carriers and controls became irritated by the task, the brain processes negative feedback differently in pre-symptomatic patients versus controls. **Markus Alexander Lindinger** and colleagues examined aggression in HD patients. They showed that aggressive behavior not only increases as the disease progresses, but also that the increase in aggression correlates with diminished cognitive function, total functional capacity, independence, and psychosocial functioning, as measured by the rating scales (UHDRS) used to diagnose HD.

The clinical features of juvenile HD (JHD) patients, i.e., those younger than 16 years of age, differ from those of adult HD patients, said **Ferdinando Squitieri**. These young people manifest more severe disease with a more rapid loss of independence and faster brain atrophy than adults, he said. Moreover, the pathogenic mechanisms appear to be different between these patients with very large CAG repeat expansions and adult patients carrying mutations in the low/mid penetrance range.

Biomarkers in HD

The importance of identifying validated biomarkers of HD was a widely discussed topic throughout various sessions of the Congress, since designing efficient therapeutic trials depends on the availability of such markers. Of course, this is a prime motivation behind the Predict-HD study, discussed earlier by Jane Paulsen and Julie Stout. The brain imaging tools discussed by Diana Rosas and the markers of immune dysfunction discussed by Edward Wild and Maria Björkqvist illuminate the pathogenic mechanisms underlying HD while also suggesting possible biomarkers.



Brain atrophy, as measured by MRI, has previously been shown to be a validated biomarker of HD progression, although better automated and quantitative methods are needed. **Andrea Ciarmiello** described studies that used an automated method to segment MR images and measure volume in gray matter, white matter, and CSF. These studies showed that in HD patients, fractional volumes of gray matter and white matter decreased and the fractional volume of CSF increased as the disease progressed. **Nicola Z. Hobbs** and colleagues used a semi-automated technique to measure atrophy in the caudate over 12 months. In comparison to a manual delineation method, the semi-automated technique provided similar atrophy rates but with consistently reduced within-group variance, suggesting greater accuracy. There was a significant trend of increasing caudate atrophy rate with disease progression when examining controls, premanifest, stage 1, and stage 2 subjects. Higher caudate atrophy rate also correlated with longer CAG repeat length and increased decline in some cognitive areas.

Asymmetrical atrophy was also addressed by **Adolf Weindl**, who assessed gray matter atrophy in a group of right-handed HD gene carriers. This study showed no asymmetry in presymptomatic gene carriers but a leftward-biased increase in atrophy as the disease progressed. Weindl hypothesized that an increase in cortical excitatory input from the dominant left hemisphere into the left striatum may account for greater neuronal loss in the left versus the right striatum.

Another imaging method, positron emission tomography (PET), assesses functional as well as structural characteristics of the brain. **Wim Vandenberghe** and colleagues used a radio-labeled ligand that binds to cannabinoid-type 1 receptors (CB1) in the brain. Post-mortem studies have previously shown a loss of CB1 in HD brains, suggesting that the endocannabinoid system plays a role in HD pathogenesis. In this study, Vandenberghe demonstrated a widespread, profound loss of CB1 across the gray matter of both early and advanced symptomatic HD patients *in vivo*. The findings indicate that the anatomic distribution of the disease process in HD is more widespread than previously suspected, and is consistent with a model where mutant huntingtin repressed CB1 transcription from very early disease onwards. Another study of function was conducted by **Robert Christian Wolf** and colleagues, who used functional MRI to assess verbal working memory (WM) in pre-HD subjects compared to healthy controls. In this study, although pre-HD subjects performed similarly on the WM task, activation of the dorsolateral prefrontal cortex was decreased at high WM load levels. This decreased activation precedes atrophy, suggesting that it may be an even earlier marker of HD pathology.

