

# Hereditary Disease Foundation

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New Therapies: Screening in Animal Models

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**Prepared by Ai Yamamoto**

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## Overview

Since the publication of the first transgenic model of Huntington's Disease (HD) by Gillian Bates and colleagues<sup>1</sup>, a flurry of work has led to the creation of a second generation of models to better understand the disease. These numerous mouse models, together with in vitro systems, provide an excellent opportunity to study possible therapeutic agents. Before this can be done, however, it is necessary to establish the phenotypes that not only simulate HD, but can be most efficiently studied. Furthermore, the differences and similarities between all of the models must be ascertained, for it is still unclear which, if any of the models, truly represent HD.

In the meeting "New Therapies: Screening in Animal Models" held on March 28, 1999, laboratories that have generated the different mouse models, statisticians and experts in clinical trials were brought together to determine how most efficiently to begin drug screening. Each mouse model, as well as a primate model, was discussed at length and the following issues were addressed:

- Which compounds should be given priority to be screened, and how should we determine which compound is worth screening?
- How should the screens be designed such that not only is the greatest amount of information extracted, but the different models can be directly compared?
- Should the screening between the various groups be formally organized?

## THE MEETING:

### Criteria For Establishing a Screen:

As summarized in Table 1, eleven mouse models were presented including the X-linked Hprt gene with an expanded polyglutamine insert presented by Dr. Peter Detloff. Each model differed in promotor, huntingtin fragment length (except between the Bates R6/2 and Tet-off models), and repeat length. Similarities and differences in behavioral, physiological and neuropathological phenotypes are seen. Before correlating the similarities to design appropriate screens, Dr. Ira Shoulson, an expert in clinical trials, and Dr. Shirley Eberly, a statistician, outlined the criteria required to assess an effective intervention. First, the natural history of the measured phenomenon or end point must be established. Then, the magnitude and variance of the intervention's effect need to be estimated.

One key attribute of the measured end point is its relative simplicity to allow for quick and efficient study. The screen will then give a first pass indication of what is going on, that is, the potential efficacy of the drug. The end point should also have relevance to the disease. The actual relevancy of the phenotypes demonstrated by these animals, however, is not yet clear.

At first glance, the many models and their diversity might give rise to the inclination to limit the number of models to be used as a means to increase efficiency. The consensus, however, was that reducing the number of models could lead to possible false negatives or false positives and therefore this should be avoided. Another issue at hand was the problem with strain and mixed genetic backgrounds of the mice. Arguably for behavioral experiments, strain differences and mixed backgrounds bring about large variability in finer tests, thus requiring a large number of animals. Most laboratories using mixed backgrounds (Dr. Jenny Morton (Bates R6/2), Dr. Neil Aronin, Dr. Ai Yamamoto) are back-crossing onto single backgrounds such as C57Bl6. A concern was raised by several participants that if everybody made congenic strains on the same background, strain specific phenotypes that have little relevance to HD could arise and be mistakenly pursued. In sum, the phenotype that occurs regardless of the differences in construct design and genetic backgrounds might be the best to study, for they best correlate with expression of the mutation. Furthermore, as pointed out by Dr. Morton, the findings where drugs have an effect are likely to be robust, and therefore should overcome minor differences.

Figure 1: Survival curve example

Significant points were raised in regards to how the data should be collected and represented. First, the data should be represented in terms of survival curves, which allows for a more powerful means to analyze the data. A survival curve takes into account the entire population in the study, including those that may drop out prematurely due to unrelated causes (e.g. mouse dies prematurely due to fighting with its cagemates). As illustrated in Figure 1, the x-axis represents the independent variable (such as age), while the y-axis represents the percent nonafflicted (phenotype) such that 100% denotes normal. According to this example, all of the animals tested are normal or 100% nonafflicted through week 3. By week 4, however, 25% of the animals display the phenotype (75% nonafflicted) and by week 7, all animals tested demonstrate the phenotype. In Figure 1B, the effect of the treatment regimen (Rx) can be seen as a right-hand shift; the onset of the phenotype is now week 4, and 100% of the treated animals are not afflicted until week 8.

