CONFERENCE REPORT

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Keith T Gagnon

Address
University of Texas, Southwestern Medical Center, Departments of Pharmacology and Biochemistry,
Dallas, TX 75390-9041, USA
Email: keith.gagnon@utsouthwestern.edu

The CHDI Fifth Annual HD Therapeutics Conference, held in Palm Springs, CA, included topics covering new therapeutic developments in the field of Huntington's disease (HD). This conference report highlights presentations on biomarkers in HD; emerging topics in drug targeting, such as the lysosomal degradation pathway and target prediction by network-based modeling; understanding phenotype and neuronal circuit dysfunction in animal models; regulation of huntingtin protein expression and function; RNAi and antisense technology to deplete the mutant huntingtin protein; and small-molecule drugs that are progressing quickly through the clinic. Investigational drugs discussed include ALN-HTT (Alnylam Pharmaceuticals Inc/Medtronic Inc), EPI-743 (Edison Pharmaceuticals Inc), LNK-754 (Link Medicine Corp) and pridopidine (NeuroSearch A/S).

Introduction

The Cure Huntington's Disease Initiative's (CHDI) Fifth Annual HD Therapeutics Conference provided a forum for translational researchers and clinicians to discuss approaches to improve drug discovery and development for Huntington's disease (HD). Presenters were primarily from academia, but the conference also had notable representation from the pharmaceutical and industrial research sectors. The conference program focused on developing and understanding disease biomarkers to inform preclinical and clinical research, *in vivo* models of HD, huntingtin (Htt) protein function, and research on drugs under development for HD. Exciting highlights of the conference included a proposal to use network-based approaches to understand HD and to identify therapeutic targets, the potential for sheep as a viable large animal model, the advent of a 'phenocube', discussions on antisense technology and allele-selective depletion of the mutant Htt protein, and reports of drugs for HD that may soon enter the market.

Biomarkers for Huntington's disease
Absolute and relative delta power biomarkers

A need for robust biomarkers in preclinical and clinical research for HD, as well as for a modality to bridge preclinical models and research in patients, has been recognized in drug development for HD. In particular, there is a need to follow the progress of disease and drug efficacy non-invasively. Andrew Leuchter (University of California Los Angeles) described the identification of a potential biomarker using quantitative electro encephalography (qEEG) in a study of patients with HD (n = 27) who exhibited both manifest and pre-manifest symptoms, and healthy individuals (n = 15). Patients with HD had an increase in global power of the delta power frequency (0 to 4 Hz), but had a distinct loss of the anterior to posterior delta gradient. Absolute and relative delta power may provide good biomarkers, as a large discrepancy in delta power exists between patients with HD and healthy individuals. The severity in loss of the delta gradient correlated with trinucleotide (cytosine, adenine and guanine [CAG]) repeat length in patients with HD, as well as with age-to-onset of disease. Subclinical changes might be detectable up to 20 years prior to clinical manifestation of the disease. Changes in delta power and gradient did not differ for medicated versus non-medicated patients with HD in a resting state. Dr Leuchter emphasized that these findings were preliminary and needed to be replicated, followed longitudinally and cross-referenced with other measurements, such as MRI, diffusion tensor imaging (DTI) and PET.

Imaging biomarkers

Nellie Georgiou-Karistianis (Monash University) described the coupling of functional MRI (fMRI) with spatial working memory tasks (n-back task) and cognitive flexibility (set shifting task) in the preclinical and early clinical manifestation of patients with HD (n = 35) in an effort to identify correlations between disease and changes in imaging markers. Differential activation patterns were observed across tasks for both pre-manifest and clinically manifest patients with HD compared with control individuals. These differences were assigned to variations in crucial timepoints in the disease, such as neuronal death. Therefore, fMRI data may be influenced by patient conditions, such as disease progression among different patient groups, and need to be combined with other techniques in order to evaluate potential biomarkers.

