

BIOMARKERS FOR HUNTINGTON'S DISEASE

By Lisa J. Bain

Abstract

A group of 17 scientists met in Playa Del Rey, California in June, 2001 to discuss biomarkers for Huntington's disease. Biomarkers would facilitate accurate evaluation of the effectiveness of new therapies and improve the safety and efficiency of clinical trials. The ideal biomarker should be readily obtained from extraneural tissue such as blood or urine. Levels should correlate with disease progression and response to treatment. Biomarkers may also be measurable using imaging techniques such as positron emission tomography (PET), magnetic resonance imaging (MRI), and nuclear magnetic resonance spectroscopy (NMRS). Cognitive testing may also provide relevant markers. Animal and human studies of biomarkers must be run in parallel and compared with each other in order to take advantage of advances being made in animal models to understand the mechanisms of disease. A protocol was designed for comparing human subjects who are gene negative to those who are gene positive asymptomatic and gene positive symptomatic. A possible new therapeutic approach using histone deacetylase inhibitors was discussed, highlighting the need for biomarkers in the drug development process.

One of the hallmarks of HDF workshops is a visit by a person or family affected by HD. Participants at the Biomarkers workshop in Playa Del Rey, California in June, 2001, met three members on one such family: a 55-year-old woman, recently diagnosed with HD, her husband, and their 35-year-old daughter. Although the woman had grown up watching her mother's steady decline from the time symptoms began in her 30s until her death at age 63; and had seen her brother manifest symptoms early and die at the age of 50, she had decided not to be tested herself to see if she had the HD gene. Following surgery for an unrelated problem, however, she began to notice balance and gait problems, difficulty swallowing, and loss of memory. She changed her mind about testing and to no ones' surprise tested positive for the gene.

The family generously shared information about their own reactions to the diagnosis as well as information about the extended family. In addition to her brother, one other sister was diagnosed in her 30s and has significant disability. One sister, at age 47, has no symptoms but is

“consumed” by the idea that she has it. At least 10 children in the next generation are at risk.

The daughter was asked whether she had thought about being tested. Her main concern, she said, was about having children. But she wondered about the many downsides of being tested. “You have to wonder how much of someone’s decline is in the knowing,” she said. How much does her mother pull herself out of depression less now that she knows she has it?

The family’s visit focused the attention of participants on the issue for which this workshop was organized: identifying markers of disease onset and progression that will guide the development of new therapies. While CAG repeat length is responsible for as much as 60% of the variance in age of onset it has little value as a predictor of progression, as was clearly shown in this family, where CAG length was presumably the same but onset and progression differed.

Defining terms

Biomarkers, according to Ira Shoulson, are generally biological or physical events you can measure with instruments, in contrast to clinical markers, which are determined by talking to or observing a patient. The ideal biomarker could be should be readily obtained from extraneural tissue such as blood or urine -- “transparent to intervention with the patient.” Other tissues such as cerebrospinal fluid and muscle might also be useful for studying biomarkers yet are less ideal because they are less easily obtained. Biomarkers may also be measurable non-invasively using imaging techniques such as positron emission tomography (PET), magnetic resonance imaging (MRI), and nuclear magnetic resonance spectroscopy (NMRS). The measurable level of the biomarker should correlate with disease progression and response to treatment. The ultimate biomarker, one for which there is a one to one correlation with a relevant clinical outcome, would then qualify as a surrogate marker.

Peter Gilbert reminded the group that a marker that is correlated with clinical outcome may not be useful as a surrogate marker. "Correlation with disease progression is a necessary, but not sufficient, condition for a marker to be a surrogate," he said. For a biomarker to be a surrogate the disease process must be mediated through the marker and not just correlated. "The history of clinical trials is replete with examples of therapies that showed a favorable effect on markers but subsequently failed to demonstrate a beneficial effect on clinical outcomes." For example, CD4⁺ cell count has been shown to be valuable for predicting the development of AIDS in a person infected with HIV and for predicting death from complications of the disease, but does not correlate with clinical outcome in response to treatment.

"You can't really validate a marker as being a useful surrogate until you have effective treatments," said Gilbert. "Biomarkers are useful in phase II trials to screen [drugs] for activity, but are not particularly useful as surrogates for clinical outcome."

Leslie Weiner argued that clinical markers should also be considered biomarkers. New clinical tests, including assessments of cognitive function, at this point are the most sensitive measures of disease progression and response to treatment. Some of these measures rely on imaging modalities, such as functional magnetic resonance imaging (fMRI). "I don't think we're going to come up with a single test but we may well come up with a battery of tests to tell us [a treatment] is working and it does correlate with the other parameters of having the disease," said Weiner.