While imaging has proven especially useful as an indicator of neuropathology in HD, its usefulness in clinical studies will depend on whether or not treatment results in a measurable change in the biomarker. Moreover, a biochemical test of plasma or another easily obtained biological fluid or tissue might prove simpler and less costly as an outcome measure in clinical studies. **Diana Rosas** discussed one such plasma biomarker, 8-hydroxy-2'-deoxyguanosine (8-OH2'dG), which has shown particular promise. This work was done in collaboration with Steven Hersch. 8-OH2'dG is a byproduct of



oxidative damage and has been detected in the brain of HD patients and in HD animal models. Rosas showed data demonstrating that plasma 8-OH²dG levels are elevated one fold in premanifest subjects, and more than three fold in early manifest subjects. Moreover, 8-OH²dG levels respond pharmacodynamically to treatment with creatine, a drug that is thought to be neuroprotective in HD.

Other markers of HD have also been proposed, including measures of both cognitive dysfunction and eye-movement abnormalities. **Matthieu Robert** combined these two measures into an oculomotor decision making paradigm that assesses performance on a cognitively-taxing oculomotor task. In a study of 28 subjects with pre-symptomatic or early HD, this task was shown to correlate with the predicted onset of clinical symptoms.

Biomarkers are also useful in preclinical animal studies to assess the effectiveness of a drug in producing the desired results. For example, drugs have been developed to modulate the aggregation process, but reliable methods for measuring the different species of aggregates in different tissues have not been available. **Gillian Bates** described an assay developed in her lab, which uses a “Seprion” ligand to capture aggregates in microtiter plate wells. She used this assay to assess aggregation in various tissues in two different mouse models at different disease stages. Then, she analyzed the aggregates to determine the various species that were captured in different tissues. This assay should be useful as an outcome measure in preclinical trials to show the effect of various treatments on the species and location of aggregate formation.

Clinical Practice: Genetic Testing, Predicting Onset and Phenotype & Guidance for Care

Although genetic testing for the HD mutation has been available since 1993, most people at risk of HD by virtue of family history forego the definitive test. **Kimberly Quaid** has been examining the reasons for declining genetic testing as part of the Prospective Huntington At Risk Observational Study (PHAROS), which enrolls subjects who have chosen not to undergo genetic testing. Of the 1001 subjects enrolled in PHAROS, 83 of these subjects later chose to have the test done. Quaid found that the main reasons people declined testing were fear of losing their insurance and because there is no medical treatment available. Those who subsequently chose to be tested thought they were more likely to carry the mutation and had greater fear of being treated differently and losing insurance. In contrast, factors that led people to continue to decline testing included greater comfort with uncertainty and the lack of an effective treatment.

Losing insurance is only one part of genetic discrimination that may be experienced by people with HD and other genetic disorders. Other aspects include discrimination within families and social relationships. Concerns about genetic discrimination prevent people at risk for genetic disorders not only from getting tested but also from participating in research, according to **Yvonne Bombard**. She studies the nature and extent of genetic discrimination. She found that genetic discrimination is common among people at risk for



HD and is associated with psychological distress. Interestingly, genetic testing was not found to increase the likelihood of discrimination. In fact, having a family history of HD but not the result of the genetic test was the reason most people felt they were discriminated against. Those who discover their family history early in life face an even greater risk of discrimination

A concern of genetic counselors has always been the repercussions of genetic testing. **Claudia Licklederer**, in cooperation with the German Huntington Association, studied the long-term consequences of genetic testing on mental health and quality of life. Not surprisingly, she found that HD patients showed poorer mental health and quality of life than either gene carriers or non-gene carriers. Among both gene carriers and non-gene carriers, poorer outcome related to depression was associated with lack of perceived social support. Predictors of depressive symptoms in gene carriers also included the expectation of an unfavourable test result and having no children. Furthermore, younger age was associated with depression in non-gene carriers.