Dr Georgiou-Karistianis also discussed the use of DTI, which measures water diffusion in the brain and enables the evaluation of white matter neuron degeneration. DTI was combined with fractional anisotropy (FA) and tract-based spatial statistics (TBSS). Patients with clinical HD exhibited widespread white matter degeneration that correlated with deficits in cognitive and motor tasks. In addition, magnetic resonance phase imaging (MRPI) revealed a significant increase in the levels of iron in the brains of patients with the disease, thereby suggesting another potential biomarker. A need for more studies to relate findings in patients to animal models and to identify common markers was emphasized.

Techniques for biomarker discovery in preclinical models

The use of preclinical mouse models to identify biomarkers for HD and to follow changes in brain activity and structure was discussed by Stephan Morairty (SRI Internationl) and Herman Moreno (Columbia University). Disintegration of the sleep/wake cycle in HD has been reported previously, and Dr Morairty proposed the combined use of qEEG, locomotor and core body temperature measurements to study this phenomenon in mouse models of HD. Data for mice treated with serotonin 5-HT$_2$ antagonists, which induce sleep but do not affect non-REM sleep, were highlighted to provide a precedence for the use of these techniques. Dr Morairty hypothesized that studying sleep patterns in mouse models of HD might expose some of the underlying causes of cognitive deficit in HD patients.

Dr Moreno described the use of fMRI using a 9.7 Tesla magnet in ten mice each from the R6/2, YAC128 and Q140 models of HD; fMRI was performed in mice at several different ages. R6/2 mice exhibited spontaneous spikes in neuron discharge and hypersynchronous brain activity, making these animals highly susceptible to seizures. Significant increases in hypermetabolism and structural changes in the brain were also noted. YAC128 mice imaged from 20 to 68 weeks of age exhibited time-dependent changes in the striatum, frontal cortex and thalamus. MRI-guided microarray on these specific tissues identified a gene (*PP1R7*) for which expression was substantially reduced at disease onset. These mice also developed late-onset age-dependent striatum hypometabolism. Q140 mice exhibited little neuronal loss, and instead had functional abnormalities. These results underscored the complexity of models of HD and the continued search for common and relevant biomarkers.

Emerging targets for drug development
Lysosomal degradation of mutant Htt protein

Various proteins and cellular pathways have emerged as potential targets for therapeutic intervention in HD. The modulation of lysosome biogenesis and activity was discussed by Marco Sardiello (Telethon Institute of Genetics and Medicine/Baylor College of Medicine) and Jianhua Zhang (University of Alabama). Dr Sardiello described the use of a genome-wide screen to identify a network of genes that were regulated coordinately with lysosome formation and function. Analysis of these genes revealed a conserved transcription factor binding site, the CLEAR element, which is recognized by transcription factor EB (TFEB). The regulation of TFEB was characterized, and overexpression of this transcription factor was observed to remove mutant Htt protein aggregates in a cell line containing an expanded CAG repeat *Htt* allele. The expression of TFEB is being further studied in a mouse model of HD using adenovirus- or lentivirus-based expression to evaluate its efficacy *in vivo*. Dr Zhang described the role of cathepsins, the primary proteases present in the lysosome, in the clearance of aggregated proteins. The mutation of cathepsin D, B or L proteins is causally linked to lysosomal storage disorders. Using Parkinson's disease (PD) as an initial model, overexpression of cathepsin D was demonstrated to degrade overexpressed α-synuclein protein aggregates efficiently. Cathepsin D had a similar effect on Htt protein aggregates in primary neurons cultured from brain tissue. Mutant Htt protein-induced cytotoxicity was strongly reduced in these neuronal cells. Dr Zhang's research group is currently investigating the effects of inducible cathepsin D expression using adenovirus constructs in a mouse model of HD that contains 200-CAG repeats.