Ira Shoulson noted that a distinction must be made between state and trait markers. For Huntington's disease, the ideal trait marker already exists, i.e., the presence of the gene with a CAG expansion. State markers, in contrast, tell you the state of the disease once it has become

manifest. The ideal state marker would be something that could be detected presymptomatically and that would reveal something that was going on physiologically. "Because if there's something going on biologically, that's the time we want to intervene," said Shoulson.

Blair Leavitt added that the optimal biomarkers one would want to assess may be different depending on the clinical trial. "If your clinical trial is a trial of prophylaxis in presymptomatic patients, your outcome measure is going to be age of onset and you're going to want a full set of markers that change when a person becomes symptomatic." In contrast, if the treatment goal is slowing down the disease, those biomarkers may be useless and another set of markers would be needed.

Identifying markers of onset – PHAROS and PREDICT

"Historically, we haven't been able to look at the change in a person who is presymptomatic at risk becoming symptomatic," said Leavitt. Two cohort studies were described that will attempt to identify markers of this transition. The PREDICT-HD (Neurobiologic Predictors of Huntington's Disease Onset) study, under the direction of Jane Paulsen, will enroll 525 presymptomatic subjects between the ages of 30 and 55 who have undergone DNA testing for the HD gene. The study will use a variety of assessments, including volumetric MRI and sophisticated neurocognitive testing, to characterize subtle changes that occur when a person becomes symptomatic. Other components may be added to the study, for example, PET and fMRI. PHAROS (Prospective Huntington At Risk Observational Study), under the direction of Ira Shoulson, will enroll adults who are at risk of developing HD but have chosen not to have gene testing done. In both studies, blood samples will be collected (in a blinded fashion for PHAROS) for correlative analysis of repeat length with clinical features. Measurement of

biomarkers in blood samples could be added to the protocol should such measurements become available.

Studying fluids and tissues

One such potential biomarker is 8-Hydroxy-deoxyguanosine (8-OHdG), a marker of free radical oxidative damage to DNA, Susan Browne discussed work being done in Flint Beal's lab to evaluate 8-OHdG as a possible biomarker. A pilot study measuring blood plasma levels of 8-OHdG in Venezuelan HD patients is currently underway. Studies in the Beal laboratory have led to the development of HPLC methods to measure 8-OHdG levels in urine and blood. Browne said levels have been measured in urine, plasma, and brain microdialysates from R6/2 and control mice and found to be increased in R6/2 striatum and urine. They have also begun extensive HPLC work in mice to try to pick up other markers of oxidative damage.

Two other areas of research were discussed which could lead to the identification of biomarkers in extraneural tissue. Jim Olson described microarray analysis for studying gene expression and Julian Watts discussed the emerging field of quantitative proteomics, which measures actual protein levels.

Olson described studies he conducted a few years ago with Ruth Luthi-Carter to study gene expression changes in the R6/2 mouse models of HD. Studies have since been conducted using various mouse models as well as cell culture systems, and plans are to continue these studies and to conduct gene expression studies of presymptomatic human brain in the near future. Olson and others who are working with microarray analysis formed a consortium, called the Hereditary Disease Array Group (HDAG) to ensure consistency of samples and quality control and to encourage collaboration (see HDF workshop report, "Microarrays, Models, and Mechanisms,"

January 26-27, 2001.)

Gene expression studies suggest that different pathways are turned on or off at different time points during disease progression. Moreover, there are different patterns of gene up- and down-regulation among the different mouse models, although within a model the fraction remains consistent. For example, in the presymptomatic R6/2 mouse, 150 to 200 genes could reliably be shown to change, with about three times as many genes decreased as those that were increased. Work done with different mouse models suggests that as the polyglutamine repeat length increases, the ratio of genes going up and down becomes more equivalent.

While gene expression studies are done primarily to study the mechanisms of disease, the results could also serve as a biomarker, said Olson. He described a possible scenario: seven years prior to onset of disease, one might be able to measure 100 reproducible changes; five years prior to onset there might be 200 changes, and three years prior to onset there might be 400 changes. At some point, a threshold might be reached and this may correlate with disease onset. He cautioned, however, that in the mouse studies, no one particular gene has been shown to be correlated with disease onset. "I think it would be improbable that we would find a gene that would be, say, 1.2 fold decreased 7 years earlier, 1.5 fold decreased 4 years earlier, 2 fold decreased three years earlier, etc."