Even when at-risk individuals decline genetic testing, their risk of developing the disease can be estimated based on a combination of factors, according to **Reinier Timman**. He presented a model that incorporates age of the subject; age of onset, CAG repeat length, and gender of the affected family member; and the number of children who tested negative. Genetic counselors can use this information to advise their patients about risk. For example, middle-aged subjects whose family members had a low age of onset are at substantially reduced risk.

In addition to predicting the risk of developing disease, it may be possible to predict clinical features of HD, according to **Lucienne van der Meer**. Her studies suggest that an individual's premorbid personality, psychological, and social characteristics predict certain aspects of HD symptomatology. For example, premorbid neuroticism and conscientiousness are related to mental rigidity and aggressive behavior in hospitalized HD patients. Individuals with behavioral symptoms at onset, rather than motor or cognitive symptoms, were premorbidly less agreeable and more conscientious.

The interpretation of the genetic test relies on the number of CAG repeats in exon 1 of the huntingtin gene. Greater than 40 repeats predicts disease, while less than 36 repeats is associated with a normal phenotype. **Carsten Saft**, however, reported on a 75-year-old man with 34 CAG repeats who manifested motor and cognitive symptoms of HD beginning around the age of 60.

In addition to predicting whether a person will develop HD, the determination of CAG repeat length has been used to predict the age of onset. **Doug Langbehn** presented data from the Predict-HD study that validated a previously published model he had co-authored showing that CAG repeat length is inversely correlated to the age of onset. Among 500 CAG-expanded, pre-manifest participants, 68 "converted" to manifest HD over a two-year period. Observed two-year onset rates matched well with predictions



based on CAG length and age from this model. In an earlier talk, **Ferdinando Squitieri** had presented data from a group of individuals with Juvenile HD. He characterized “true” JHD individuals as those with CAG repeat numbers exceeding 70 who manifest symptoms before the age of 15. In addition to early onset, these individuals show differences in cellular and subcellular characteristics, suggesting that different pathogenic mechanisms are involved.

While there are many excellent clinics that provide care for HD patients, guidelines that incorporate best practices have not been developed. **Sheila Simpson** and the European Huntington Disease Network have set out to use develop clear, uniform, and evidence-based standards that can be applied internationally. For patients in the terminal phases of the disease, effective treatment strategies are sorely needed, said **Martha Nance**. She conducted a retrospective study of 56 HD patients who died while residents of a single nursing home. Her study showed that many of these patients experienced distressing terminal symptoms and would benefit from systematic palliative care.

Recent and Upcoming Clinical Studies/Trials in HD

A new international observational biomarker study called Track-HD is set to begin in early 2008, according to **Sarah Tabrizi**, principle investigator of the trial. The goal of the study is to determine what combination of measures is most sensitive for detecting change over the natural course of premanifest and early HD; and then to validate these assessments as potential outcome measures for use in clinical trials. An intensive battery of novel assessments will be conducted over the 2-year enrollment period, including some of the most advanced MRI techniques; a novel quantitative motor assessment; tests of cognitive, oculomotor, and neuropsychiatric function; biochemical analysis of “wet” biomarkers; quality of life and family history questionnaires, and genetic studies to determine genotype/phenotype correlations. Data will be fed into a Track-HD database based in Ulm, biological samples will be sent to Biorep in Milan for long-term storage, and MR images will be sent to the neuroimaging center in Los Angeles for storage. With full electronic data capture and intensive dynamic live data analysis, the study is designed to be fluid and dynamic, with updates to the assessment protocol instituted when deemed necessary. The first four study sites have been identified, in London, Vancouver, Paris, and Leiden and will begin to conduct baseline visits in January, 2008, with follow up visits and 1 year and 2 years, or more frequently if robust changes are identified that require more frequent assessment.

Several therapeutic trials were also discussed. **Tiago Mestre** and colleagues conducted a systematic review of therapeutic interventions to evaluate the efficacy and safety of available treatments. In order to be included in this analysis, the trials had to be randomized, placebo-controlled trials with more than 10 HD patients. Only three trials identified positive effects on valid clinical measures. Tetrabenazine produced the most robust results, affecting both chorea and clinical global impression (CGI) in one trial. Amantadine showed positive effects in one trial, but conflicting results in another trial.



