

Somatic Mosaicism in Huntington's Disease

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Abstract:

To explore the role of somatic mosaicism in Huntington's disease, the Hereditary Disease Foundation (HDF) convened a diverse group of investigators at a workshop in October, 2001, in San Diego, California. Both human and animal data reveal somatic instability of the HD mutation, with significant expansions in CNS tissues, especially the cortex. However, many questions remain unanswered, including what the relationship is between repeat instability and pathogenesis; whether specific cell types (e.g., neurons or glia) are affected; whether translation of the repeat expansion is a prerequisite of toxicity; and whether somatic instability is specific to CAG repeats. Participants at the workshop discussed possible mechanisms, including an important role for mismatch repair enzymes; and the possibility that repair mechanisms are generating instability in response to DNA damage. Possible triggers of DNA damage and instability include depletion of brain-derived neurotrophic factor (BDNF) and oxidative stress. Therapeutic implications of these mechanisms were discussed, including the need for high-throughput drug screens. Finally, participants outlined a number of important experiments needed to resolve some of the unanswered questions.

Somatic expansion of the CAG trinucleotide repeat of the HD mutation has been proposed as a mechanism of HD pathogenesis. As early as 1994, Michael Hayden demonstrated somatic mosaicism in human tissue from HD patients¹. In that study, larger expansions were found in areas of the brain most vulnerable to cell death. Studies since then have confirmed this observation and demonstrated somatic mosaicism in animal models of HD². Yet, the importance of these observations remains unclear. Researchers have speculated that somatic mosaicism may explain cell-selective vulnerability and the progressive nature of the disease. In order to explore this phenomenon further and plan future experiments to clarify the role of somatic mosaicism, the HDF convened a group of some 20 investigators from diverse disciplines at a workshop in October, 2001, in San Diego, California.

Allan Tobin opened the discussion with a focus on the therapeutic implications of somatic instability. If somatic mosaicism results in a subpopulation of cells with a very large number of repeats, different strategies for intervention will be needed than those that are targeting more modest sized expansions.

There is no question that somatic instability exists and that the greatest level of instability occurs in tissues with the greatest degree of pathology, continued Blair Leavitt. Yet many questions remain to be answered before the importance of this observation will be clear. Among these questions: Is there a relationship between the size of the repeat and the level of pathology? Does it hold for all cells and if not, why is it that certain cells with very large repeats are fine and others are not? Is there any direct evidence that instability in DNA leads to expanded protein, and do you need the protein to get pathology? Is it a sudden or gradual phenomenon; or is somatic expansion a “last gasp” from already dying cells? Which comes first, instability or damage? Does instability occur in neurons or glia? Does it affect the striatum directly or indirectly? Is this phenomenon specific to the CAG repeat locus, or is instability seen at other loci; and if it is specific to CAG repeats, is it unique to the HD gene, or is it also observed in other triplet repeat diseases?

Human evidence for somatic mosaicism

Peggy Shelbourne presented preliminary human data from the brain of a girl with a repeat length of 75 who died at age 15 from juvenile onset HD. The extent of damage to the striatum was extensive, with little tissue remaining to be examined. However, in the cortex, Shelbourne saw “massive instability,” with some cells having as many as 800 repeats. The small amount of striatal material examined also showed expansions, but not at the level of the cortical material. Highest levels of repeat instability were observed in the temporal and parietal cortex, with lower levels in the hippocampus and substantia nigra. Repeat instability was also observed in the analysis of brain tissue from a pre-symptomatic individual with 41 repeats on the mutant allele. In this individual, one cell in the striatum had over 1,000 repeats.

Norman Arnheim presented similar data from human temporal cortex tissue of an individual with 50 repeats on the mutant allele. Arnheim did not see the huge expansions that Shelbourne saw, but noted that this could have been due to technical limitations of the single molecule analysis technique he used.

