Hereditary Disease Foundation (HDF) conference 2022 – Day 4

Read updates from clinical trials and scientific research on Huntington's disease from Day 4 of the 2022 HDF Milton Wexler Biennial Symposium #HDF2022

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DNA repair and CAG repeat instability

The effect of HTT lowering on CAG repeat expansions

Welcome to last day of the @hdfcures conference! We'll only be sharing a few talks from today's sessions, which focus on DNA repair. The first is from HDBuzz's very own Jeff Carroll!

The editorial board of HDBuzz gathered for a photo op during a break to commemorate the inaugural event of their partnership with HDF.

Jeff will be sharing his work on HTT lowering and how this might influence the stability of the CAG number in mouse models of HD. This is part of a process called somatic instability which we previously wrote about on HDBuzz.

Scientists have found that buildup of HTT within a cellular compartment called the nucleus, where our genetic material is stored, might be driving aspects of HD. This might be because of interactions HTT has with that genetic material - the DNA

It seems that the huntingtin protein molecule is binding to genes which we know are very important in HD. Interestingly, it looks like huntingtin is binding on to the end of genes, where expression of the gene ends. Very spooky!

When they looked to see which groups of genes huntingtin seems to be hanging out near, it looks like these are mainly genes with lower expression in HD and HD animal models. While cool, it's not clear what this all means just yet.

Now Jeff is switching gears to look at somatic expansion in HD mouse models. His team found that when HTT levels are lowered, the amount of expansion is reduced when they looked in the liver, but in the brain, they don't see the same effect.

It turns out that the HTT-targeting ASO causes the machinery involved in gene expression, a process called transcription, to be thrown off the DNA at the HTT gene. Scientists have found that transcription is important for somatic expansion so Jeff thinks this might be why the ASO reduces expansion.

Lowering HTT using a different tool, Jeff's data shows that lowering only the expanded form of HTT prevents expansion of the CAG repeat - somatic instability. This is great news since there's been a lot of talk at this meeting about the contribution of somatic instability to HD and what it could mean for therapeutic development

It turns out that the HTT lowering ASO also reduces somatic instability at other genes which have lots of CAG repeats. It's not quite clear what's going on just yet but Jeff and his team are on the case to follow up on this interesting data.

The role of modifiers in CAG repeat expansion

Our next talk is from Anna Pluciennik, who will be sharing her work on DNA repair and CAG expansions. Anna's work is focused on understanding how mistakes in reading DNA can lead to additions of CAG repeats.

When the gene has lots of CAGs, like HTT, DNA slips out forming a little loop. This little DNA loop is recognized by molecular machines in the cell that can increase those repeats.

Normally, cells can repair this, but it seems in diseases like HD there are also problems with the proteins that repair these slip outs. Understanding more about these DNA slip outs at the CAGs and proteins that repair DNA could tell us something about the cause of HD.

Interestingly, many genes that modify the age of onset of HD - "modifiers" - also happen to be these proteins that repair DNA. It's all connected!

One of those modifier proteins that Anna is interested in is called FAN1. Anna and her team can make FAN1 protein in the lab and look to see what other molecules it might be working with. They found that FAN1 interacts with DNA only when CAG slip outs are present. Her lab is doing lots of experiments to find other proteins that are required for this process.

Understanding exactly what's going on and what proteins are involved will help the team understand if they can disrupt this process to reduce the slip outs. Ultimately, they hope this could help them reduce CAG expansions in HD.

Different forms and fragments of the HTT protein

The last talk of the conference is by Gill Bates, who will put HTT splicing into perspective for HTT-lowering therapeutics. HTT splicing is something we've heard a lot about lately with recent trials around PTC-518 and branaplam.

Splicing is the fancy science name for the process by which genetic messages are processed and chopped up before they get turned into protein molecules. If the huntingtin genetic message is spliced differently, then different forms or fragments of the huntingtin protein molecule will be made.