One trial of riluzole also showed positive effects but raised safety concerns at a dose of 200 mg; in another study, a lower dose proved ineffective.

A hybrid observational/therapeutic trial was suggested by **David Craufurd** and **Josef Priller**. This trial would evaluate the effect of drugs that might be prescribed for other reasons on two of the most common characteristics of HD: apathy and irritability. The draft protocol that has been developed for this trial would use a combination of self assessments, carer ratings, and physician ratings to assess effect of the treatment on irritability and apathy. The trial would allow a preliminary assessment of the most promising agents, piloting of assessment trials, and data collection on effect size that would inform power calculations for definitive trials. Some of the drugs that might be evaluated in this trial would include selective serotonin reuptake inhibitors (SSRIs), Bupropion, other antidepressants, and Methylphenidate.

Other therapeutic trials that were discussed included those for Citalopram and Atomoxetine, Ethyl EPA, ACR16, high-dose Creatine, Nabilone, and Coenzyme Q10.

- **Stacie Vik** presented in the place of Leigh Beglinger on the use of Citalopram and Atomoxetine in early HD. Both of these drugs target the neurotransmitters serotonin and epinephrine and are thought to have possible benefits in terms of improving cognition. These trials have begun recruiting and enrolling subjects.
- **E. Ray Dorsey** and **Anthony Clarke** described two studies in the United States and Europe to evaluate Ethyl-EPA, also known as Miraxion or Lax-101, an omega-3 fatty acid that was thought to improve motor function. The drug had a good safety profile in the both studies, but although there was a slight improvement at 3 months, no change was evident after 6 months.
- **Joakim Tedroff** discussed ACR16, which represents a new class of CNS drugs called dopamine stabilizers. Preclinical studies showed that the drug had a tendency to stabilize psychomotor activity and also had antidepressant, anxiolytic, and antipsychotic activities. In an initial open-label study in patients, normalization of gait and a reduction in falls was seen, leading to a small randomized phase II study in Sweden and Norway giving 50 mg. q.d. This study showed an improvement in voluntary movement, a tendency toward a reduction in chorea, improved gait, improved trail making performance (a test of cognitive impairment), and some indication of benefits on anxiety and depression. The drug showed a good safety profile. Future tests are planned to determine the effect of a larger dose and extended duration of treatment.
- **Diana Rosas** presented data from a trial of high dose Creatine, a putative neuroprotective agent that is converted in the body to phosphocreatine. In mouse models, the drug has been shown to correct energy imbalance. Early small pilot studies of low-dose creatine had failed to produce symptomatic benefits,



prompting Steven Hersch (P.I.) and colleagues to design a dose-finding study using higher doses in patients with early-stage HD. In addition to monitoring brain levels of the drug, safety, cognitive function, and regional brain atrophy, the investigators measured levels of 8-OH²dG, a serum biomarker of oxidative injury. At a dose of 30 grams/day, patients showed a reduction in 8-OH²dG, a slowing of brain atrophy, and a slowing of cognitive decline. A larger study of high dose creatine (CREST-E) was recently approved by the FDA, and this study should also provide validation data about the use of 8-OH²dG as a biomarker.