Collection of the data and the design of the screens must also be considered. Most importantly, the test groups need to be assigned randomly. Random sampling protects against a distortion or misrepresentation of a population by treating each member in the population equally. Another way to protect for bias is the use of a blind observer to collect the data. Proper collection of the data will allow for better chances of seeing a real effect of the drug increasing reproducibility. As summed by Dr. Shoulson "If it's randomizable, its analyzable."

A question raised by Dr. Aronin was how many animals should we expect to use per group per treatment. Dr. Eberly demonstrated how the number could be determined by using power analysis. Power is the probability that the test will reject the null hypothesis when it is false. In other words, it denotes the confidence that the difference measured, such as a change incurred by a drug, is real. It relies on knowledge of the history of the placebo effect and an estimate of the variability and strength of the response. A rough estimate is as follows:

- If the probability of an effect occurring is 90% in mutant and 50% in wild type;
- And if the power of the test is 80% (a reasonable level);
- Then the number of animals required per group (n) = 11.
- If the probability of an effect occurring is lowered to 80% in mutant and 50% in wild type;
- And if the power is 80%;  
Then n = 30.
- Else if the power is 90%  
Then n = 40.

Therefore, the weaker the effect of the drug, the number of animals necessary per group increases dramatically, demonstrating the importance of a robust effect by the tested treatment.

## Potential Screens

With this information in mind, participants of the meeting

suggested a variety of markers and indicators that may prove to be an effective screen. As an early screen, Dr. Morton suggested cognitive tests like those used in her studies on the R6/2 line<sup>3</sup>. In the R6/2 line back-crossed onto a C57Bl6 background, Dr. Morton found that at 3.5 weeks of age (before the appearance of visible nuclear aggregates) both wild type and transgenic mice can perform on a two-choice swim test. This test is a simple choice paradigm that consists of a swimming tank, in which there is a visible escape platform on one side by a cue such as light. Each mouse is placed on one end of the tank and must learn to associate the cue (light) with escape (the platform). Transgenic mice are indistinguishable from control mice in this paradigm. However, when the location of the platform is placed on the side opposite of what they learned, the R6/2 mice have greater difficulty learning that now the platform is on the other side. Further, in alternation tasks such as the T-maze and Y-maze, the transgenic mutants cannot learn to alternate. The appeal of these tests is that they demonstrate a cognitive deficit which is part of the human HD phenotype, and it is seen prior to the onset of the motor phenotype. Unfortunately, these tests have not been applied toward models other than the R 6/2. Also, the necessity of a training period makes the tests more cumbersome.

Dr. Danilo Tagle proposed using cell death as an endpoint. However, it was quickly pointed out that although the screen would be quite clear, it required killing mice and processing tissue, which are both laborious and costly. Some participants suggested rotarod, grooming and death as other possible endpoints. Several participants believed that death as a possible endpoint would have potential drawbacks, particularly since it is unclear what causes death in any of the mouse models. Dr. Detloff cited the Hprt mice as an example. In the initial study, these mice were reported to have a median age of death of 45 weeks, with all dying by before 53 weeks. In more recent observations, Dr. Detloff reported that the lifespan of the mutants were now beyond 60 weeks of age. Although the exact reason for the increase is unknown, he hypothesized that the decreased handling of the mice might allow these seizure-prone animals to live longer. He further surmised that small alterations in somatic mosaicism and germline alterations might also contribute, since the effect of mild shortening of the repeat length is not known as well. A difference in the mean age of death of the other models was also reported to vary between colonies.