Commenting on this scenario, Marie-Francoise Chesselet said, "I think it's a very interesting idea. And it fits with the idea that it's not a particular gene but a general inability for transcription in whatever situation."

Proteomics, though a few years behind microarray technology in development of hardware and software, also holds promise both for elucidating mechanisms and for identifying biomarkers.

At the Institute for Systems Biology in Seattle, Julian Watts and colleagues use a mass spectrometry technique with isotopic labeling to analyze the proteins in tissue and cell culture samples. Unlike mRNA array analysis, proteomics is not limited to cellular material but can be applied to body fluids. Currently, their system is only able to compare two samples in any single experiment; but hundreds to thousands of proteins can be identified and quantified in a single typical experiment, depending on the complexity of the original sample. These measurements are used to determine, for example, which proteins are upregulated and by how much. Watts, for instance, is studying signaling processes in T cells, looking at which proteins are recruited to protein complexes.

Gene array and proteomics studies of various mouse models could yield promising clues about mechanism and potential biomarkers. Allan Tobin suggested that parallel genomics/proteomics studies be run on a common tissue set. Blair Leavitt commented that in order for animal studies to be applicable to humans, the tissue of choice should be one that could also be obtained from human patients. In other words, brain tissue would not be optimal. Lymphocytes would be the easiest tissue to collect with muscle tissue, collected via biopsy, a second choice. The cell physiology of muscle is more advanced than that of lymphocytes, particularly in terms of transcription regulation, making it potentially more comparable to the post-mitotic neurons that are affected in HD. However, some of the workshop participants questioned the use of non-neuronal tissue for this neurodegenerative disease.

Imaging

While proteomics and genomics are potentially very useful, Jeff Alger noted that imaging currently offers the only existing means for directly studying the brain tissues that are affected in

HD. Volumetric whole brain studies would give a sense of whether tissues were showing subclinical signs of micronecrosis, while NMR spectroscopy and PET have the potential to reveal and, in some cases, quantify the biochemical changes going on in the brain.

One of the proposed mechanisms of HD pathogenesis is that polyglutamine expansion leads to a defect in energy metabolism. Jane Paulsen said that she has data from presymptomatic HD patients showing hypometabolism in caudate and putamen bilaterally, and some hypermetabolism in the cortex. These data will be presented at the Society for Neuroscience meeting in November. Susan Browne reported that 2-deoxyglucose studies with in vivo autoradiography, done in transgenic mice with 82 repeats (Borchelt mice), have demonstrated an increase in glucose utilization at about 2 months of age. This is before any of the pathological changes are seen and before deposition of neuronal intranuclear inclusions (NII), which occurs at about 2 ½ to 3 months of age. Hypermetabolism is seen in the cortex and striatum, but not in cerebellum. Movement abnormalities appear in these mice at about 3-4 months and the mice live about 4-6 months, said Browne. Similar observations have been made in Marcy MacDonald's mice. These are knock-in mice with a 111CAG repeat inserted into the mouse *htt* gene. In this model, hypermetabolism was seen at 4 months of age, with the extent of increase in glucose use seemingly dependent on the number of affected alleles and the CAG repeat length.

Using PET, Beal's group and collaborators have also demonstrated an elevation in lactate in symptomatic HD patients. Browne noted that the elevation in lactate implies that there is a defect in energy metabolism somewhere, probably downstream. The bulk of energy production in cells takes place in the mitochondria, many of which are localized at the nerve terminals, she added, suggesting that changes in lactate levels in the striatum probably reflect something going on

in terminal activity.

Beal's group has also worked with Bruce Jenkins at Massachusetts General Hospital to measure levels of N-acetyl aspartate (NAA), a marker of neuronal health, using nuclear magnetic resonance spectroscopy. Working in transgenic mice, they have shown a correlation between decreased NAA levels and increased CAG repeat length. Leslie Weiner noted that NAA is currently used routinely as a clinical marker in demyelinating diseases like multiple sclerosis. It is thought to be a marker of neuronal density and thus, decreases may indicate nothing more specific than neurodegeneration. He added that it would be important to study changes in the level of NAA when elevated lactic acid levels have been reduced (which has been demonstrated in humans after chronic administration of Coenzyme Q10.) "If NAA were to stabilize and not continue to progress as lactic acid is reduced, I think that would be a very good indication," he said.