Vulnerability of different cell types

If, in fact, somatic mosaicism does turn out to be a real phenomenon, as data from both human and animal studies suggest, it will be important to determine whether specific cell types are affected by instability of CAG repeats and if the pattern of instability correlates with the pattern of pathology. The answers to these questions should help clarify the mechanism of mutation expansion in somatic tissues and how expansion contributes to the death of medium spiny neurons. Although death of neurons is assumed to be the primary event in HD pathogenesis, there is some evidence from a group in Japan³ using laser capture microdissection that glia, rather than neurons, are the site of the most instability. This group studies DRPLA, another triplet repeat disorder. Jenny Morton noted that there is one glial cell supporting every 40 neurons in the striatum, suggesting that instability in a glial cell could be quite significant.

There is also significant evidence implicating neurons. In the adolescent HD patient cited above, the absence of cells with very large repeats in the striatum, despite the accompanying gliosis, suggests that neurons carry the large alleles. Further, while repeat expansions appear to accumulate over time, glia are thought to divide only one or two times, implying that if somatic instability in glia was the critical event, expansions would likely be occurring post-mitotically, as in neurons.

“If glia carry the largest mutations,” said Peggy Shelbourne, “then it is possible that neurons are murdered, perhaps through withdrawal of trophic support. Conversely, a model of suicide or assisted suicide may be more likely if neurons carry the largest mutations.” Very large expansions in the cortex, as have been suggested, might also “murder” striatal neurons, added Marie-Francoise Chesselet.

The question of striatal compartmental vulnerability was also raised. Might the repeat tract be less stable in “older” patch neurons than in “younger” matrix neurons? Jenny Morton commented that in the hippocampal formation, the appearance of intracellular inclusions correlates with neuronal maturation, suggesting that the maturity of the cell may affect repeat length instability.

In some animal models, repeat expansions have also been observed in non-CNS tissue although in Shelbourne’s mice, mutation expansions were most unstable in CNS tissue. Striatum showed the most instability in terms of mutation load and magnitude of size change, followed by cortex, hippocampus, cerebellum, and then all non-CNS tissue. She has also studied another knock-in mouse model developed by Peter Detloff, which has 150 repeats. In these mice, striatum was again most affected, followed by liver, cortex and cerebellum. Shelbourne speculated that the pattern of instability was related to repeat length.

Are repeat expansions transcribed and translated?

Peggy Shelbourne showed preliminary data from her lab suggesting that *Hdh* transcripts containing very long repeat stretches are present in the striatal tissue of mice. This raises the possibility that such transcripts may be translated into protein with enormous polyglutamine stretches. However, at this point, there are no data to confirm

this possibility, nor is there any indication of the possible consequences of the presence of such a protein.

Is somatic instability CAG specific?

Larry Marsh wondered if repeat instability was specific to the CAG repeat locus, or if it might also occur at some other hypervariable tandem repeat locus. Darren Monckton presented data indicating that a non-transcribed CTG repeat can expand significantly in the striatum of an otherwise normal mouse brain. Monckton's mice contain a transgene from the human DM1 (myotonic dystrophy type 1) locus, which includes a CTG repeat in the 3'-untranslated region (3'-UTR.) This gene shows the highest levels of somatic expansion in the striatum, yet produces no protein and no pathology in the brain.

The question also arose as to whether a pure CAG repeat was required or if a mixed CAA/CAG repeat (which would also code for polyglutamine) would also exhibit mutation instability and pathology. Blair Leavitt said that the first mouse developed in the Hayden lab did have a mixed CAG/CTG and showed instability. Mice developed in Dan Tagle's lab have pure CAG and also showed a lot of instability.

