Genetic testing in HD—
the winds of change...

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Ground Rules

• You are all muted (sorry)
• There are SO MANY of you (!), we had to do it this way
• If you have a question, go to the “chat” function on the side of your screen, and write it down; we hope to have 10 minutes at the end for questions
Introduction

- Describe complexities of the repeat sequence that are emerging as clinically important (Matt)
- Discuss research studies that at-risk patients might ask about (Martha)
- Discuss the implications of all of this on the work that genetic counselors do
- Ask whether this is a useful activity

My background (Matt)

- The molecular diagnostics laboratory at the University of Minnesota began testing for Huntington disease in 1994

- I have been involved with predictive genetic testing for HD in our adult neurology clinic since 2001.
Huntington disease as a ‘simple’ genetic disease

• I begin teaching our laboratory rotation using Huntington disease as an example of a deceptively simple test.
  • We are evaluating a single genetic ‘variant’ to make the diagnosis
  • Clear interpretive guidelines

Interpretive guidelines

• Normal [9-26 CAG repeats]
  • Expansion of 26 repeat allele [PMID:23946314]
• Intermediate [27-35 CAG repeats]
  • Symptoms in intermediate range [PMID:27402890]
• Reduced penetrance [36-39 CAG repeats]
  • Why do some individuals remain asymptomatic?
• Full penetrance [40+ repeats]
  • Patients with late/early onset relative to repeat number
• The reported repeat number does not fully explain age of onset or meiotic instability.
Changing guidelines

- Is there sufficient evidence to warrant revision?
- What are the downstream consequences?
  - Duty to recontact

Current theories

- Tissue specific somatic expansion of repeat may be a critical factor in disease onset and/or progression.
- Somatic expansion of the repeat may be mediated by both cis and trans acting factors
- Hot topic at recent European Huntington Disease Network
**Cis acting factors**

- Somatic and/or meiotic instability of the repeat may be mediated by:
  - Interruptions (or loss of interruptions) in CAG repeat
  - Extension of the pure CAG repeat due to single nucleotide variants
  - Variation in size of adjacent (CCG) repeats.
- I will focus on these for today’s talk

**Trans acting factors**

- Evidence suggests that in intact mismatch repair system (MSH2/MSH3) is required for somatic expansion.
  - Knocking these genes out in mice appears to preclude expansion.
- Variants in DNA repair genes identified as modifiers
  - FAN1
- This will not be the focus of today’s talk
How does the HD test actually work?

Clinical view

Counting CAG repeats

Laboratory view

Amplifying a DNA fragment and inferring repeat number based on several assumptions

The HD molecular test

1. Amplify a fragment containing the CAG repeat

2. Subtract the non-CAG repeat portions of the fragment

3. Divide the remaining fragment size by 3

4. Technical adjustments based on laboratory validations
What assumptions are we making?

- There are no variations interfering with our primers
- The size of adjacent non-CAG repeats is irrelevant
- There are no SNPs present that would extend the CAG repeat sequence into the regions we are subtracting
- The content of the remaining fragment is purely CAG repeats

Taking a closer look

There is a 12 base (4 codon) sequence between the “pure” CAG repeat and “pure” CCG repeat. This sequence contains two “interruptions” that also encode glutamine or proline, respectively.
Taking a closer look

The HD diagnostic test determines the size of the ‘pure’ CAG repeat sequence

Polyglutamine tract

Q Q Q Q Q Q

Polyproline tract

P P P P P

CAG.CAG.CAG.CAG.CAG.CAG CAA.CAG.CCG.CCA CCG.CCG.CCG

Taking a closer look

We are not actually sequencing this region. We are assuming it is composed of CAG repeats

CAG.CAG.CAG.CAG.CAG.CAG CAA.CAG.CCG.CCA CCG.CCG.CCG

These two alleles appear identical on molecular testing

CAG.CAG.CAG.CAG.CAG.CAG CAG.CAG.CAA.CAG.CAG.CAG
Taking a closer look

CAG.CAG.CAG.CAG.CAG.CAG  CAA.CAG.CCG.CCA  CCG.CCG.CCG

CAG.CAG.CAG.CAG.CAG.CAG  CAG.CAG.CCG.CCA  CCG.CCG.CCG

A single base variation in this codon can extend the CAG repeat by two - but this is always subtracted from the fragment size.

CAG.CAG.CAG.CAG.CAG.CAG  CAG.CAG.CAG.CAG.CAG.CAG  CCG.CCG.CCG

3

American Journal of Medical Genetics 87:01-02 (1999)

Letter to the Editor
Expansion of a 27 CAG Repeat Allele Into a Symptomatic Huntington Disease-Producing Allele

CAG.CAG.CAG.CAG.CAG.CAG  CAG.CAG.CCG.CCA

CAG
27

The original family with an unstable 27-repeat allele had a SNP that extended the ‘pure’ CAG repeat.
Taking a closer look

The initial HD diagnostic test assumed this CCG repeat sequence was the same size in everyone.

Original (HD12) primers

The CCG repeat actually varies from 7-12 repeats.

If we subtract a fixed number of CCG repeats (7), we may overestimate the CAG repeat number in cases where there are more CCG repeats present.
HD 1/3 primers

Current diagnostic primers now exclude the polymorphic CCG repeat sequence

| CAG.CAG.CAG | CAA.CAG.CCG.CCA | CCG.CCG.CCG |
| CAG.CAG.CAG | CAA.CAG.CCG.CCA | CCG.CCG.CCG.CCG.CCG |

We still utilize HD ½ primers to resolve homozygosity

HD 1/3 primers yield a single fragment size

- Most likely due to homozygosity for a common CAG repeat size (e.g. 17)
- Can’t exclude the presence of an extremely large expansion (>90) that failed to amplify
- If two different sized CCG repeats present, we can use the old ½ primers to demonstrate two distinct normal-sized alleles.
We still utilize HD $\frac{1}{2}$ primers to resolve homozygosity

Utilizing $\frac{1}{2}$ primers in cases of apparent homozygosity will often allow us to visualize two distinct normal-sized alleles.