Cognitive/neuropsychological markers

While molecular studies of the brain may ultimately reveal useful markers that indicate cell dysfunction or death, Barbara Knowlton suggested an alternative approach of studying the brain at a systems level to detect changes that predict disease onset. "An advantage of looking at a systems-level measure is the fact that it may relate better to functional capacity of patients than molecular markers," she said. Possible tests include functional MRI as well as a variety of neuropsychological and cognitive tasks that detect subtle deficits in people with basal ganglia disorders, such as HD. For example, she is developing tasks to assess implicit learning; that is, the ability for a person to learn things in a more habit-like rather than cognitive manner. In one task, subjects are manipulated into thinking that they are guessing at the correct response when in actuality they are learning. "It's implicitly learned. By hiding the structure of the task, we kind of

trick the more declarative conscious memory so they can't really use that strategy and they go on gut feeling. And when we set up the task this way we can show normal performance in patients with amnesia, patients with Alzheimer's disease even; but in patients with basal ganglia disorders - - we've tested patients with HD in collaboration with Jane Paulsen, and also in patients with Parkinson's disease -- we've shown pretty severe deficits."

Linking behavioral studies in humans with those in experimental animals will allow researchers to make better use of the many animal models of HD. Julie Stout said that in designing the PREDICT study, they carefully reviewed the animal literature. Then, guided by what was already known about HD in areas such as cognition, they were able to identify several tasks would be useful. "It's tricky," she said. "You have to figure out what those behavioral descriptions in the animal literature might correspond to in humans. We clustered together animal studies and then mapped those to cognitive domains in humans and chose tasks in humans that we thought would test those domains."

Jane Paulsen added that for the PREDICT study, they started out by studying the neuropathology of people who died early in the course of HD to try to understand the progression of the disease. "What we want to map in the PREDICT study is if initial changes are in the tail of the caudate, then that circuitry should be first and the most sensitive test. And then within the head of the caudate we would look at dorsal medial paths or circuitries first and ventral lateral circuitries next. What we hope to map is if we can really take a healthy person with a healthy brain and follow them from their progression of health to a diagnosis of HD. What we should be able to see is that exact progression of the brain disease through both imaging and behavior."

Next steps

While animal studies such as those described earlier have been and will continue to be instrumental in identifying appropriate biomarkers and surrogate markers, there was enthusiasm among workshop participants for running parallel experiments in humans. After some discussion of the optimal study parameters, an experimental design emerged that would involve three groups of human subjects: 1) non-gene carriers -- individuals who have undergone gene testing and found not to have a CAG expansion ($CAG_n < 30$); 2) asymptomatic/presymptomatic gene carriers with confirmed CAG expansions; and 3) symptomatic individuals with confirmed CAG expansions in the early stages of the disease. Ideally, participants in groups 2 and 3 would have similar or identical CAG repeats. As noted by Blair Leavitt, there is less variability in age of onset as CAG repeat length increases, and there are many possible subjects available with repeat sizes of about 50. Leavitt also suggested that a more valuable and productive experiment would involve following a single patient with all these various measures over time. However, this would be a 5-10 year experiment.

Discovery

The whole purpose of the discussion about biomarkers, said Ira Shoulson, is to consider to what extent they will facilitate the discovery process for new therapies and provide useful outcome measures for therapeutic trials. With that in mind, Bob Hughes began a discussion of histone deacetylase inhibitors. Hughes noted that a "growing body of evidence suggests that enzymes that affect gene expression at the level of chromatin structure seem to be perturbed in various models of polyglutamine disease." He explained that DNA is wrapped around a core of proteins called histones, which packages the genetic material into chromatin. The ability of

transcription factors to access genes is partly regulated at the level of association between histones and DNA; and this association is regulated, at least in part, by two counterbalancing enzymatic activities: histone acetylases, which put modifications onto histones; and histone deacetylases, which remove them.

“So, given the thought that histone acetylation is deficient in cells that are expressing expanded polyglutamine, one might somehow want to pharmacologically intervene in that process.” A number of compounds exist that are known to inhibit the activity of deacetylases, he continued. If polyglutamine is inhibiting acetylases, one might be able to compensate by inhibiting the deacetylases.

Histone deacetylase inhibitors are a hot topic in oncology and clinical trials are already underway. However, Susan Browne noted that the mechanism of HD pathology is very different from cancer. Deacetylase agents in cancer seem to act by arresting the cell cycle, while in HD the relevant mechanism would be to counteract polyglutamine-mediated transcription interference. Several papers are soon to be published which should further elucidate the potential of histone deacetylase inhibitors to interfere with polyglutamine induced neurotoxicity.