Possible mechanism

Anne Messer presented evidence showing suppression of somatic mosaicism in Msh2 knockout mice; and Darren Monckton presented data showing partial suppression of repeat instability with Pms2 knockouts. Msh3 has also been implicated. These experiments indicate that expansion is actually a repair process, depending on DNA repair enzymes, rather than a slippage during mitosis. Both Msh2 and Msh3 appear to be required for somatic mosaicism to occur in the R6/1-2 models of HD. Darren Monckton

also discussed data that implicates mismatch repair gene mutants found in hereditary non-polyposis colon cancer patients as being *trans* acting genetic modifiers of expanded repeat stability in humans. Christopher Pearson pointed out that the role of Msh2 in CAG instability may be related to processes that are independent of post-replication mismatch repair, for example, DNA recombination. Not all the functions in which mismatch repair proteins participate are known, he said. Binding of mismatch repair proteins to mutagenic intermediates may actually block them from being correctly repaired, thus permitting mutation fixation.

Pearson presented data that showed no repeat instability in stationary primary cells from a myotonic dystrophy type 1 patient. However, data from other studies in the sperm and brains of transgenic mice suggests a cell-division independent mechanism.

Does repeat expansion occur gradually or suddenly? Evidence suggests that the problem of somatic instability worsens with age. Peggy Shelbourne said she has looked for, but not seen, any evidence of repeat instability during embryogenesis or neurogenesis. Moreover, in mice, she has not seen any evidence of repeat instability before three months of age and then sees a gradual rise after that point. “My gut feeling is there are a large number of small events,” she said. Anne Messer agreed, based on her work with R6/1 mice. Darren Monckton said it is also clear from human data that repeat length variation is a gradual process. The question remains, how gradual is gradual?

Carl Johnson asked whether one can deduce, from the kinetics of the change in the distribution, at what point repeat expansion is likely to become toxic. Monckton said it is a difficult problem to address experimentally.

Nonetheless, somatic instability may help explain some of the repeat length inconsistencies observed in humans. Blair Leavitt said that in the database his group maintains, no one with fewer than 35 repeats has ever developed HD. The mean repeat length in this cohort is 44. Nancy Wexler added that while there are some people with a repeat length of 37 who go on to live long lives, almost everyone with a repeat length over 40 develops the disease. Greater than 60 repeats ensures onset by the age of 20. However, at repeat lengths of 44 or so, she said, there is a lot of variability in age on onset, suggesting there is something beyond repeat length that is affecting pathogenesis.

Christopher Pearson concluded that it is probably impossible to address at the present time whether somatic mosaicism contributes to disease severity and to what degree. Somatic mosaicism needs to be studied in age-matched and repeat-length-matched human samples in order for correlations with clinical severity to be determined.

The chicken and egg problem. Blair Leavitt suggested, however, that any correlation raises the “which came first” question: instability or pathology. One of the likely reasons for instability is that repair mechanisms are generating instability in response to DNA damage, he said. But why is the DNA being damaged? “There is no evidence that I’ve ever heard that would suggest anything except that cells that are damaged show more instability. So I would suggest that the instability is probably secondary to the toxic effects of the protein.”

Peggy Shelbourne agreed with Leavitt, suggesting three events in the process: First, the mutant huntingtin protein triggers instability of the mutation; second, the mutation expands; and third, the larger repeat produces protein with a longer

polyglutamine chain. What is not known are the pathological consequences of that increased polyglutamine length and whether there are complicated feedback mechanisms.

Shelbourne suggested that the trigger of instability may be depletion of brain-derived neurotrophic factor (BDNF) in cortical cells expressing mutant huntingtin, which has been demonstrated by Elena Cattaneo and colleagues⁴. This may result in an increased excitotoxic challenge of striatal cells that leads to mutation expansion, which, in turn, could have detrimental downstream effects. However, as noted previously, there is still no clear evidence that expanded protein is a mediator of pathogenesis.

Oxidative stress. “The striatum is a time bomb in terms of oxidative stress,” said Marie-Francoise Chesselet. So, if you were to think about where in the brain DNA damage might be most severe, the striatum would be a likely candidate. “This is the one place where you have a convergence of an extremely powerful and abundant source of glutamate and a source of dopamine,” she said. She also wondered whether huntingtin protein getting into the nucleus might be part of the equation; either by contributing to instability or altering repair mechanisms in a way that favors expansion. Huntingtin going into the nucleus is regionally specific, she said, which may be related to oxidative stress.

