

World Congress on Huntington's Disease 2005

by Lisa J. Bain

The World Congress on Huntington's Disease 2005 convened in Manchester, England from September 11th to 13th, 2005, attracting more than 450 scientists, clinicians, researchers, advocates and family members from around the world. The Congress opened with Sir Peter Harper discussing the history of HD and what can be learned from that history as the HD community moves forward. Since HD was first described by George Huntington in 1872, knowledge about the disease has been built on the efforts of countless clinicians, scientists, patients, and family members. HD has been a paradigm for progress in genetics for several reasons: It was one of the first diseases recognized to show clear dominant inheritance, and was the prototype for late-onset dominantly inherited diseases. Later, in the 1980s and '90s, it was the test case for new approaches in gene mapping and isolation, culminating in the discovery of the gene in 1993.

Professor Harper noted that the process of isolating and mapping the gene would not have been possible without the efforts of the Hereditary Disease Foundation, which spearheaded an international collaboration of key scientists, nurtured that collaboration for over a decade, and brought those scientists and clinicians together with the people of Lake Maracaibo, Venezuela, where the data was collected that led to the gene discovery.

HD has also been a model upon which the field of genetic counseling has been built, owing to the special challenges it presents in terms of late onset, variable severity and onset, effects on the extended family, and stigmatization. In 1981, Dr. Harper wrote about the severe burden imposed by the disease and how the lack of preventative or therapeutic measures makes HD particularly difficult for genetic counselors to deal with. In a 2004 edition of the same book, he wrote that his opinion was essentially unchanged, and that HD "represents the most difficult genetic counseling problem among the mendelian disorders of adult life." (Practical Genetic Counseling)

HD has also been the prototype on the issue of genetic prediction. Recognizing that gene testing had the potential to cause serious harm to patients and families, family organizations have worked closely with clinicians to draw up guidelines aimed at protecting those who do not wish to be tested. Nonetheless, there continue to be social and ethical challenges in genetic prediction. While geneticists and genetic counselors are

well aware of the risks associated with testing, clinicians may not be, particularly if they treat only one or a few cases. There is also a need for comparable standards across the world and a continuing danger from commercial interests and “enthusiastic” researchers. However, according to Dr. Harper, HD has been a model of how to live with these pressures, particularly because of the activism of lay organizations that have forged close links with scientists and clinicians.

Pathogenic Mechanisms

Several sessions at the Congress were devoted to discussing pathogenic mechanism that result in Huntington's Disease (HD). **David Rubinsztein** introduced the topic, providing an overview of the multiple pathways to pathogenesis that have been proposed. HD represents a gain-of-function mutation, which may be modulated by loss-of-function effects, said Rubinsztein. While the mechanism remains unclear, he favors a model in which the mutation induces a series of parallel and largely independent pathways at the protein level, including producing elevated reactive oxygen species, abnormal calcium metabolism, and transcriptional changes. The implication of this multiple pathway model is that in developing treatments, correcting one abnormality will only have a big effect if that pathway is a major driver behind the disease. Only by doing experiments will we know if there are dominant pathways in HD, said Rubinsztein.

Rubinsztein's lab has been investigating the toxicity of the protein, including the role both of the expanded polyglutamine and the fragments produced during the disease process that lead to the accumulation of protein aggregates in neurons. The root of the problem, he said, is expanded polyglutamine, and the level of the mutant protein is regulated by production and degradation of the protein. Based on this assessment, possible targets for treatment include reducing the cleavage of the protein into toxic fragments, which is accomplished with enzymes called caspases; or speeding up the removal of toxic fragments. One of the strategies for removing fragments and aggregates is to block inhibitors of a process called autophagy, which cells use to get rid of unwanted proteins. However, there is also confounding evidence suggesting that aggregates in some form may play a protective role.

Michael Hayden's group suggests that toxicity is exposed when the protein is chopped up into fragments. If this is the case, the formation of fragments may be the rate-limiting step in the toxic pathway. Hayden's work has been done in the YAC128 mouse model of HD. This mouse expresses the full length Huntingtin protein¹ (designated Htt) with a CAG² repeat length of 120. According to Hayden, the model essentially recapitulates human HD in terms of behavior, motor abnormalities, nuclear localization of Htt, and pattern of neuronal loss. However, he has shown that the mice can be essentially cured of all features of HD by inhibiting cleavage of the Htt protein at one particular site (the caspase-6 cleavage site), which prevents the production of one particular Htt fragment. YAC128 mice that are unable to produce this fragment because they are resistant to caspase 6 show no signs of the motor dysfunction, striatal volume loss, or neuronal loss that afflict mice with normal caspase-6 cleavage sites. Hayden's group also serendipitously came across another mouse, dubbed "short-stop," in which the Htt protein produced is much shorter than normal Htt protein. Like the caspase-6-resistant mice, short-stop mice have no features of HD, suggesting again that the size of the fragment is important.

Unfortunately, the caspase-6 pathway is complicated and may be difficult to inhibit pharmacologically in a manner that does not produce adverse consequences. Hayden, however, is exploring another pathway. His lab has shown that both caspase-6-resistant mice and short-stop mice are resistant to NMDA excitotoxicity, and he believes that excitotoxicity may be a key pathogenic pathway in HD.

In humans, neurodegeneration occurs primarily in the striatum, the part of the brain that is responsible for planning, modulation of movement pathways, and many cognitive processes. Understand what makes striatal neurons especially vulnerable to the damaging effects of mutant Htt might reveal therapeutic targets. Several possible mechanisms were suggested that may be responsible for regional variability and specificity:

¹ The gene that causes HD is designated *htt*, and produces an abnormal protein called Huntingtin, which is designated Htt.