SIRT1 in metabolic manifestations of Huntington's disease

Metabolic dysfunction in mouse models of HD has long been recognized as a potential contributor to this disease. Wenzhen Duan (Johns Hopkins University) described a connection between this phenomenon and sirtuin 1 (SIRT1), a protein that induces neuroprotection during caloric restriction. Overexpression of SIRT1 in PC12 cells, a model cell line for HD, protected the cells against the toxicity of mutant Htt. Moreover, crossing SIRT1-overexpressing mice with the N171-82Q mouse model of HD led to improvements in motor function and an attenuation of weight loss, brain atrophy and hyperglycemia when compared with the N171-82Q mouse model. Interestingly, SIRT1 did not affect the survival of mice with HD, suggesting a separation between the metabolic phenotype and other phenotypic manifestations. Overexpressing SIRT1 in a bacterial artificial chromosome (BAC)-mediated transgenic mouse model of HD (BACHD), which expresses the full-length human mutant Htt, provided similar results to the N171-82Q HD/SIRT1-overexpressing mouse model, including improved motor skills. The BACHD/SIRT1-overexpressing mice are currently being characterized at later disease stages.

Predicting targets with a network-based modeling approach

A network-based modeling approach to improve the understanding of HD and to identify putative therapeutic targets was proposed by Jonathan Derry (SAGE Bionetworks). Network-based models of disease

are designed to address an exponential increase in biological data and to help investigators select suitable therapeutic targets. Network models need to integrate genotypic, gene expression and trait variation data into descriptive (correlation-based) and predictive (probability-based) models to predict target genes. Predictions based on this network-based approach have been successful using gene expression data from adipose tissue of mice fed high-fat diets or from breast cancer tissue samples. In an HD network model, genes with high prediction scores included homeobox transcription factors, synaptic transmission genes and protein-folding pathway genes. CAG repeat length, age of disease onset and death also correlated with the expression of these groups of genes in HD.

New developments in studying preclinical models

Advances in understanding phenotype and neuronal circuit dysfunction

Cognitive and psychiatric disturbances often precede motor dysfunction in HD by several years. To understand these changes at the neuronal circuit level, Elizabeth Abercrombie (Rutgers University) reported on the investigation of the function of dopaminergic (DA) neurons in different parts of the brain in the R6/2 mouse model of HD. By employing single-unit electrophysiology readings, EEG and microdialysis, a substantial reduction in DA neurons in the substantia nigra region was observed. These DA neurons demonstrated spontaneous activity and small decreases in pacemaker, random and burst signal modes. Intraperitoneal administration of 5 mg/kg of tetrabenazine, a small-molecule agent used in the treatment of HD, resulted in small improvements in motor skills, such as rotarod performance. The activity of the subthalamic nucleus neurons in R6/2 mice was also altered, resulting in fewer and more erratic burst signals. However, because the prefrontal cortex is not developed in rodents, studying frontal-striatal-thalamic circuits and executive function in mouse models may not be informative.

George Rebec (Indiana University) described HD as a communication problem between brain regions, specifically a dysregulation of corticostriatal processing. Microdialysis demonstrated a poor uptake of glutamate by cortical-basal ganglia, a process that drives synaptic communication. The GLT1 glutamate transporter was identified as a candidate target, and was shown to be upregulated in R6/2 mice following treatment with ceftriaxone sodium (Rocephin), a β-lactam antibiotic. Increased GLT1 expression improved glutamate uptake and resulted in less clasping, better climbing and more turns in a choice maze. Therefore, glutamate transport may be a viable therapeutic target and *GLT1* may represent an important gene for further investigation in HD.

Phenotypic data are time-consuming to collect from mouse models and are often limited in scope. Dani Brunner from PsychoGenics Inc reported results from the company's 'phenocube', which was designed to address this issue. The phenocube should provide a comprehensive method for correlating phenotype to changes in neurochemicals and brain function, as well as a method to screen drug effects rapidly. The small cubicle houses several mice and is monitored by a high-definition camera positioned above the cubicle. Continuous 24-h surveillance can be recorded for up to 6 days, and data are analyzed in real-time by computer programs. The phenocube can currently collect 40 phenotypic features, and researchers at PsychoGenics aim to expand this collection to 2000 features to produce microarray-like datasets.