Therapeutic implications

The data presented on the role of mismatch repair enzymes suggest obvious possible therapeutic targets. In addition, Richard Sinden, Christopher Pearson, and Darren Monckton presented preliminary data using several model systems and treatment regimes, indicating that repeat instability can be modified in vitro using various chemical treatments. Sinden noted that while they have been looking for DNA damaging agents that are very specific for CAG repeats, effective agents may be very different depending

on repeat length. He said his lab has found some things that seem to work in bacterial model systems. Pearson stated that the chromosomal context may contribute to the effectiveness and specificity of certain compounds to modulate CAG stability. Al LaSpada noted that even if it's true that large repeat expansions are driving pathogenesis, it may be possible to intervene downstream, but proximal enough to cure disease.

Blair Leavitt suggested two additional therapeutic approaches: first, ameliorating with drugs a deficiency of BDNF in the cortex; and second, interfering with increases in excitotoxicity to protect the medium spiny neurons in the striatum.

Key to identifying new therapies will be developing high-throughput drug screens. In order for somatic expansion itself to be a candidate for a drug screen, cell-based systems would be useful. Darren Monckton described some of the curious mutation behaviors his group has observed in *ex vivo* cell cultures derived from transgenic mice carrying extended trinucleotide repeat tracts. Christopher Pearson also mentioned some of his cell culture experiments with DM1 patient cells. Further characterization of the behaviors observed in these systems may yield a cell-based screening approach.

Future Studies

Much of the meeting was devoted to discussing the most important experiments needed to resolve some of these unanswered questions.

- Generating mice that are conditional knock outs for Msh2, Msh3, and/or Msh6 could help clarify the role of repair mechanisms as well as provide a model to study the consequences of somatic instability. Anne Messer also suggested sequencing a series of these enzymes to look for polymorphisms in the human HD population that might affect the rate of somatic expansion.

- Messer also suggested crossing SCA1 mice, which have the CAG repeat but no striatal pathology, to HD mice to try to uncouple the pathology from the repeat expansion.
- One experiment already underway is a cross between Peggy Shelbourne's HD knock-in mice and Darren Monckton's DM transgenic mice with a large expanded repeat as a reporter for a trans effect on DNA stability.
- In order to study whether the striatum is particularly sensitive to somatic expansions because of increased oxidative stress, Bob Hughes proposed injecting 3-NP into the striata of SCA1 PcP transgenic mice to test whether this normally unaffected brain region would exhibit somatic instability when subjected to increased oxidative stress.
- Al LaSpada suggested trying to get a sense of the repair replication profile of the cerebellum versus the striatum to try to determine why the striatum is a more mutable substrate.
- In order to clarify the relationship between somatic instability and cell type, Sarah Augood and others proposed capturing individual cell populations using laser capture microdissection (LCM), extracting the DNA, and then comparing the extent of CAG repeat instability among the different cell types. These studies could be done in both human and mouse tissue. Augood commented that she is already providing Peggy Shelbourne with test mouse striatal LCM samples. Richard Sinden suggested that this technique could also be used to study whether cells with moderate expansions or cells with massive expansions are associated with toxicity. Cells captured with LCM could be

examined using a variety of biochemical and genetic techniques, including histochemistry, immunofluorescence, and DNA microarrays. Peggy Shelbourne also suggested studying function of single cells using electrophysiology and correlating the results to repeat size.