² CAG stands for three of the nucleotides (DNA building blocks), Cytosine, Adenine, and Guanine. The Huntington mutation consists of multiple repeats of CAG in the DNA. During protein synthesis, these three nucleotides form a template for an amino acid called glutamine. Thus, at the DNA level, the mutation may be discussed in terms of the number of CAG repeats, but at the protein level, HD is called a polyglutamine (many glutamines) disease.

Michael Hayden suggested one mechanism that might explain regional differences in neuronal degeneration. His lab has focused on excitotoxicity as a key pathogenic pathway in HD. While the distribution of neuropathology is not mirrored by the presence of Htt protein expression, it is mirrored by the distribution of cell surface molecules called glutamate receptors. Glutamate is a neurotransmitter that is involved in excitotoxicity. Thus, an elevation in the number of glutamate receptors could make the brain more susceptible to excitotoxicity. Hayden's group has shown that one particular type of glutamate receptor, called NR2B, is over-expressed in the striatum compared to other brain regions. This, he said, may in part explain the heightened sensitivity of the striatum to excitotoxicity.

Jeremy Van Raamsdonk postulated that another process, nuclear localization, may contribute to the distinctive pattern of selective degeneration in HD. Working in Hayden's lab with the YAC128 mouse model, Van Raamsdonk has demonstrated that in these mice, as in humans with HD, while mutant Htt protein is expressed throughout the brain, nuclear localization only occurs in those areas that experience degeneration: the striatum, globus pallidus, and cortex, with relative sparing of the hippocampus and cerebellum. In contrast, in the R6/1 mouse model, which shows unselective, widespread neuronal degeneration, there is also unselective, widespread nuclear localization.

Simon Warby, also a member of Michael Hayden's lab, suggested another mechanism that could play a role in the selective loss of a particular type of neurons, called medium spiny neurons, from the striata of people with HD. Studies have shown that one particular amino acid on the Htt protein is phosphorylated (meaning a phosphate group is chemically linked to the protein) and that this phosphorylation is protective against the toxicity of mutant Htt. Warby showed that the amount of phosphorylation in different brain regions is inversely correlated with pathology, i.e., it is higher in the striatum as compared to the cerebellum. This suggests, he said, that decreased phosphorylation of Htt may play a role in pathogenesis and thus may be a target for potential therapies.

Roman Gonitel presented evidence suggesting that somatic instability may play a role in selective striatal neurodegeneration. Somatic instability in HD refers to the tendency for the number of CAG repeats to expand in some cells over a person's lifetime.

In mouse models, Gonitel and others have shown that instability is progressive and highest in the striatum in comparison to other brain regions. Moreover, at least in mice, somatically expanded repeats are preferentially expressed compared to unexpanded repeats, which could contribute to neurodegeneration. Somatic instability thus may be a therapeutic target, said Gonitel.

While studies of regional specificity have focused primarily on the area of the striatum that shows the most extensive degeneration, recent studies have suggested cell loss in cerebral cortex may also be important. **Richard Faull** and colleagues have conducted studies to determine whether the variable pattern of cell loss in the cerebral cortex correlates with variable patterns of symptomatology. They studied patterns of cell loss in 115 HD brains from human brain banks around the world. Meanwhile, they worked closely with HD families to collect detailed information on symptoms. What they found is that patients could be grouped according to whether their symptoms were mainly mood or mainly motor; and that these groupings correlated with whether there was more cell loss in the cingulate cortex (mood) or the primary motor cortex (motor). Patients with mixed patterns of cell loss tended to have mixed symptomatology patterns as well.

The question of regional specificity was also addressed by **Angela Hodges**, who has been looking at transcriptional changes (changes in gene expression) in different areas of the brain in patients with early stage HD. Transcriptional dysregulation has been proposed as one of the key early events that leads to pathology in HD. Hodges and colleagues examined changes in gene expression in 44 brains from individuals with early (grade 0-2) HD in comparison to 33 control brains. They found that gene expression changes, both increases and decreases, were greatest in the areas of the brain most affected by neurodegeneration, especially the caudate and secondarily, the primary motor cortex (Brodmann's area 4). Moreover, the genes that were most altered appeared to be genes associated with neuronal transmission and homeostasis. Hodges (and others) have postulated that mutant Htt may interact abnormally with the cell's transcriptional proteins, possibly by inhibiting histone acetylation. This hypothesis underlies the current efforts to test histone deacetylase (HDAC) inhibitors as treatments for HD.

Lesley Jones has examined this hypothesis further. She proposed that mutant Htt represses transcription by binding to transcriptionally active proteins, including a protein called NCOR1 (nuclear receptor co-repressor 1), which regulates the transcription of many hormone receptors including thyroid receptors. Through further examination of the thyroid hormone receptor system in human HD brain samples and cells transfected with the HD gene, Jones and colleagues have shown that thyroid receptors may be important mediators of HD transcriptional changes. These studies could uncover additional therapeutic targets.

H. Phuc Nguyen has also been looking at gene expression changes, but in a different model. Working in Stephan von Hörsten's lab, Nguyen uses a transgenic rat model of HD that carries a truncated huntingtin gene fragment with 51 CAG repeats. This model exhibits emotional, motor, and cognitive behaviors that closely replicate what is seen in humans with HD, he said. Moreover, the lab has identified a very early behavioral difference in 10 day old rats -- decreased ultrasonic vocalizations -- and they have shown that at the same time, there is reduced expression of genes involved in neurotransmission and cell signaling, processes that could affect cell survival and neural connectivity.

