Genetic testing in HD— the winds of change…

Martha A. Nance MD
Hennepin County Medical Center
Matt Bower, LGC
University of Minnesota

September 20, 2018

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

HUNTINGTON STUDY GROUP
seeking treatments that make a difference

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.

HUNTINGTON STUDY GROUP
seeking treatments that make a difference

Changing guidelines

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

HUNTINGTON STUDY GROUP
seeking treatments that make a difference

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

HUNTINGTON STUDY GROUP
seeking treatments that make a difference

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	Laboratory view
↓	↓
Counting CAG repeats	Amplifying a DNA fragment and *inferring* repeat number based on several assumptions

11

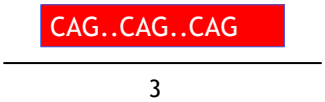
HUNTINGTON STUDY GROUP
seeking treatments that make a difference

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

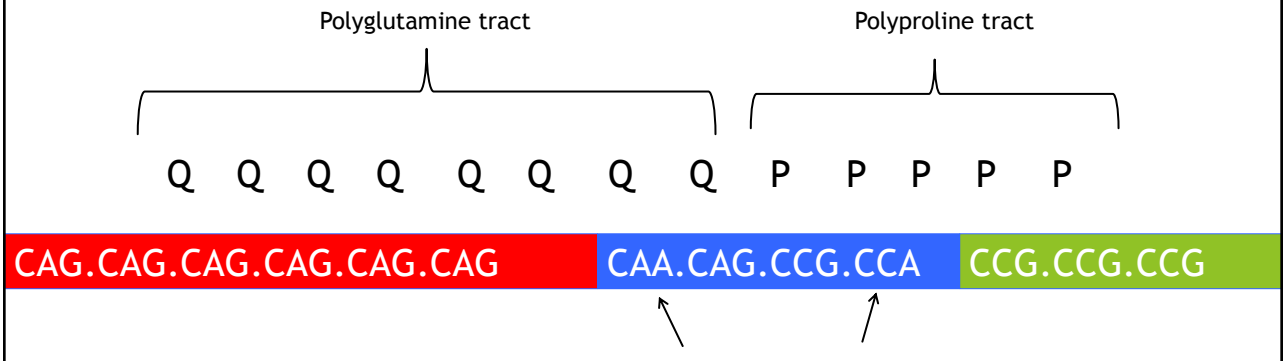
12

HUNTINGTON STUDY GROUP
seeking treatments that make a difference

What assumptions are we making?

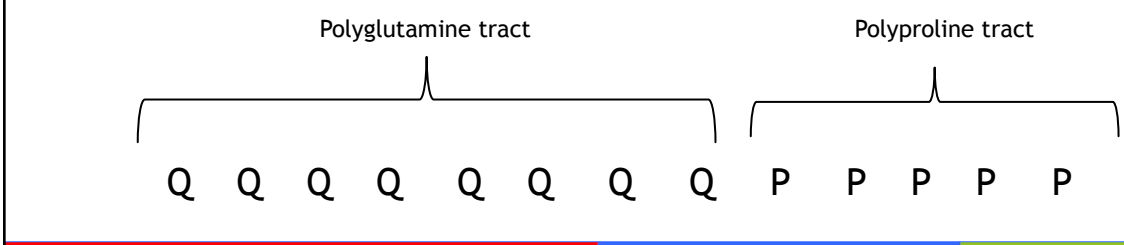
- There are no variations interfering with our primers
- The size of adjacent non-CAG repeats is irrelevant
- There are no SNPs present that would extend the CAG repeat sequence into the regions we are subtracting
- The content of the remaining fragment is purely CAG repeats

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



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

- The HD diagnostic test determines the size of the 'pure' CAG repeat sequence

15

HUNTINGTON STUDY GROUP
seeking treatments that make a difference

Taking a closer look

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

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

CAG.CAG.CAG.CAG.CAG.CAG

CAG.CAG.**CAA**.CAG.CAG.CAG

- These two alleles appear identical on molecular testing

16

HUNTINGTON STUDY GROUP
seeking treatments that make a difference

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. | CCG.CCG.CCG

3

HUNTINGTON STUDY GROUP
seeking treatments that make a difference

American Journal of Medical Genetics 87:91–92 (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.

HUNTINGTON STUDY GROUP
seeking treatments that make a difference

Taking a closer look

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

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

Original (HD12) primers

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

HUNTINGTON STUDY GROUP seeking treatments that make a difference

The CCG repeat actually varies from 7-12 repeats.

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

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

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.

