Genetics and Huntington disease

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Presenter Disclosures

Matthew Bower, MS

The following personal financial relationships with commercial interests relevant to this presentation existed during the past 12 months:

No relationships to disclose
Roadmap:

- Ancient history (aka before 1993)
  - Mapping the HD gene
- Modern history
  - The era of predictive testing
- Life in a molecular lab
- Molecular explanations for HD mysteries
HD history--Making genetics interesting!

\[
\text{Var}[LR(G)] = \text{Var}(\alpha_{cc} + \beta + \gamma X^T + \delta)
\]

\[
= \text{Var}(\alpha_{cc}) + \sum_{i=1}^{n} \text{Var}(\beta_i) + \sum_{i=1}^{p_1} X_i^2 \text{Var}(\gamma_i) \\
+ \sum_{i=1}^{p_2} \text{Var}(\delta_i) + 2 \sum_{i=1}^{n} \text{Cov}(\alpha_{cc}, \beta_i) + 2 \sum_{i=1}^{p_1} X_i \text{Cov}(\alpha_{cc}, \gamma_i) + 2 \sum_{i=1}^{p_2} \text{Cov}(\alpha_{cc}, \delta_i) \\
+ 2 \sum_{i=1}^{n} \sum_{i=1}^{p_1} X_i \text{Cov}(\beta_i, \gamma_i) + 2 \sum_{i=1}^{n} \sum_{i=1}^{p_2} \text{Cov}(\beta_i, \delta_i) + 2 \sum_{i=1}^{p_1} \sum_{i=1}^{p_2} X_i \text{Cov}(\gamma_i, \delta_i) \\
+ 2 \sum_{i<i'} \sum_{i'=1}^{p_2} \text{Cov}(\beta_i, \beta_{i'}) + 2 \sum_{i<i'} \sum_{i'=1}^{p_1} \text{Cov}(\gamma_i, \gamma_{i'}) + 2 \sum_{i<i'} \sum_{i'=1}^{p_2} \text{Cov}(\delta_i, \delta_{i'}) 
\]
History of HD in America

Huntington G. (1872) The Medical and Surgical Reporter 26(15)
What was notable about Dr. Huntington’s description?

- Published when he was only 22 years old!

- His only medical publication.

- Drew on 78 years of records from his family’s medical practice on Long Island.

- Accurate description of the hereditary nature of the disease
  - Gregor Mendel had only described dominant and recessive patterns of inheritance in 1865 (using peas!)
Key points on **autosomal dominant** inheritance:

**Autosomal** - Both males and females can be affected with HD. Both males and females can pass HD to their children.

**Dominant** - If a person has Huntington disease, there is a 50% risk for each of their children.

If a person does not inherit HD from their parent, they **cannot** pass it to their children.

Each child of a person with HD has an **independent** 50% risk. (i.e. their risk is not changed by whether or not their brothers’ or sisters’ test results).
While we understood the inheritance pattern for many years, we did not have the necessary tools to find the actual gene:

- Important groundwork from the 20th century
  - The discovery of DNA
  - Formation of patient advocacy groups
  - Most notably the contributions of the Wexler family.
1979- The US-Venezuela HD Collaborative Research Project

Why look for genes?
• Understand the mechanism of disease
• Potential treatments
• Answers for families
• Scientific curiosity
Why Venezuela?

• Lake Maracaibo region of Venezuela has the highest incidence of HD in the world.
• All cases can be traced to a single European ancestor.
• This founder has ~18,000 descendants.
Searching for genes - A brief detour to define genetic terms.
Where is our genetic information stored?

- Cell
- Nucleus
- Chromosomes
What are chromosomes?

- Packages of genetic information
- We have two copies of each chromosome (one from mom and one from dad)
What is a gene?

- A gene is a series of genetic letters (A, C, G, T) that spells out a specific instruction for the body.
- Genes encode proteins.
- The Huntington disease gene tells the body how to make “Huntingtin protein”-nobody knows the function of this protein.
The HD story - finding the gene

Perspective:

In 2011, finding the HD gene would be a relatively simple undertaking.
The HD story - finding the gene

1979
- No modern scientific techniques
- No human genome sequence
- No catalogs of normal variants to use for mapping
- No clues from the normal structure or function of the gene product.
- Late age of onset - difficult to assign individuals to “Affected” or “unaffected” groups
How do you map a gene?

- You need large families who are willing to be clinically evaluated and to give a blood sample.
- Researchers then look for parts of chromosomes that are shared by family members affected with the condition.
- Conversely- exclude areas of the genome that are not shared by affected individuals.
- Initial results usually highlight several areas of interest (see next slide)
What comes next?