The rotarod test, on the other hand, is a simple task that requires no training. In all models tested, the HD mice demonstrate early and progressive changes. Other screens were proposed by Drs. David Borchelt and Stephen Davies such as clasping and body weight. Although the cause of the body weight change nor the neural circuit for clasping is known, these measurements are easily procured under defined conditions. Dr. Morton pointed out that monitoring for glucose levels

via urinalysis is also a possible screen. The final consensus was that the following tasks can be potential screens:

1. Rotarod
2. Clasping
3. Body weight
4. Urine glucose levels

One problem arose during the discussion of the rotarod results. Differences in the protocols exist between the various groups, even if the same published behavioral protocols were followed. As the published protocols tend to be very general, subtle differences that affect analysis arose. Though discrepancies between laboratories can be as important as similarities, these subtle differences might grossly increase variability of the behavioral end point, making comparison masking effects that other groups see as "real." To be certain the groups can cross-compare their results, it was decided that protocols must be either standardized or at least made readily available to all labs conducting drug screening.

Table 2: Summary of Cebus Monkey With Systemic 3-NP Stage

Motor Phenotype

Site of neuronal degeneration

I

Hyperactivity

Dorsolateral caudate and putamen

II

Upper limb choreoform and dystonia

Larger region of DL Cpu

III

Hypoactivity and dystonia

Increased lesion size and Globus Pallidus

#### Closer to Humans: A Monkey Model

Dr. Jeffrey Kordower presented a model he is developing in cebus monkeys. The animals are given a systemic 3-NP (3-nitropropionic acid) treatment until the onset of symptoms. Then, the 3-NP is removed and the animals are analyzed. The animals progress through three phenotypic stages summarized on Table 2. This progression, particularly stage III, is not seen in models which rely on striatal injection of quinolinic acid. Examining the brains of the afflicted animals using MRI demonstrates that the progress of the motor phenotype correlates with an increase degeneration of the basal ganglia. Dr. Flint Beal added similar findings in monkeys administered MPTP (1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine). Using PET scanning and MRI, MPTP treatment causes a decrease in the number of dopamine terminals, 6 to 8 months after treatment. 8 Dr. Beal further reported that studies conducted by Dr.

Bill Langston and colleagues showed that patients treated with MPTP actually progressed faster.

Dr. Kordower also presented a short glimpse into the newer studies he is conducting in collaboration with Dr. Fred Gage. In these studies he is applying direct viral transfer into monkey brain polyglutamine tracts of 97 and 15.

Which Drugs to Screen? That is the question.

Interventions fall into three general classes: 1) Symptomatic, which extend palliative treatments; 2) Neuroprotective, which slows functional impairment and degeneration; and 3) Restorative, which have a general effect on neuronal viability. A treatment of ramacimide (MK-801), an ionotropic glutamate noncompetitive antagonist, in conjunction with CoEnzyme Q, which affects with mitochondrial permeability and also acts as an antioxidant, is currently in Phase III clinical trials. A glutamate release inhibitor and GABAB receptor agonist riluzole is in Phase II.

Another interesting compound that will be entering Phase II next year is creatine. Creatine increases both ATP efficiency and levels of phosphocreatine, leading to increased muscle strength and heart force. Creatine also was shown to have a neuroprotective effect in amyotrophic lateral sclerosis (ALS) mouse model, and 3-NP and MPTP treated animals. Though the phase II study is for the treatment of ALS, interesting studies using the R6/2 line were discussed by Dr. Beal and Dr. Morton. Their data showed that the life span of the R6/2 mice was extended several weeks when treated with systemically administered creatine. Dr. Morton added that the mice also performed better on the rotarod after treatment. The data was still preliminary with a small n value, but promising nonetheless.