HUNTINGTON STUDY GROUP seeking treatments that make a difference

HD 1/3 primers

Current diagnostic primers now exclude the polymorphic CCG repeat sequence

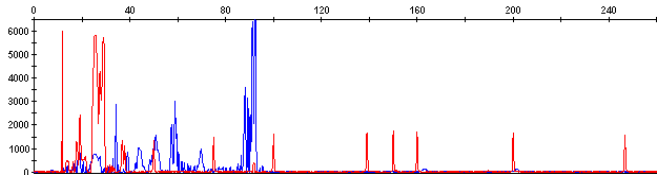
→

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

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

←

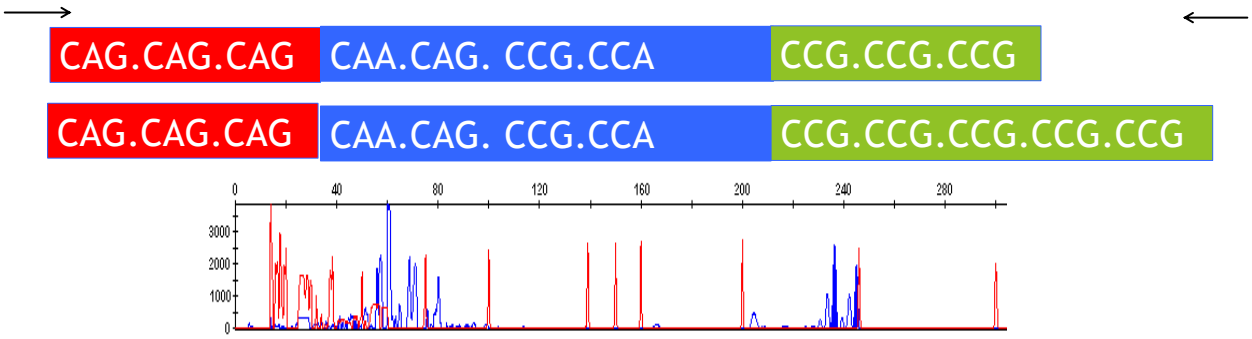
We still utilize HD ½ primers to resolve homozygosity



HD 1/3 primers yield a single fragment size

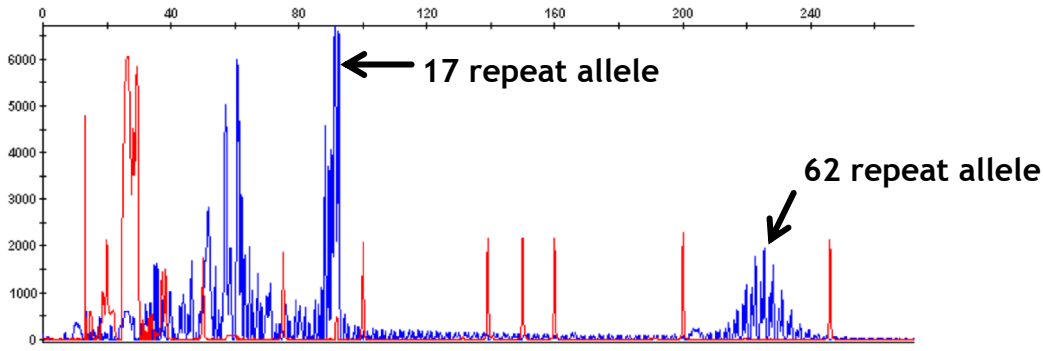
- Most likely due to homozygosity for a common CAG repeat size (e.g. 17)
- Can't exclude the presence of an extremely large expansion (>90) that failed to amplify
- If two different sized CCG repeats present, we can use the old ½ primers to demonstrate two distinct normal-sized alleles.

We still utilize HD ½ primers to resolve homozygosity



Utilizing ½ 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.

HUNTINGTON STUDY GROUP
seeking treatments that make a difference

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.

HUNTINGTON STUDY GROUP
seeking treatments that make a difference

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?

HUNTINGTON STUDY GROUP
seeking treatments that make a difference

HD Clinical Research

- Enroll-HD
- Precision HD1 and Precision HD2 (Wave Life Sciences
- Generation HD1 (Roche)

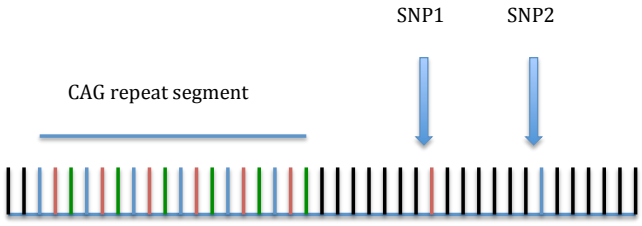
HUNTINGTON STUDY GROUP
seeking treatments that make a difference

Enroll-HD

- 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_1

- 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

35

HUNTINGTON STUDY GROUP
seeking treatments that make a difference

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)

36

HUNTINGTON STUDY GROUP
seeking treatments that make a difference

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

39

HUNTINGTON STUDY GROUP
seeking treatments that make a difference

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

40

HUNTINGTON STUDY GROUP
seeking treatments that make a difference

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

HUNTINGTON STUDY GROUP seeking treatments that make a difference

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

HUNTINGTON STUDY GROUP
seeking treatments that make a difference

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?

HUNTINGTON STUDY GROUP
seeking treatments that make a difference

Email addresses

- Martha Nance
  - martha.nance@parknicollet.com
- Matt Bower
  - MBOWER1@Fairview.org

HUNTINGTON STUDY GROUP
seeking treatments that make a difference