- **Adrienne Curtis** discussed the use of Nabilone for symptomatic relief of HD. Nabilone is a synthetic cannabinoid. Cannabinoids have demonstrated beneficial effects in reducing motor symptoms in Tourette's syndrome and also are known to affect mood, decrease insomnia, enhance appetite, and prevent weight loss. In a randomized, double-blind, placebo-controlled study in HD patients, no significant effects were seen in the UHDRS motor, cognitive, or behavior ratings, although there was a trend toward improvement in psychiatric symptoms. The drug was well tolerated. A larger trial will be needed to provide the power necessary to fully assess the possible benefits of the drug.
- **Kevin Biglan** discussed a proposed trial of Coenzyme Q10 in pre-manifest HD subjects. This trial, dubbed PREQUEL, would be the first drug trial in pre-manifest subjects and thus would be a model for future pre-manifest trials. In a previous study of manifest HD subjects (CARE-HD), CoQ showed a tendency towards improving total functional capacity. The PREQUEL trial will focus on safety and tolerability as its primary outcome, since pre-manifest subjects would presumably have to take a drug for an extended period of time in order to see benefits. Other secondary outcomes will also be monitored.

HD Related Disorders: Neuroacanthocytosis & HDL2

One session of the Congress was set aside to discuss genetic neurodegenerative diseases that mimic HD but are negative for mutations of the huntingtin gene. **Ruth Walker** summarized the clinical features of these disorders, including HDL2 and the neuroacanthocytosis syndromes, chorea-acanthocytosis (ChAc) and McLeod syndrome (MLS). Since they are all extremely rare and most clinicians will have never knowingly seen a case, an international database has been established to document the features of the diseases and encourage collaboration in research protocols. In addition, Walker emphasized the need to raise awareness about these disorders in order to ensure appropriate diagnoses, counseling, and treatment. Huntington's disease-like 2 (HDL2) resembles HD both clinically and pathologically, although the genetic mutation is a CTG/CAG expansion in the gene for junctophilin-3. Walker presented evidence from studies by Rudnicki and Margolis suggesting that the pathogenic mechanism for this disease is RNA toxicity mediated by a splicing protein called MBNL1 (muscleblind-like protein 1). This protein not only rescued cells with the HDL2 mutation, but also from

toxicity induced by a fragment of the huntingtin protein, suggesting that it may also play a role in HD.

Alexander Storch introduced the procedure for acanthocyte determination developed in his institution (heparin or EDTA dilution, phase microscopy) and discussed one new neuroacanthocytosis syndrome that he and his colleagues were able to discover, FAPED: Familial Acanthocytosis with Paroxysmal Exercise-induced Dystonia.

Hans Jung discussed McLeod Syndrome, an X-linked multisystem disorder with hematological, neuromuscular, and CNS involvement. Patients experience disabling muscle weakness and atrophy, as well as variable chorea, psychiatric disorders, cognitive decline, and seizures. Jung studied muscle pathology in a group of 10 patients with McLeod syndrome and showed that muscle weakness and atrophy are primarily due to motor neuropathy. Brain pathology demonstrates striatal neuronal loss and gliosis. In contrast to HD, however, McLeod syndrome patients have only minor cortical pathology.

The neuropathology of ChAc was described by **Benedikt Bader**. Bader said that neurodegeneration in ChAc differs from that seen in HD brains in terms of the degree of tissue loss and the amount of astrogliosis seen, although similar brain regions are affected. Mutations in a protein called chorein have been identified in patients with ChAc. While this protein is expressed ubiquitously in healthy brain, it is absent in the corresponding areas of ChAc brains.

Adrian Danek described the neuroacanthocytosis syndromes database mentioned by Walker, which has a portal on the Euro-HD Network website (www.euro-hd.net/html/na/submodule/). A protocol for filming patients has been developed, and preliminary observational data are available for 22 MLS and 108 ChAc patients. The next step, he said, will be to use this resource in observational treatment studies for these disorders, which because of their rarity will require small scale and geographically dispersed trials.

A large genetic screening of individuals with clinical signs of HD revealed that HD phenocopies occur in 1-3% of these individuals. However, according to **Jean-Paul Vonsattel**, the lack of sensitive diagnostic tools makes the identification of HD phenocopies difficult and may influence the incidence of these disorders. He suggested that as novel markers are identified, hitherto unrecognized tissue alternations will allow better diagnosis of HD phenocopies.