- Researchers try to hone in on which of these regions actually contains the gene.
- Larger families (and a little luck) are needed for more precision.
- In 1983, researchers pinpoint the approximate location of the HD gene on chromosome 4.
Fig. 1 Pedigree of an American Huntington’s disease family.

Fig. 2 Pedigree of the Venezuelan Huntington’s disease family. This pedigree represents

Huntington’s Disease Society of America
Gene location is mapped to 4p16.3

A polymorphic DNA marker genetically linked to Huntington’s disease

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Mary Anne Anderson*, Rudolph E. Tanzi*, Paul C. Watkins*‡, Kathleen Ottina‡,
Margaret R. Wallace‡, Alan Y. Sakaguchi§, Anne B. Young‖, Ira Shoulson‖,
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‖ Venezuela Collaborative Huntington’s Disease Project*
Ancient history of HD

• Some descriptions may date back

HD gene location

Presumed location of the HD gene
The frustrating search for the gene

- The gene’s approximate location was found in 1983
- Linkage testing could give a likelihood of being affected, but not a certainty.
- The actual gene was not found until 1993
- Required world-wide collaboration of scientists and families
A novel gene containing a trinucleotide repeat that is expanded and unstable on Huntington's disease chromosomes. The Huntington's Disease Collaborative Research Group.

The Huntington's disease (HD) gene has been mapped in 4p16.3 but has eluded identification. We have used haplotype analysis of linkage disequilibrium to spotlight a small segment of 4p16.3 as the likely location of the defect. A new gene, IT15, isolated using cloned trapped exons from the target area contains a polymorphic trinucleotide repeat that is expanded and unstable on HD chromosomes. A (CAG)n repeat longer than the normal range was observed on HD chromosomes from all 75 disease families examined, comprising a variety of ethnic backgrounds and 4p16.3 haplotypes. The (CAG)n repeat appears to be located within the coding sequence of a predicted approximately 348 kd protein that is widely expressed but unrelated to any known gene. Thus, the HD mutation involves an unstable DNA segment, similar to those described in fragile X syndrome, spinobulbar muscular atrophy, and myotonic dystrophy, acting in the context of a novel 4p16.3 gene to produce a dominant phenotype.

PMID: 8458085 [PubMed - indexed for MEDLINE]
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Welcome to the National Institutes of Health.

At NIH, we are dedicated to improving the health of Americans by conducting and funding medical research.

We also train scientists, and communicate medical and health sciences information to patients, their families, health care providers and the general public.

NIH guides America's efforts in medical research. Our goal is to uncover new knowledge that will help prevent, detect, diagnose, and treat disease and disability, from the common cold to the rarest genetic disorder.

Our investment in understanding such diseases as AIDS, diabetes, heart disease and cancer returns dividends in longer, healthier, and safer lives.

We continue to make major inroads in fighting humanity's most enduring illnesses. And we are working to confront new threats to our health and safety, like bioterrorism.

We encourage you to explore the wealth of medical research on the NIH Web site.
Discovering the HD gene

- The HD gene was called “IT15”
- The gene contained a repeated series of letters- a CAG repeat.
- Affected individuals from 75 different families consistently had > 40 CAG repeats
- Unaffected individuals consistently had <30 repeats
- “Intermediate” range of 30-40 repeats- uncertain significance

- Please note, these repeat ranges are not currently accurate!!!!
Discovery of HD gene opened the door for accurate predictive testing

Rather than a “probability” of being affected based on sharing genetic information, individuals received their own “CAG” repeat number
Discovery of HD gene opened the door for accurate predictive testing
Predictive Testing Guidelines
Key Points- Autonomy

“2. The decision to take the test is the solely choice of the individual concerned. No requests from third parties - family or otherwise - shall be considered.”

“2. The individual must choose freely to be tested and must not be coerced by family, friends, partners or potential partners, physicians, insurance companies, employers, governments, or others.”

*Journal of Medical Genetics* (1994) 31(7) 555-559
Predictive Testing Guidelines

Key Points - Juveniles

“2.1 The test is available only to individuals who have reached the age of majority (according to the laws of the respective country).”

Journal of Medical Genetics (1994) 31(7) 555-559
Predictive Testing Guidelines

Key Points - Setting

“2.9 The counselors should be specifically trained in counseling methods and form part of a multidisciplinary team.”

“2.9 Such multidisciplinary team should consist of, e.g., a geneticist, a neurologist, a social worker, a psychiatrist and someone trained in medical ethical questions.”
Lessons from 15 years of predictive testing

**Uptake of predictive genetic testing**—What percentage of the “at-risk” population chooses to have predictive genetic testing.