Dr. Bates' team looked at lots of these different forms and fragments so that they could systematically ask what each is doing. Interestingly, they found that there is one particular fragment - called "exon 1" - which may be super important.

This exon 1 fragment contains the CAG repeats, but is missing much of the rest of the HTT gene. So it seems that this particular fragment may be causing much of the trouble in HD.

Since scientists like to give molecules specific names once they know they're important, this exon 1 fragment of the huntingtin protein has been named HTT1a.

Using various tools in lab, they have shown that HTT1a is also made into a little protein fragment and can be found in different mouse models of HD. When they looked in brains generously donated from people with HD, they also found this little HTT1a fragment there.

It seems that the HTT1a protein fragment is important for beginning the formation of toxic protein clumps, called aggregates. Aggregates are a common feature in HD in both people and our animal and cell models of HD.

Dr. Bates has focused on developing tools to specifically look at the small HTT1a protein. This has been tricky because HTT protein fragments, like HTT1a, are hard to handle and make in the lab as they are rather sticky.

Interestingly, when they look in certain mouse models of HD over time, they find full length HTT levels go down as the mice age but levels of the HTT1a clumps go up. This suggests the HTT1a fragment becomes more prevalent as the HD mice get more sick.

Gill's team is also looking at measuring the really enormous full-length HTT protein molecule. There are lots of different ways to do this but nearly all of these experiments get confused by a mixture of expanded and unexpanded HTT.

All of this work is very important because all of the HTT lowering clinical trials rely on these tests to work out if their drug is working or not by measuring changes in the HTT levels in different samples.

One important thing Gill's work points out is that it's really critical to measure various forms of the HTT protein - both full length and fragments that seem to be very toxic and contribute to disease.

An interesting question Gill asked was, what happens if we could make a mouse that doesn't produce the toxic HTT1a fragment given how important it appears to be in HD?

Gill's team have used some clever genetics tricks to make a mouse which only makes the full-length HTT protein but not the HTT1a fragment.

When they compare these mice to the same strain that DOES express HTT1a and look at protein clump formation in the brain, they find they do eventually form, just much later than expected and to a lesser degree.

While this might seem to suggest that even without HTT1a, mice can form toxic protein clumps, the caveat with this interpretation is that these mice did have a very small amount of HTT1a still present. So that small amount may be driving this pathology.

No experiment is perfect, but these results strongly suggest that a significant amount of the toxicity associated with the HTT protein is because of the HTT1a fragment.

That's all for our reporting from the @hdfcures conference! HDBuzz looks forward to tweeting future HDF symposia. We hope you all enjoyed following along and we look forward to sharing more HD research with you soon!

To learn more about the Hereditary Disease Foundation, visit their website. To learn more about the science discussed at #HDF2022, tune into a live webinar on September 15th at noon EST! Register here You can also follow HDF on Facebook, Instagram, and Twitter to ensure you don't miss future webinar updates.

Sarah Hernandez is an employee of the Hereditary Disease Foundation.For more information about our disclosure policy see our FAQ...

GLOSSARY

huntingtin protein The protein produced by the HD gene.

transcription the first step in making a protein from the recipe stored in a gene. Transcription means making a working copy of the gene from RNA, a chemical messenger similar to DNA.

therapeutics treatments

CAG repeat The stretch of DNA at the beginning of the HD gene, which contains the sequence CAG repeated many times, and is abnormally long in people who will develop HD

aggregate Lumps of protein that form inside cells in Huntington's disease and some other degenerative diseases

splicing the cutting up of RNA messages, to remove non-coding regions and join together coding regions.

nucleus A part of the cell containing genes (DNA)

somatic relating to the body

Exons The small fraction of our DNA that is directly used to instruct cells how to make proteins

ASOs A type of gene silencing treatment in which specially designed DNA molecules are used to switch off a gene

HTT one abbreviation for the gene that causes Huntington's disease. The same gene is also called HD and IT-15