Sheep as a promising large animal model
The relatively small brain size of rodent models of HD presents several challenges, including difficulty in modeling changes in executive functions, as well as in psychiatric or social behavior. Scaling of drug dosages and prediction of drug distribution and penetration in the human brain are also challenging. Jenny Morton (University of Cambridge) suggested that sheep may provide an excellent large animal model, as they are affordable and easy to maintain in a field setting, can recapitulate complex social behaviors, can be trained to perform tasks, and have a brain volume and convolution similar to that of humans. In addition, no obvious ethical concerns exist for sheep. Sheep models of HD with a 73-CAG-repeat human *Htt* gene have been developed at the South Australian Research and Development Institute. Testing to establish pathology and techniques to study brain function and social, cognitive and motor changes are ongoing.

Modulating Htt expression and function
Reducing or removing the Htt protein
The mutant form of the Htt protein, which contains an expanded polyglutamine tract, causes disease by competing with the normal function of Htt and by acquiring noxious functions, such as aggregation. Ruth Luthi-Carter (Swiss Federal Institute of Technology Lausanne) discussed the many known facets of mutant Htt function and how the mutant protein interferes with normal Htt activities. Strategies for mitigating these effects have mainly focused on specific activities of mutant Htt. However, Dr Luthi-Carter concluded that the most straightforward approach may be to eliminate the mutant Htt protein or to reduce levels of both the normal and mutant Htt protein. Using siRNAs targeted to the mutant and wild-type Htt mRNA, a neuroprotective effect with no toxicity was observed in cultured cortical neurons during a study period of 9 months.

An approach to silence the mutant *Htt* allele specifically was discussed by Neil Aronin (University of Massachusetts Medical School). SNP sequences unique to the mutant *Htt* allele were targeted with siRNAs, resulting in allele-specific Htt mRNA cleavage. Although SNPs vary among patients and alleles, it was estimated that more than 75% of the patient population

could be amenable to this approach using only four SNP-specific siRNAs. Effective *Htt* allele-specific silencing with siRNAs in patient-derived fibroblasts was demonstrated using SNP-specific primers and quantitative PCR to observe mutant and wild-type Htt mRNA levels.

To ascertain the level of endogenous wild-type Htt necessary for normal functioning, Andrea Kudwa from PsychoGenics reported that the company had generated a conditional knockdown (KD) mouse. The technology involved the incorporation of a siRNA into the genome; the expression of the siRNA was activated by doxycycline fed to the mice. Dr Kudwa indicated that a 40 to 80% KD of Htt levels was sufficiently tolerated for normal development to occur. Increased KD of Htt resulted in phenotypes similar to those of other mouse models of HD. Crossing these conditional KD mice with the YAC18 mouse model of HD, which contains a full-length wild-type human *Htt* allele, ameliorated the deficits produced by the KD of Htt in the presence of doxycycline. Thus, these experiments demonstrated that the effects were caused by the loss of normal Htt protein, and provide an approach for evaluating the lower limits of tolerability for the reduction of Htt levels in animals.

The N-terminus of Htt

Recent research aimed at understanding the function of Htt has focused on the post-translational modification status of the first 17 amino acids of the protein. Leslie Thompson (University of California Irvine) discussed the balance between SUMOylation and phosphorylation of this highly conserved motif. The N-terminal domain, which also includes polyglutamine and polyproline tracts, regulates nuclear localization, protein and membrane interactions, and protein stability and aggregation. Dr Thompson identified the E3 ligase PIAS1 as a candidate Htt SUMOylation enzyme, and SENP1 and SENP2 as potential isopeptidases that remove attached SUMO peptides. Phosphorylation appears to regulate SUMOylation, and is mediated by the IκB kinase (IKK) complex. Phosphospecific antibodies and phosphomimetic/phosphoresistant mutants of Htt have allowed phosphorylation to be tracked and its effects characterized. Dr Thompson noted that X William Yang (University of California Los Angeles) had recently reported that mice with phosphomimetic mutants of this 17-amino acid motif did not develop disease in a BACHD background. Dr Thompson is collaborating with the CHDI to identify compounds that could modulate the phosphorylation of Htt.