Kerry Murphy investigates the mechanisms underlying cognitive decline, one of the three predominant aspects of HD symptomatology. Even asymptomatic patients, he said, often show subtle impairments in recognition memory, which has been mapped to an area of the brain called the perirhinal cortex. Murphy has been examining this part of the brain in HD mouse models in comparison to wild-type mice. He has shown, using electrophysiological recordings of neuronal firing, that HD mice have altered patterns of neuronal firing that may explain the impairment in recognition memory. Asymptomatic HD patients have also been shown to have reductions in the activity of the neurotransmitter dopamine, which is a potent neuromodulator in the perirhinal cortex. Murphy showed that normal neuronal firing could be restored in HD mice by treating them with a type of drug called a D2 agonist, which acts similarly to dopamine in the brain. These data suggest that dopaminergic therapy may be useful in treating cognitive decline in HD patients.

Neuroendocrine changes have also been observed in HD patients, and could contribute to cognitive and metabolic dysfunction. **Åsa Petersén** described studies in

R6/2 mice, where she has demonstrated a loss of several types of neurons that are involved in the regulation of the hypothalamus and endocrine systems. The loss of these neurons was accompanied by decreases in the levels of hormones, peptides, and other factors that are part of these regulatory pathways. Petersén suggested that these hypothalamic peptides may serve as biomarkers of disease that could be measured in CSF or plasma and could provide a means of objectively monitoring disease progression. In addition, therapies that target these neuroendocrine pathways may be useful in treating cognitive function, weight loss, and sleep disturbances in HD patients.

While efforts to understand the pathogenic mechanisms of HD have focused mostly on the toxic effects of mutant Htt, **Elena Cattaneo** suggested that some features of the disease may result from a loss of normal Htt function. Evidence suggests that the normal Htt protein is both anti-apoptotic (meaning it prevents cell death) and neuroprotective. The mechanism for this neuroprotection appears to be that wild-type, but not mutant Htt stimulates the production of a factor called BDNF (brain derived neurotrophic factor), which promotes survival of neurons in the striatum. Thus, loss of wild type Htt function could contribute to the vulnerability of striatal neurons. Cattaneo's lab has shown that wild type Htt increases BDNF production by inhibiting a molecule that silences gene transcription, thus allowing gene transcription to proceed. Mutant Htt, however, appears to interfere with this pathway, thus preventing transcription of BDNF and other neuronal genes. These studies have thus identified two potential molecular targets that may lead to novel therapeutic agents.

Genetics

James Gusella opened a session of the Congress devoted to discussing the genetics of HD. Genetics, he said, has identified the essential mutation that defines HD: a variable expansion in the number of consecutive glutamine codons (CAGs) in the huntingtin coding sequence. In addition, genetics has shown that the size of this expansion is highly correlated with age of disease onset. Moreover, while the mutant gene is present in all cells throughout the lifetime, symptoms do not appear until mid-life, and only certain cell types are damaged; suggesting that there are other mechanisms that modulate the pathogenic process. In order to uncover these mechanisms, geneticists have

developed a number of model systems that recapitulate certain pathogenic phenotypes. These models have helped elucidate mechanisms and are also being used to search for treatments. However, Gusella warned that relying on the end stage phenotype in these models (for example, looking at survival of mice) when developing treatments may be problematic unless one can be sure that the root cause of the phenotype is the same in the model as in human patients. Development and acceptance of model systems, he said, must be guided by human phenotypes. Thus, he identified criteria for model systems. They should: 1) display a threshold polyglutamine tract that results in a normal lifespan, with progressivity of disease beyond that threshold length; 2) the mutation must be dominant (i.e., only requires inheritance from one parent); 3) the phenotype must be more sensitive to polyglutamine length than to Htt protein concentration; and 4) neuronal specificity must be based not just on polyglutamine length but also on the protein context.

Gusella expressed concern about models that contain only fragments of the *htt* gene, and thus produce only protein fragments. HD appears to be one of a group of disorders caused by a conformational change in the protein that leads to accumulation of protein aggregates. While isolated polyglutamine or Htt protein fragments also produce aggregates, studies in full-length models suggest that the initial trigger that leads to pathogenic phenotypes occurs when the glutamine tract is still contained within the full-length protein. Thus, he said, when looking for inhibitors of aggregation, it is critical to demonstrate that they work in full-length models.

While the length of the polyglutamine expansion is clearly the primary determinant of age of onset, accounting for up to 70% of the variance, other factors also play a role. Studies of the disease within families (where the repeat length is similar) suggest that other inherited factors account for most of the remaining variance. Thus, geneticists have been searching for genetic modifiers, reasoning that these modifiers may be targets for intervention. Gusella described two different approaches to search for modifiers: a biased approach, where scientists identify candidate genes that may affect pathogenesis, or an unbiased approach, where the entire genome is scanned to look for genetic markers that occur more frequently among individuals with a common phenotype. Using the biased approach, David Rubinsztein and others have identified a gene called *GRIK2*, a subunit of a glutamine receptor, which has a significant effect on

age of onset in a small number of people. Using the unbiased approach, another area of the genome has been identified that appears to contain genetic modifiers of age of onset. These studies, emphasized Gusella, require the participation of large numbers of people from HD families, but could lead to new therapeutic approaches.

One factor that has been suggested as possibly contributing to age of onset is the CAG repeat length of the non-expanded allele. However, **Peter Kraus** presented data that refutes this hypothesis. In his studies of 230 unrelated patients, the size of the unexpanded allele was not shown to have any significant influence on age of onset, nor were they able to show any significant interactional effects of the unexpanded and expanded alleles.