Caspase inhibitors also might be a potential drug treatment for HD. Caspase I has been shown to mediate neuronal death in stroke, head trauma and ALS. Dr. Robert Friedlander created a caspase I dominant negative via introduction of a point mutation of a cysteine on an active site. Preliminary data of crosses between HD models and the dominant negative was presented by Drs. Friedlander and Tagle. Dr. Friedlander's work with the R6/2 line showed an increase of life span by approximately 3 weeks. The age at which mice failed on the rotarod was also delayed by a similar amount of time. Dr. Tagle crossed the dominant negative with his full length cDNA model. He reported changes in the neuropathology of the mice. At the same time point at which the animals normally exhibit diffuse intranuclear staining, 75% of the mice crossed with the dominant negative strain demonstrated no nuclear staining. When intranuclear aggregates were seen, they were smaller than those in control mice.

## CONCLUSION

Though only one day, this session was very productive not only for establishing putative screens, but also for learning more

about the various mouse models and the latest results. The primate model developed by Dr. Kordower was intriguing, and the excitement and hope brought about by delaying death in the ALS mice and treatment with creatine as discussed by Dr. Beal added an extra dimension. In summary the following was decided:

The rotarod test, body weight and clasping phenotype make potential screens for testing novel therapeutic agents;

The protocols for the behavioral paradigms used should be standardized if not at least available to all groups;

Though it is still early to name which are the best drugs to be used for HD, as a starting point, some drugs to be screened were decided. These drugs were creatine, remacimide and CoEnzyme Q, and riluzole;

Caspase I inhibition (via crossed with transgenics or drugs) is an area to be actively explored; and

The group should reconvene in several months' time.

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## BIBLIOGRAPHY

1. Mangiarini L. Sathasivam K. Seller M. Cozens B. Harper A. Hetherington C. Lawton M. Trottier Y. Lehrach H. Davies SW. and Bates GP. "Exon 1 of the HD gene with an expanded CAG repeat is sufficient to cause a progressive neurological phenotype in transgenic mice." *Cell* 87(3):493-506, 1996 Nov 1.
2. DiFiglia M. Sapp E. Chase KO. Davies SW. Bates GP. Vonsattel JP. and Aronin N. "Aggregation of huntingtin in neuronal intranuclear inclusions and dystrophic neurites in brain." *Science* 277(5334):1990-3, 1997 Sep 26.
3. Carter RJ. Lione LA. Humby T. Mangiarini L. Mahal A. Bates GP. Dunnett SB. and Morton AJ. "Characterization of progressive motor deficits in mice transgenic for the human Huntington's Disease mutation." *The Journal of Neuroscience*. 19(8): 3248-57, 1999 April 15.
4. Ross CA. "Intranuclear neuronal inclusions: a common pathogenic mechanism for glutamine-repeat neurodegenerative diseases?" *Neuron*. 19(6):1147-50, 1997 Dec.
5. Ordway JM. Tallaksen-Greene S. Gutekunst CA. Bernstein EM. Cearley JA. Wiener HW. Dure LS 4th. Lindsey R. Hersch SM. Jope RS. Albin RL. and Detloff PJ. "Ectopically expressed CAG repeats cause intranuclear inclusions and a progressive late onset neurological phenotype in the mouse." *Cell* 91(6):753-63, 1997 Dec 12.
6. Reddy PH. Williams M. Charles V. Garrett L. Pike-Buchanan L. Whetsell WO Jr. Miller G. and Tagle DA. "Behavioural abnormalities and selective neuronal loss in HD transgenic mice expressing mutated full-length HD cDNA." *Nature Genetics*. 20(2):198-202, 1998 Oct.
7. Yamamoto A, Lucas JJ and Hen R. "A reversible mouse model of Huntington's Disease." *Society for Neuroscience Abstracts* 1999 (in press).
8. Beal MF. "Excitotoxicity and nitric oxide in Parkinson's Disease pathogenesis." *Annals of Neurology* 44(3 Suppl 1):5110-4. 1998 Sept.
9. Langston W. "Astrpocytes, brain aging, and neurodegeneration." *Neurobiology of Aging*. 17(3): 483-5. Discussion 488-90. 1996 May-June.