Antisense oligonucleotides

Don Cleveland (University of California San Diego) summarized ongoing studies with antisense oligonucleotides (ASOs) targeted indiscriminately to both wild-type and mutant Htt mRNA. The ASOs used an RNase H cleavage mechanism and were infused directly into the CSF of mouse models of HD. Dosing typically involved a daily infusion of an ASO (50 to 75 μg) for 2 weeks. An ASO targeting the human *Htt* transgene prevented disease progression in R6/2 and BACHD mice. For BACHD mice, treatment was initiated at 6 months, and partial phenotypic reversal of motor activity loss and hypoactivity was observed by 8 months. The delay in improvement was attributed to the long half-life of aggregated Htt protein. Unexpectedly, a reduction in Htt mRNA levels was sustained for up to 4 months after treatment was withdrawn, and partial phenotypic reversal was observed for at least 5 months. The ASOs had deep brain penetration and broad distribution in the mice. Dr Cleveland indicated that ongoing research involved characterizing the duration of ASO effects and, through collaboration with Isis Pharmaceuticals Inc, Genzyme Corp, and David Corey (University of Texas Southwestern Medical Center), the testing of novel ASOs that selectively inhibit the expression of mutant Htt by targeting the CAG repeat itself.

ALN-HTT

Progressing siRNA-mediated inhibition of Htt expression from the bench to the clinic was described by Dinah Sah from Alnylam Pharmaceuticals Inc. In collaboration with Medtronic Inc, Alnylam has developed a device for the direct intraparenchymal delivery of siRNAs, involving a pump, cranial anchor and catheter. Using a rigorous siRNA selection process and RNA modification scheme, a single, potent and stable siRNA, ALN-HTT (Alnylam/Medtronic), was identified as suitable to progress into animal testing. ALN-HTT was infused into rat striatum at varying concentrations for 7 days, followed by an analysis of Htt mRNA levels. The siRNA inhibited Htt expression for 2 to 4 weeks, with no observable toxicity. Treatment was scaled-up and siRNA distribution and efficacy were assessed in monkeys. Dosing at 8 or 12 mg/ml at a flow rate of 0.1 to 0.3 μl/min suppressed levels of Htt mRNA by 45%, with an siRNA distribution of 12 mm outward from the infusion site in monkey brains. Flow rates and siRNA concentrations are currently being optimized prior to progression to phase I clinical trials.

Small-molecule drugs for the treatment of HD
EPI-743

Guy Miller from Edison Pharmaceuticals Inc described the reversal of disease by the company's EPI-743, a co-enzyme Q_{10} analog, in children with Leigh syndrome (LS). LS is caused by a mutation in the *SURF1* gene, which is essential for cytochrome C oxidase function in mitochondria. Children affected by LS usually die by the age of 5 years. Edison was granted special access to children with LS, who were administered a 50-mg/kg/day dose of EPI-743. The clinical trial is ongoing, and Dr Miller reported that participants had demonstrated continued improvement in all disease aspects, as well as increased brain volume and an approximately 50% decrease in lesion burden, indicating that neurons may be dividing and compensating for the impact of the mutation. A video taken of one child with a prognosis of only months to live prior to treatment and at 6 months after treatment revealed remarkable

improvements in speech, chorea and cognitive abilities. No adverse events were noted during more than 500 days of dosing. Deficits in cellular energy in HD are similar to those observed in LS. Edison will complete an HD biomarker screen and co-enzyme Q_{10} analog screen in 2010, and expects to progress these investigations into patients with HD by 2011.