Marcy MacDonald described studies her lab is doing in mice to identify genetic modifiers of the HD mutation. She emphasized that these experiments are not designed to make predictions in humans, but rather to use the mouse as a tool to identify genetic modifiers that can then be tested in humans. She has designed a genetically accurate knock-in mouse model (where the entire human HD gene, with 111 CAG repeats, is inserted into mouse genome), designated HdhQ111. These mice show behavioral abnormalities as early as one month of age and exhibit a cascade of presymptomatic pathogenic events more than two years before neuronal cells start to die. By crossing these mice with non-HD mice that have mutations in genes thought to be related to the development of HD (i.e., possible genetic modifiers) they can identify the effect of those genes on the disease phenotype. Using this approach, MacDonald's lab has shown that genes that modify late stages of the disease may not modify early presymptomatic disease. They have also shown that one candidate early modifier gene, a mutation in a mismatch repair gene (Msh2) that normal cells use to repair genetic defects, delays the onset of the phenotype. In order to identify further modifiers, the lab has inserted the HdhQ111 gene into several different inbred genetic backgrounds. Early analysis of these mice shows that the genetic background plays a major role in determining age of disease onset. Further study of these mice will allow identification of additional genes that modify early disease events.

Somatic instability of the CAG repeat is another phenomenon that may modify the expression of disease. **Sarat Vatsavayai** has been looking at somatic instability in

two lines of mice (R6/1 and R6/1 del) that are identical except for the fact that one has a shorter CAG repeat length (115 vs. 85). Both lines exhibit a progressive disease phenotype but in the deleted line, onset is delayed and the disease is somewhat milder. In both lines, somatic instability is evident before onset of the overt phenotype, suggesting that it may contribute to the disease process. These two lines of mice, identical except for the CAG repeat number, offer a unique tool to study the effects of repeat length instability, said Vatsavayai.

While the CAG repeat expansion in the huntingtin gene on chromosome 4 is responsible for most cases of HD, **Amanda Krause** presented data from another group of patients who have a disease that clinically appears similar to HD, but that is caused by a CAG/CTG expansion in another gene, called the junctophilin-3 gene, which is on chromosome 16. The expansion does not encode polyglutamine. This disorder has been designated HD type 2 (HDL2), and accounts for a significant number of HD cases among patients of African ancestry. These patients tend to have a later age of onset and age of diagnosis and a higher frequency of parkinsonian symptoms. Genetic studies have strongly suggested that there was a common founder for all known cases of HDL2 and that the mutation originated in Africa between 300 and 2000 years ago.

Experimental therapeutics

Although the pathogenic mechanisms responsible for disease pathology in HD are far from clear, scientists are using the knowledge they have to design therapeutic strategies. While new therapies are the ultimate goal of this work, the search for compounds that ameliorate the disease process will also provide additional clues about mechanisms.

Erich Wanker has focused his attention on the protein aggregates that are seen in HD and related disorders. Several lines of evidence indicate that the process of aggregate formation correlates with toxicity, however the mechanism of this phenomenon is not clear. Because Htt is a huge protein, it interacts with many other proteins that are involved in many cellular processes. Aggregation may lead to sequestration of essential proteins and autophagy of the aggregates could remove essential proteins from the cell.

There is also evidence that aggregates may be protective in cells. These seemingly conflicting ideas led Wanker to the conclusion that aggregates need a susceptible cellular environment to cause toxicity and the hypothesis that toxic aggregates display an abnormal surface that leads to deleterious protein-protein interactions. Reducing the toxicity of aggregates may thus involve changing the conformation or blocking the accessibility of the surface of the aggregate to other cellular factors. Wanker's lab has developed an automated assay to screen compounds that inhibit aggregation of Htt. They have screened more than 180,000 compounds. Results from these screens are then confirmed by other assays and then tested in cellular and fly models to see if they also reduce toxicity. These assays have led to the identification of several compounds that will be further analyzed for efficacy in HD animal models.

Adriana Valera presented data from Jose Lucas' lab that further explores the role of aggregates in HD. The process of aggregation involves multiple steps and several intermediate aggregate forms, ending with the inclusion body (IB) found in the nucleus of cells that express mutant htt. IBs also contain components of the ubiquitin proteasome system (UPS), machinery the cell uses to degrade proteins. One possible mechanism for HD pathogenesis is that the mutant protein inhibits the UPS. Lucas' lab has been studying this process with mouse model of HD that expresses mutant htt with 94 CAG repeats, which leads to IBs in cortex and striatum. The lab purifies IBs from these mice, isolates huntingtin filaments, and then studies the effect of these IBs and filaments on proteasome activity. What they have shown is that IBs from these mice do not affect proteasome activity; however, mutant htt filaments do inhibit one component of the proteasome. This suggests that proteasome inhibition depends on the degree of Htt aggregation.

Leslie Thompson discussed cell and fruit fly (*Drosophila*) models she uses in her lab to search for compounds that will counteract the pathogenic mechanisms of HD. These models have been useful in identifying pathways to target and in prescreening drug effectiveness before preclinical trials proceed to more expensive and time-consuming mouse testing. Pathology in the *Drosophila* model mimics many aspects of the human disease: dominant inheritance, late onset, progressive neurodegeneration, formation of aggregates and inclusions, loss of motor control, dependence on the number of

polyglutamine repeats, and early death. Thompson's lab has used this model to test compounds that target a variety of mechanisms, including aggregation, transcriptional dysregulation, abundance of the Htt protein, and signal transduction. The fly model can also be used to test combinations of drugs, since combination therapy may be needed as it is in other diseases such as cancer and AIDS. This model has been particularly useful in studying a class of drugs called histone deacetylase (HDAC) inhibitors, which target transcriptional dysregulation. Several HDAC inhibitors have been shown to arrest neurodegeneration in the HD flies and clinical trials have already begun for at least one of these (phenylbutyrate). In the meantime, Thompson's lab has shown that there are three classes of HDAC inhibitors that have different effects on neurodegeneration; and they are continuing to examine how best to target HDACs pharmacologically.