Can we detect evidence of somatic repeat size mosaicism with the standard diagnostic test?

- Standard PCR does not generate a single clean ‘peak’ for larger alleles
- Identifying somatic mosaicism on this noisy background is difficult.
Summary

- The ‘CAG repeat’ in HTT is actually a complex repeat structure.
- Variations in the structure of these repeats may be important in mediating somatic stability/instability.
- Somatic instability may be a determinant in disease onset and progression.

Why not sequence?

- Sequencing is costly relative to fragment analysis.
- Analyzing polymorphic repetitive sequence is extremely messy.
- Setting phase of CAG repeat size with interruptions may not be possible in a single sample.
- Any signal of somatic mosaicism in blood may be overshadowed by the noise of the PCR reaction itself.
Should diagnostic testing include more information about the repeat

- Is there sufficient evidence that this information is clinically useful in an individual patient.
- Can these variations in repeat structure be reliably detected in an individual sample?
- Can these variations in repeat structure be detected in a cost-effective way to patients?

HD Clinical Research

- Enroll-HD
- Precision HD1 and Precision HD2 (Wave Life Sciences)
- Generation HD1 (Roche)
Global observational study of HD, began 2012
- Goal 20,000 subjects, currently at 19,000
- 170 sites, 19 countries involved
- Includes gene positive, gene negative, not tested, affected, (not at risk)
- Researchers can (freely) access data and samples
- Companies look to high-enrolling Enroll sites for their drug trials
- Enroll-hd.org

Wave LifeSciences
- Precision HD1 and Precision HD2
- Phase 1b-2a trials of SNP-based ASO, delivered by monthly spinal infusion
Why target the SNP?

- To selectively reduce production of mHTT
- About 70% of Caucasians with HD have SNP1, SNP2, or both on the chromosome with the expanded allele
- Establishing the presence and phase of the SNPs has been challenging
- The company is looking for less common, but relevant, SNPs to target

Precision HD1 and Precision HD2

- US sites are planned
- Currently enrolling in Toronto and Poland

Wavelifesciences.com, hdtrialfinder.org, clinicaltrials.gov
IONIS-HTTRx study

- Completed 4th quarter 2017
- 46 subjects, Canada, Germany, England
- First in-human trial of ASO for HD; delivered by monthly spinal infusion for 3 months
- Goal was to reduce production of huntingtin (wild type and mutant), reflected in reduced CSF Htt levels

Results of IONIS-HTTRx

- CSF Htt levels reduced by 40%, more lowering with bigger doses of the drug
- NO safety issues arose
- Study subjects are continuing to receive the drug on an open-label basis
- Roche (who bought Ionis) is skipping to the Phase 3 trial described above
- (Hints that some subjects had a little improvement in some clinical measures)
Generation HD

- Phase 3 trial of RG6042, a nonselective ASO, to reduce huntingtin production
- 660 subjects WITH MANIFEST HD (early stages), about 90 sites, multiple countries
- The concept of the CAP score (CAG-Age Product)
  - Age x (CAG-33.66)=CAP score
- Monthly spinal infusions for 2 years
- Planned start 1st quarter 2019
- Roche is also organizing a “natural history study”, of about 100 additional subjects

What does this have to do with genetic counseling/testing for HD?

- What can the patient do if he/she tests positive, or negative, or decides not to be tested?
  - Enroll in Enroll-HD
  - Be active in the HD community (HDSA, HDYO, etc)
What does this have to do with genetic counseling/testing for HD?

- Can the patient get SNP testing to see if he/she is a candidate for the Wave trial?
  - NO
  - SNP testing is not clinically available
  - Wave is performing SNP testing AFTER patients have signed initial consent to be in their trial
  - The WAVE trials are targeting people who have “manifest” (diagnosable motor symptoms of) HD; gene-positive but unaffected individuals cannot enroll in the study

What does this have to do with genetic counseling/testing for HD?

- Should an at-risk patient get a gene test “so that I can enroll in that gene therapy trial”?
  - NO
  - All of the currently proposed gene silencing trials are targeting diagnosed/affected individuals with manifest (diagnosable motor symptoms of) HD
What does this have to do with genetic counseling/testing for HD?

- What research study can a person be in if they have tested positive but do not have symptoms of HD?
  - Enroll-HD

Will there ever be a drug trial for gene positive people who do not have HD symptoms?

- Maybe, we don’t know when. There are no such studies currently in the works.
  - CAP score
Can a person get RG6042 as part of “right to try”?

- Roche will not approve any such requests.

In summary

- The indications for predictive testing have not changed---yet.
- We need to educate and support patients who misunderstand the relationship between having a gene test, being in a research study, having a drug available in the clinic, etc
- Patients (and centers) will be disappointed if they are not selected for these trials
In summary

► In the future...
  ► Clinical labs may need to add tests for the CAA interruption or the SNP (which will be expensive)
  ► We need to prepare for the time when disease-modifying treatments are available, as the floodgates will open for “predictive testing” at that point

In summary

► Matt and Martha are happy to talk to health professionals 1:1 to make sure all this is clear
► Does this group think that this presentation was helpful?
► Are there other topics that the HD genetic counseling community would like to discuss in future webinars or in-person meetings?
► Are there people in the group who would like to be a leader rather than a participant in such activities?
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