Given that there is some suggestion that these drugs might be useful in treating HD, and that toxicity studies have already been conducted, the question arose as to whether it might be the right time to begin a human clinical trial, bypassing mouse studies. Ira Shoulson argued for doing both mouse and human studies in parallel for promising agents with known safety and tolerability. Jim Olson argued strongly for going through the more classic drug discovery mechanisms.

Jeff Alger added, “it seems that HD is a rare disease and it’s going to cost a lot of money to bring a drug to market, so for that reason you want to be pretty sure before you start that you

have a hit. I think that argues for spending more time on the in vitro and mouse studies than going directly to trials.”

But Ira Shoulson noted, “On the other hand, patients want and need to have something NOW.” The CARE-HD¹ study is a good example of how a clinical trial has demonstrated benefits (of coenzyme Q), albeit modest, in slowing the functional decline in HD patients.

It became clear that one of the factors limiting the ability to move quickly from animal studies to human trials is the lack of biomarkers. Biomarkers would not only accelerate and improve the efficiency of clinical trials, but could also make trials safer by providing data about safety and tolerability at the same time.

Conclusion

At this time, cognitive and gross motor changes appear to be the most relevant markers of disease progression. Robust, quantitative, and clinically relevant biomarkers that are predictive of disease onset and progression are clearly needed to facilitate the drug discovery process. As the mechanism of pathogenesis in Huntington's disease is not yet clear, markers will be needed at all stages of disease, and combinations of markers will likely be required. Participants in the workshop discussed a number of avenues to be explored in the pursuit of biomarkers, which must be done in parallel with studies aimed at better understanding the mechanisms of disease. Markers of energy metabolism and oxidative damage are available but require validation.

Imaging technologies offer great promise in identifying biomarkers. These modalities include volumetric MR, whole brain quantitative T2 imaging, PET, nuclear magnetic resonance spectroscopy of both brain and muscle, and functional MRI. Using the MR modalities, patients could be serially imaged over months or years with minimal adverse side effects, potentially

revealing a great deal of information about the natural history of the disease.

Gene arrays and proteomics also look extremely promising in terms of both identifying biomarkers and revealing clues about mechanism. Blood and/or muscle tissue could be readily collected for these studies which could be run in parallel.

Cognitive studies are being developed to challenge prefrontal circuitry and perhaps detect some of the earliest, subclinical signs of neuropathology. Mapping animal to human studies will greatly augment the usefulness of cognitive approaches. For all other approaches discussed, going back and forth between humans and animals is also necessary in order to take advantage of the discoveries being made in animal models.

Mechanistically, participants designed a human study which would search for biomarkers by comparing patients who are gene negative with those who are gene positive but asymptomatic and those who are gene positive with symptoms. Another idea floated would be to collect tissues from patients undergoing elective surgery and autopsy tissue. Said Jim Olson, "If we're doing some fishing, we may want to fish a few different holes."

END

Addendum: 'to do' list from participants in workshop:

HDAC Inhibitors

Sue Browne – testing in mouse models (R6/2, Borchelt) for the following:

Crossing blood brain barrier

Acetylation in brain

Acetylation in lymphocytes

Jim Olson – study gene expression

Other studies needed – bioavailability, pharmacokinetics, pK, safety and tolerability, dosing

Proteomics

Julian Watts –

Variability between mouse models and humans
Body fluid or CSF

8-OHdG

Sue Browne – test in various mouse models

Metabolism

Jane Paulsen – PET studies in humans

Sue Browne -- PET studies in mice

[Tony Shapiro looked at metabolism in muscle of HD patients?]

[Lucy Browne, metabolism in Yamamoto mice?]

Gene regulation

Jim Olson – get muscle tissue samples from HD patients undergoing surgery for anything

Les Weiner – said there are labs that can image analysis muscle – will try to let Olson know; will help Olson contact people

Proteomics

[Greg Folz coming to Julian's lab – has worked with CSF and could give info about albumin-like problems]

Imaging

Jeff Alger - Need to design protocols – volumetric whole brain studies, quantitative T2 (look for indications of microgliosis), MRS study of slices in basal ganglia and frontal cortical matter, fMRI procedure in anterior and posterior limbs of caudate – this would be about 1 hour of patient time.

Also - Muscle MR, p31, PET (FDG). NAA

Protocols for tissue collections (Jim Olson)

General biomarkers study protocol – bank tissue

Gather tissues when patients undergoing surgery; bank tissue

Collect autopsy tissue with standardized protocol

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