Compounds that emerge as promising from high throughput screening of compound libraries, such as the screening that Erich Wanker described; or from cell-based or drosophila testing such as that described by Leslie Thompson; or from a variety of other model systems, are generally tested next in a mammalian system. The most widely used HD model is the R6/2 mouse, developed by **Gill Bates**. The R6/2 mouse models juvenile HD, said Bates, because the number of polyglutamine repeats is so high. This produces an early onset, rapidly progressive phenotype with measurable endpoints that can be efficiently used in the laboratory. Thus, while the model may not recapitulate human HD faithfully, it can be used effectively to test agents against a variety of molecular targets, including protein expression and processing (e.g., production of fragments, aggregates, and inclusions), transcriptional dysregulation, excitotoxicity, and mitochondrial dysfunction. Bates' lab has been working to establish rigorous standards and testing protocols for using the R6/2 mouse in preclinical testing. These protocols include making sure the drug reaches the brain; testing stability, tolerability, and toxicity; determining the appropriate dose in mice; measuring tissue concentrations; and assessing outcome through behavioral testing. Since mice metabolize drugs much faster than humans, administration must be adjusted to best mimic the drug exposure that would be seen in humans. Usually this means providing compounds in food or water. All tests are also done in environmentally enriched conditions, which have been shown to significantly improve performance on motor tests.

Using these standardized protocols, Bates' lab has tested a number of compounds that have been proposed as potential treatments. Several agents that looked promising in *in vitro*, cell, and fly systems have shown little effect in HD mice. However, at least one of the HDAC inhibitors has emerged as promising in alleviating motor problems, although it did exacerbate weight loss.

Clinical markers

The search for new therapeutics would be greatly enhanced if biomarkers of the disease were available. Measurement of such markers could be used to demonstrate whether a treatment was having its desired effect, and also to determine at what point an individual should begin treatment or be enrolled in a treatment trial. Neuroimaging has, to this point, provided the most reliable markers of disease progression in HD, said **Elizabeth Aylward**. In particular, she presented data which demonstrates that measurement of striatal volume by magnetic resonance imaging (MRI) fulfills most of the requirements of a biomarker: It can be reliably and objectively measured; it changes in a predictable manner over time; it can predict onset of symptoms; and it is associated with a known mechanism of pathology. Data from the ongoing PREDICT-HD study indicates that a linear decline in striatal volume begins about twenty years prior to disease onset, suggesting that treatment would be most effective if initiated before symptoms are apparent. However, for striatal volume to be used as a "surrogate" marker for disease progression it must be shown that treatment halts or reverses the decline in striatal volume. Since no effective treatment is available now, this essential point cannot be proven. In the meantime, Aylward proposed that MRI striatal volume be used provisionally as an surrogate endpoint to screen candidate treatments for full clinical trials; followed by full clinical trials with onset of diagnosable symptoms as the outcome measure. This two-stage trial would allow not only evaluation of a potential treatment, but also provide validation of MRI measures as surrogate endpoints.

Other measures should also be considered as potential biomarkers. In addition to meeting the criteria cited above, other considerations include the cost and time required to collect data, level of invasiveness, level of stress to the subject, reliability across sites,

rate of change, linearity of change, and the stage of disease during which change is observed.

Andreas Bauer described a potential marker of HD progression that would assess the level of neuronal stress in the brain. Bauer's approach used positron emission tomography (PET), an imaging technology that is currently used more for research than for clinical applications. Though not widely available, PET has generated interest among neuroscientists because it yields 3-dimensional images of the brain based on biochemical and functional, rather than strictly structural, characteristics. In this case, Bauer wanted to study the distribution of receptors for a neurotransmitter called adenosine, which is thought to be a "natural" neuroprotective compound. Post-mortem studies of humans with HD has shown that there is a loss of these A₁ adenosine receptors (A₁AR) in the HD brain. Using a PET scanner and a radioactively-labeled compound that binds to A₁AR, Bauer showed that there is a significant reduction in striatal A₁AR density in the brains of HD patients in comparison to age-matched controls, suggesting that this marker has the potential to serve as a marker of the progression of HD.

Yen F. Tai used PET to assess microglial activation. Microglia are immune cells that become highly activated in the brains of patients with neurodegenerative and other brain diseases. When microglia become activated they express a certain binding protein. Using a radio-labeled compound that binds to that protein, scientists have developed a method for imaging microglial activation with PET. They have shown increased binding of this marker in the striatum and cortex of HD patients. Tai presented data collected in a group of 11 presymptomatic HD gene carriers compared to age-matched controls, which demonstrated elevated microglial activation. particularly in subjects who were nearing the predicted onset of symptoms. This study suggest that PET imaging of microglial activation may be useful in evaluating agents (such as minocycline) that act to delay onset by inhibiting microglial activation.

The markers most commonly used in assessing progression at this point remain clinical ones. **Adam Rosenblatt** presented data suggesting that the CAG repeat length determines more than age of onset, and that it may need to be taken into account when evaluating how well a patient is responding to therapy. He and his colleagues conducted a

study of 512 individuals examined at the Baltimore HD Center. To be included in the dataset, each person had to have been examined two or more times. A total of 3,402 examinations were completed; individuals were seen a mean of 6.64 times and followed for 6.74 years. This longitudinal study allowed the investigators to follow progression of disease. What they saw was that the course and severity of the disease varied among the participants; and that the CAG repeat length correlated with the rate of progression as measured by the quantified neurological examination and the motor impairment score. Rosenblatt concluded that in therapeutic trials, CAG length may need to be factored into the analysis; and that clinically, CAG length may need to be considered in making prognoses.