- Mark Erlander proposed another approach that could help clarify the mechanism underlying differential cell vulnerability. He suggested investigating the circuitry coming in and out of the striatum to determine whether mosaicism is circuitry-dependent. Using retrograde analysis, one could identify the cortical cells that are innervating the striatum, laser capture those cells, and look at repeat length and profiles of gene expression.
- In order to test whether pure CAG is a requirement for repeat instability, Larry Marsh proposed comparing transgenic models with large pure CAG to models with mixed repeats. Allan Tobin proposed using a construct developed by Alex Kazantsev, which has a mixed CAG, CAA repeat on exon 1. Transgenic mice with this construct vs. mice with a pure CAG construct could be analyzed for stability. A possible hypothesis would be that the mixed construct would be more stable and probably express the same protein.
- Another possibility would be to knock in CAA into expanded CAG repeats to see if that leads to stabilization of somatic expansion. Participants agreed that it would be accepted as good evidence that instability isn't critical if you had two constructs with the same polyglutamine tract, one of which was pure and one of which wasn't, and they both caused the same degree of pathology.

- Al LaSpada suggested further studies of other polyglutamine diseases to determine whether somatic mosaicism is implicated in diseases other than HD.
- Mark Erlander suggested learning more about somatic mosaicism in humans by doing biopsies and/or developing some sort of whole body scans, such as those that are used to diagnose cancer. For example, peptides that recognize very long CAG repeats and have some sort of detectable and quantifiable tag might be possible to develop.
- Blair Leavitt suggested experiments to look at the proteins in cells with massive CAG expansions using a proteomics approach.
- Christopher Pearson suggested experiments to identify potential therapeutic targets that might alter somatic repeat mosaicism. These would include proteins or metabolic systems that contribute to either the stabilization or destabilization of the repeats. He also suggested identifying potential targets for genetic therapy of somatic mosaicism. Such targets might include cis-elements flanking the unstable CAG repeat tracts, which may predispose those tracts to high levels of instability.
- Pearson also suggested an educated fishing trip to screen pharmacologic agents for their potential to modify repeat instability, looking especially for specific induction of repeat deletions from only expanded disease alleles. Because such a fishing trip may take some time before a fish is caught, he said, it would be worthwhile investing in developing a high-throughput assay for such agents. For such an assay, it would be important to know in which

exact cell types the pathologically relevant instability occurred, so that agents could be identified that will act specifically on the target tissue and spare the other tissues from undue exposures.

- Michael Andresen suggested using drugs as a way to uncouple pathology from repeat expansions. If, for instance, expansion is msh2 dependent, it might be possible to knock out msh2 in the brain and then see if that stabilizes somatic expansion and if it changes the course of the disease. Mark Erlander added that it may be useful to screen for drugs that inhibit expansion, regardless of the mechanism, and then test those drugs in HD models for effectiveness against HD pathology.
- Jody Corey-Bloom suggested looking for a way of restoring BDNF underproduction and then looking at the effect on oxidative stress, the ability of cells to handle stress, mutation instability, and inclusions. She further suggested examining how the phenomenon of intranuclear localization of huntingtin relates to expansion.
- Larry Marsh noted that all experiments can be done in *drosophila*. His lab has seen some instability with non-perfect repeats. One possible experiment would be to put a stop codon into a perfect repeat, which would result in no translation, and then see if you get instability.
- Norman Arnheim noted that mice, humans, and flies are all very different in terms of repeat instability. Thus, it will be important to interpret studies in mice and flies carefully.

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¹ Telenius H et.al. (1994) Somatic and gonadal mosaicism of the Huntington disease gene CAG repeat in brain and sperm. *Nature Genetics* 6, 409-414.

² Kennedy L and Shelbourne PF (2000). Dramatic mutation instability in HD mouse striatum: does polyglutamine load contribute to cell-specific vulnerability in Huntington's disease? *Human Molecular Genetics* 9,2539-2544

³ Watanabe H et.al. (2000) Differential somatic CAG repeat instability in variable brain cell lineage in dentatorubral pallidoluysian atrophy (DRPLA): a laser-capture microdissection (LCM)-based analysis. *Human Genet* 107,452-457

⁴ Zuccato C et.al. (2001) Loss of huntingtin-mediated BDNF gene transcription in Huntington's disease. *Science* 293:5529,493-498