LNK-754

Robin Meray from Link Medicine Corp discussed the potential benefit of using farnesyltransferase inhibitors (FTIs) in HD. Originally developed as anticancer compounds targeting the *Ras* oncogene, FTIs have been comprehensively characterized. Chronic administration of a lead FTI, LNK-754 (OSI-754; Link Medicine; Figure 1), at 640 mg/day for 1 year resulted in no side effects in phase I clinical trials in patients with advanced solid tumors. Treatments for neurodegenerative disease are expected to begin at doses of approximately 10 to 40 mg/day. The upregulation of autophagy (a natural bulk degradation pathway that helps cells control stress, toxicity and disease) was observed in cells treated with LNK-754. A mouse model of PD, APP(SL), treated with LNK-754 (0.9 mg/kg) at 6 months of age for 3 months demonstrated a significant reduction in α-synuclein plaques. The FTI was also tested at the same dose in two mouse models of Alzheimer's disease (AD), APP/PS1 and Tau transgenic, and displayed a trend toward a reduction of plaques. LNK-754 has displayed excellent tolerability and safety, and has been investigated in a phase I trial for the potential treatment of AD. Link Medicine is also investigating the drug for the potential treatment of PD and for reducing Htt plaques in HD.

Pridopidine

Pridopidine (ACR-16, Huntexil; NeuroSearch A/S; Figure 2) was discussed by Joakim Tedroff from NeuroSearch, who reported that the drug had demonstrated favorable results in the European MermaiHD phase III clinical trial. Pridopidine is a DA stabilizer that can enhance or inhibit DA-dependent functions. Unlike neuroleptic drugs, pridopidine can stabilize dysregulated psychomotor functions, and appears to act by partially inhibiting dopamine D_2 receptors, activating D_1 receptors and enhancing NMDA receptor-mediated synaptic responses. The MermaiHD trial consisted of patients (n = 437) randomized for treatment with pridopidine (45 mg qd or bid) or placebo (bid) for 26 weeks. Patients were primarily from the UK or Germany and were 30 to 86 years of age (mean = 50.6 years). A total of 92% of enrolled patients completed the trial. The CAG repeat region in the *Htt* gene of the patients ranged from 36 to 63, with a mean of 44.7. The average time since diagnosis was 4.8 years, and the most common symptom among patients with HD was depression. A de-escalation of treatment dose was necessary for 34 patients (evenly distributed across treatments), and two deaths occurred. The primary endpoint of improved motor function, which was based on the modified motor score (mMS) and the
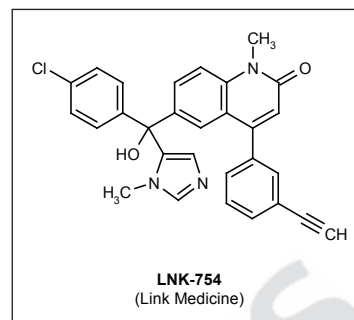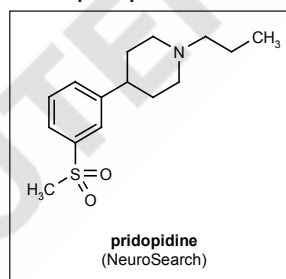
Figure 1. The structure of LNK-754.

LNK-754
(Link Medicine)

Figure 2. The structure of pridopidine.

pridopidine
(NeuroSearch)

total motor score (TMS), improved by almost 40 points (p < 0.005). Cognitive and behavioral symptoms improved, as did involuntary movements such as dystonia and eye movement. However, chorea did not change when assessed by both the mMS and the TMS. Pridopidine resulted in no adverse side effects and could likely be used with or without neuroleptics. Interestingly, patients administered tetrabenazine, which was prescribed to treat chorea, were excluded from the trial.

Summary

Although substantial progress has been made in drug development efforts for HD, significant challenges remain. The HD research community still lacks robust biomarkers that correlate well in most animal models and patients, complicating the interpretation of research in models of HD and the development of improved drugs. In addition, the various functions of the Htt protein remain uncharacterized. Disease intervention and treatments based on wild-type Htt function or mutant dysfunction rely heavily on understanding the roles of these proteins. Treatments to reduce Htt protein levels by targeting the gene product itself are in development. The intended application of these approaches in an allele-selective manner is promising, but lacks proof of principle in animal models. Finally, small-molecule drugs, particularly pridopidine, may provide relief for disease symptoms and improve or prolong quality of life, but do not address the underlying genetic or molecular cause of HD directly. Research and drug development has advanced at a considerable pace, but a cure for HD will rely on solving these fundamental problems.