Clinical trials

As scientists seek to identify and confirm the appropriateness of various therapeutic targets, and others search for ways of improving the efficiency of clinical studies, trials of promising compounds are underway or have been proceeding. The progress of several of these studies was reported to the Congress.

Berry Kremer, representing the Euro HD network, presented recent findings from the European HD Initiative (EHDI) study of riluzole. This multinational, multicenter, double-blind, placebo-controlled phase III trial assessed the effectiveness of riluzole over a 36-month treatment period. Riluzole works by inhibiting glutamate excitotoxicity. It has been shown to modify disease progression in patients with amyotrophic lateral sclerosis (ALS) and showed some beneficial effects in HD mouse models. Over 500 patients with enrolled in the study, but over 30% dropped out for various reasons. Results from 379 patients who received treatment for at least 24 months and had no neuroleptic medication were analyzed, revealing that riluzole produced no significant benefits in terms of total functional capacity or UHDRS motor score. Interestingly, however, all patients who completed treatment with either riluzole or placebo improved, suggesting that something about being enrolled in the trial was beneficial, and pointing to the necessity for blinded trials to accurately assess the effectiveness of a specific compound.

Harald Murck next discussed a double-blind, placebo-controlled trial of Ethyl-EPA. Ethyl-EPA is thought to protect neurons by stabilizing nerve cell membranes, although the exact mechanism of how ethyl-EPA works is not known. In this preliminary trial, 121 of the 135 patients enrolled completed 12 months of treatment, and 83 of these subjects did so with no protocol violations (e.g., a subject could be considered in violation of the protocol if he or she missed follow up or fell outside the inclusion criteria). Total motor score assessed at 12 months was compared to total motor score prior to treatment (baseline.) When results from all 121 subjects were analyzed, there was no significant treatment effect; however, when results from only the “per-protocol” subjects were analyzed, a significantly higher number of subjects treated with ethyl-EPA showed stable or improved motor function. Further analysis suggested that CAG length was a factor in whether or not a patient responded to the treatment. This study pointed to the need for further clinical trials of Ethyl-EPA, which are currently underway in the TREND-HD study sponsored by the Huntington Study Group (HSG) and a parallel study sponsored by Euro-HD.

Another HSG study was discussed by **Phil Nicholls** and **Frederick Marshall**. This trial, called TETRA-HD was a randomized, double-blind, placebo-controlled trial of Tetrabenazine in people with HD who have chorea. The study concluded that Tetrabenazine was effective in treating chorea and resulted in global improvement scores, but may be associated with adverse effects including sleepiness, insomnia, restlessness, or depressed mood. The drug had no significant effect on total motor score.

New treatments for HD

Development of new treatments for HD is proceeding on multiple fronts, as a clearer understanding of the mechanisms of the disease gives way to a wide range of strategies for interfering with the disease process. These strategies include pharmacologic approaches, some of which are currently in human clinical trials, as well as genetic and cell-based approaches that are in earlier phases of development.

Hank Paulson led off the session with a discussion of a relatively new treatment strategy called RNA interference (RNAi), which takes advantage of a naturally occurring biological pathway for turning off a gene. In order for a gene to exert its effects, the chromosomal DNA must first be transcribed into a complementary molecule called

messenger RNA (mRNA). The mRNA provides the template for assembly of the proteins that will carry out the functions of the gene. In RNAi, a small interfering segment of RNA (siRNA) is synthesized complementary to the mRNA. This siRNA binds to the mRNA, thus preventing the production of the offending protein.

RNAi has generated a great deal of enthusiasm among scientists searching for treatments for genetically based diseases because it uses evolutionarily conserved machinery already present in the cell and because it is catalytic, meaning that one molecule of siRNA can act on many mRNAs over and over. While it appears to have great potential, Paulson warned that the novelty of the strategy means that many unanswered questions remain. In mouse models, RNAi has shown “remarkable” results in treating ALS and Alzheimer's disease; and in HD mouse models, the strategy has been shown to slow disease progression and to silence the HD gene. However, many challenges remain, particularly with regard to the actual reagent that will be used and how, where, and when to deliver the reagent.

Steve Dunnett followed Paulson with a description of transplantation cell therapies for HD. This strategy has been used for treatment of Parkinson's disease with some success; however, progress in this area has been slow. In animal models of HD, embryonic striatal tissue has been transplanted into diseased brains, demonstrating the ability of grafts to grow, differentiate, and form connections with the host brain cells. Animals also showed functional improvements in their ability to complete complex tasks and relearn lost abilities. Based on these studies, a limited number of clinical trials have been conducted in North America and Europe. One of these studies (in France) showed good survival and functionality of the grafted tissue as well as improvements in some electrophysiologic measures. A second study (in the United States) also showed good survival and differentiation of the grafted tissue, but no significant change in clinical measures of HD symptoms or in PET imaging results. Moreover, there was one death (from a non-transplant related cause) among 7 patients enrolled in this trial and 3 brain hemorrhages. The trial was stopped based on the apparent elevated risk.

Currently, a multicenter collaborative study (NEST-UK) is underway in Europe to develop the basic experimental, neurosurgical, assessment, and follow-up procedures needed to conduct efficacy trials of neural transplantation. Dunnett said that while initial

data suggests a proof of principle, it is too early to conclude whether this approach is practical and feasible. Long-term development will require identification of alternative sources of cells (such as stem cells) as well as further data regarding preparation and placement of the graft.

Karl Kiebutz provided a broad, but not exhaustive overview of pharmacologic therapies, dividing these agents into those that are palliative, i.e., treating symptoms; and those that are preventive, i.e., aimed at halting or modifying the disease process.

Currently, there are a few palliative therapies that provide some symptomatic relief, particularly in terms of treating the mood disorders such as depression. However, there remains a need for agents that treat the motor impairments associated with HD, and an even greater unmet need for treatments that address cognitive and behavioral impairments that have perhaps the greatest impact on quality of life. In evaluating these and other agents listed in the table below, clinicians are also considering whether combination therapy may be beneficial.

Drug	Type of studies	Effectiveness	Comments
Treating motor symptoms			
Tetrabenazine	Double blind	Reduce chorea	Side effects
Olanzapine	Open label	Most impact on chorea and behavior	
Clozaril	Open label	Reduced chorea	
Donepezil	Open label and double blind	No effect on chorea	
Ethyl-EPA	Double blind	Improved motor function	TREND-HD underway
Amantadine	Double blind	Reduced chorea	
Riluzole	Open label and double blind	Reduced chorea	
Treating behavior: irritability/aggression or paranoia			
Olanzapine			
Sertraline	Case series		
Treating cognition: memory loss and impaired judgment			
Donepezil			
Memantine			
Preventive therapies: slowing progression			
Baclofen	Double blind	No effect on TFC	
Lamotrigine	Double blind	No effect on TFC,	

		reduced chorea	
Riluzole	Double blind	No effect on TFC	In progress
Remacemide	Double blind	No effect on TFC ? on chorea	
Tocopherol	Double blind	No effect	
Idebenone	Double blind	No effect	
Coenzyme Q10	Double blind	Trend toward TFC benefit	Current trial increasing dose
Creatine	Open label and double blind	Mixed results - some signs of stabilizing disease	New trial designed with increasing dose
Phenylbutyrate	Safety and tolerability studies underway		
Minocycline	Interim futility study		

Genetic counseling and testing

As noted by Sir Peter Harper in the opening session of the Congress, the field of genetic counseling has been built largely around HD. Since the HD gene was identified in 1993, making reliable predictive testing available, people at risk for HD and their family members have been faced with the difficult decision of whether or not to get a test that could tell them that they have a relentless progressive, fatal disease for which no treatment is available.

According to **Aad Tibben**, “unbearable uncertainty” is the main reason that people at risk for HD consider predictive testing. A second important consideration is family planning. Yet although predictive testing is widely available, a minority of individuals choose to learn their gene status. Genetic counseling for at-risk individuals involves not only supporting them in making the best personal decision, but in helping them process the information once they receive it. Sometimes this includes uncertainty raised by intermediate CAG repeat lengths, and it almost always includes educating them about recent research discoveries and clarifying misconceptions that have arisen. Genetic counselors also may be faced with children born after predictive testing became available but whose parents declined testing. These children bring to the counseling table another set of parent-child issues that need addressing.

While predictive testing is offered with few reservations to adults at risk for HD, there is considerable controversy if such testing should be available to young people, and international guidelines currently advise against it. **Rony Duncan** and colleagues conducted a survey to examine adherence to this guideline. They uncovered 22 cases in which an “immature” person under the age of 14 years was tested. Two of these young people were informed of the results. In three cases, parents of children who tested positive experienced considerable anxiety in deciding how and when to inform their child of the results. Another 27 cases were identified where the young person was considered “mature”. Results were disclosed to 26 of these individuals, and 2 experienced an adverse event. Respondents to the survey generally agreed that existing guidelines against predictive testing of young people are appropriate, but that each case must be considered individually.

Predictive testing is stressful not only for the individual being tested, but for his or her partner as well. **Marleen Decruyenaere** and colleagues conducted a study to examine psychological distress and coping strategies in partners of people five years after testing. They found that partners of carriers have as much or more distress as the carriers themselves and that they tend to adopt more passive coping strategies in comparison to the more active strategies adopted by the carriers. Moreover, the study suggested that adopting passive coping strategies seems to increase the amount of distress experienced. The researchers surmised that the grief of partners is not socially recognized, leading to increased distress.

For couples in which one partner is a gene carrier, preimplantation genetic diagnosis (PGD) combined with embryo transfer offers the opportunity to conceive a child without fear that the child will carry the gene. **Cindy Zaitsoff** reported on the PGD program for HD at Guy's Hospital in London. Twenty-nine couples received consultation about PGD and 24 went on to initiate the process. Sixteen of these procedures reached embryo transfer and 9 pregnancies were achieved, resulting in the births of three sets of twins and three singletons. As part of the program, the gene carrier was given a neurologic examination and asked to discuss the welfare of the child. The non-gene carriers had their allele sizes measured and the issue of prenatal diagnosis was explored. Following initial consultation, participants were asked to complete questionnaires about

these discussions. Results from this small study suggest that couples were unconcerned about the non-carrier being tested and many thought the discussions about the welfare of the child were intrusive. For the most part, they seemed to have come to the clinic aware of and having fully considered their options.

Clinical management

Caring for patients with HD involves much more than providing drugs that will either relieve symptoms or intervene in the progression of the disease, as was made evident by the numerous presentations in the clinical management concurrent sessions. As outlined by **Anne Rosser**, a broad, multidisciplinary approach, using non-pharmacologic as well as pharmacologic treatment is necessary; and this approach must take into account family and domestic issues as well as the specific physical and psychological symptoms of the patient. Among the non-pharmacologic treatments that should be considered, physiotherapy to address motor symptoms and speech therapy to address swallowing and speech impairments are often required. As with pharmacologic treatments, development of non-pharmacologic therapy requires a thorough understanding of pathogenic mechanisms.

Psychiatric disorders are a common problem in HD and may become apparent even before the appearance of motor symptoms. **Erik Von Duijn** and colleagues investigated the incidence of psychiatric disorders among both early-stage symptomatic and pre(motor)-symptomatic patients, and among relatives with unknown gene status but who appeared to be gene negative. Their results suggest that the incidence of depressive disorder and generalized anxiety disorder is high both in pre-symptomatic and symptomatic HD patients, while non-gene carriers had rates of psychiatric illness similar to the general population.

Mental health problems among at-risk subjects who do not know their gene status are also under investigation in the PHAROS (Prospective Huntington At-Risk Observational Study) project. **Elise Kayson** reported early data, which shows that adverse life events, depression, and the prospect of developing HD predispose at-risk individuals, particularly women, to an elevated risk of mental health problems, which

require prospective assessment and clinical vigilance, particularly as these subjects enter clinical studies aimed at delaying the onset of HD.

Those who care for individuals with HD also face risks to their quality of life (QoL). This group of “carers” has been the focus of research for **Aimee Aubeeluck** and colleagues. In three exploratory studies, they demonstrated that spousal caregivers of HD patients have specific difficulty maintaining their QoL; experience loneliness, a need to escape, and a sense of loss; and often neglect their own needs as the needs of their spouses become more demanding. As a result of these studies, the team developed and validated a specific QoL measure for spousal carers of HD patients, which will help clinicians identify when the carers themselves need therapeutic intervention.

As HD progresses, patients and their families come face-to-face with issues of late-stage care. **Sheila Simpson** reviewed the literature on the clinical problems associated with late stage HD. Unlike many other terminal illnesses, HD patients often have previous experience within their families of the progressive nature of the disease, and thus are aware of the challenges they will face in the future. This awareness can exacerbate anxiety about how their own care should proceed, yet clinicians have few guidelines at this point regarding how to help families through this transition. Simpson proposed guidelines for management, including strategies for discussing advance directives for feeding and treatment and placement outside the home.

Jonathon Bisson continued the discussion of advanced directives, particularly with regard to the Mental Capacity Act, which received Royal Assent in the United Kingdom in April 2005. This act provides a framework both for those who cannot make decisions themselves and for those who want to plan for the future. Bisson pointed out that no clear consensus exists about when is the appropriate time to consider advance directives for individuals with HD, particularly since they require making decisions about circumstances for which a person may have no personal experience. Nonetheless, if an advanced directive is to be considered, it must be done when the individual is competent and can effectively communicate his or her wishes.

As HD progresses into its fifth stage (according to the Shoulson Scale), patients generally have severely impaired capacity to perform activities of daily living, and often require residential nursing care. **Dirk VanDerWedden** described a psychogeriatric

nursing home "Overduin" in the Netherlands, where 70 HD patients currently reside and another 15 patients at stages 3-4 receive day care. The average patient stays there for 5-10 years, receiving multidisciplinary care from a team of specialists. For as long as possible, the patient is included in decision making regarding his or her care; however when communication with the patient breaks down, the staff and the patient's family must have documented knowledge of the patient's moral values and convictions/beliefs so that appropriate decisions can be made. VanDerWedden noted that this is a largely unexplored area of research, but one that needs more attention.

James Pollard discussed how cognitive changes impose particular challenges on nursing home care of patients with HD, and strategies that are available to help staff manage impulsive and disruptive behavior and minimize confusion, frustration, anger, and aggression. Problematic experiences can be minimized, said Pollard, through the application of research gathered over the past 20 years regarding environmental accommodations, medication management, and staff interactions.

Martha Nance provided a comprehensive overview of the clinical care and management of patients with HD through all stages of the illness. A multidisciplinary team is needed, she said, to provide optimal care aimed at reducing the burden of symptoms, maximizing function, and optimizing quality of life. While no one care team has all of the necessary components, the ideal health care team would include a neurologist, psychiatrist, general physician, dentist, nurse, case manager, research nurse, psychologist, neuropsychologist, physical therapist, occupational therapist, and speech therapist. Augmenting the health care team is the patient's broader community, including family, friends, and other community resources.

As patients progress through the different stages of their disease, their needs change in terms of the medical evaluations and treatments they receive, attention to wellness and quality of life issues, and management of symptoms, functional difficulties, cognitive problems, and other complications. In later stages, appropriate management of the illness will broaden to include attention to legal and financial matters, including health care directives.

Research is also a component of clinical care and management, said Nance, noting that many people "fail to appreciate the depth of the hope that accompanies or that

grows in patients when research trickles down to a clinical trial.” Clinicians and researchers can never thank patients and families enough for participating in clinical trials, she said, and must be sensitive about reporting back result

Future directions

Anne Young concluded the Congress with an overview of the scientific sessions and comments about the future. She called this a time of “paradigm shift,” where the HD community is no longer looking only at what might be the mechanisms of the disease but also at biomarkers that will facilitate more efficient development of treatments, and at effective therapies to delay onset as well as treat symptoms.

As the Congress clearly demonstrated, there is a great deal of activity currently underway ranging from genetic and neuropathologic studies to clinical therapeutic trials and clinical studies aimed at developing a clearer understanding of the natural history of the disease. “The future is now,” she proclaimed.. “We’re already on our way. We know the path. We just have to work hard and we’ll be there for curing this disease.”