- Prior to the availability of predictive testing, 60-85% of at-risk individuals said they would use a predictive test.

- Large study of Canadian experience reflected worldwide trends that only 10-20% of at-risk individuals have chosen to have predictive testing.

Lessons from 15 years of predictive testing

**Who uses predictive testing?**

- Females tend to outnumber males 2:1
- Average age in two studies (37-39)

Life in a Molecular Diagnostics Lab
My life in a Molecular Diagnostics Lab
Life in a Molecular Lab

• The molecular lab at the University of Minnesota performs testing for hospitals throughout the country
• >4000 tests have been performed since the 1990’s
Receiving the sample

- Most of our testing is done with blood samples.
- We occasionally test other tissue from autopsy (skin, brain).
- Testing can be performed on any tissue containing DNA (no hair or fingernails...sorry CSI fans).
Receiving the sample

- One of the most important steps is identification of the sample.
- 2 identifiers must be matched—usually name and date of birth.
- Samples are assigned a unique number which is used to track it through the testing process.
Cell Lysis - A controlled explosion!

Before lysis

After lysis
DNA precipitation -

DNA is “pulled out” of the solution by using alcohol.
Question- How can we visualize 2 tiny pieces of DNA?

Answer- Make millions of fluorescent copies!
PCR- molecular “xeroxing”

CAG\textsubscript{x17}

CAG\textsubscript{x40}

Fluorescent primers
15 and 20 CAG repeats

17 and 63 CAG repeats
Molecular Diagnostics Report

Test(s) Requested:
Huntington Disease Molecular Analysis

Specimen Description:
DNA

Clinical Comments:
CAP

Results:
PCR:
CAG Repeat Sizes:
Allele 1 18
Allele 2 59

Methodology: Total cellular DNA was extracted from the above patient’s sample and was subjected to amplification using primers specific for regions flanking the CAG trinucleotide repeat segment of the HTT(previously named IT15) gene. The PCR fragments were analyzed on an ABI 3130xl Genetic Analyzer and results were analyzed using Genemapper software. Repeat sizes are accurate +/-2 repeat units.

Interpretation:
There is an expansion of an HTT allele to a size greater than 35 CAG repeats. These results indicate that this individual has the HTT CAG repeat expansion that causes Huntington disease. Genetic counseling regarding these results is recommended.
Molecular Diagnostics Report

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Technical results:

“CAG” repeat numbers

**RESULTS:**

**PCR:**

**CAG Repeat Sizes:**

<table>
<thead>
<tr>
<th>Allele</th>
<th>Size</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>18</td>
</tr>
<tr>
<td>2</td>
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Interpretation- what does the technical result mean for the patient?

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Official repeat ranges for HD

• **9-26 repeats= Normal**
  • No risk for HD and no known risk to children.

• **27-35 repeats=Intermediate**
  • No risk for HD, but a small risk to children

• **36-39 repeats=Reduced penetrance**
  • May develop HD and a 50% risk to children

• **40+ repeats=Full penetrance**
  • Will develop HD and a 50% risk to children

Discovery of HD gene answers many of the “mysteries” of HD

- Anticipation- The observation that the age of onset becomes consistently younger in some families

- Prior to the discovery of CAG repeats, many scientists discounted this observation and attributed it to “hyper-awareness” of families and physicians.
CAG repeats explain anticipation

Diagnosis-65
17,40

Diagnosis-40
17,44

Diagnosis-25
17,50

Juvenile HD
17,68
Anticipation is due to expansion of CAG repeats

- CAG repeat numbers can expand when passed to offspring.
- Expansion occurs more often with male transmission.
- Expansion occurs more with larger repeat numbers.
- Some genes are more stable than others
HD without a family history—
as many as 20% of cases

HD-Diagnosed
age 45
HD without a family history

A molecular explanation:

- Expansion of an intermediate repeat number

![Genetic diagram with repeat numbers: 16, 35 to 17, 18 and 42, 18]
HD “skips generations”

Died age 85-HD

Died 76-No HD

HD diagnosed age 44
HD “skips generations”

Died age 85-HD

17,37

Died 76-No HD

17,37

HD diagnosed age 44

17,42

A “Reduced penetrance” mutation explains this family history
In *Mapping Fate*, Alice Wexler tells the story of a family at risk for a hereditary disease, once called Huntington's chorea. That her mother died of the disease, that her own chance of inheriting it was fifty-fifty, that her sister and father directed much of the extraordinary biomedical research to find the gene and a cure, make Wexler's story both astonishingly intimate and scientifically compelling.

Recording her own emotional odyssey, Wexler sifts through memories, dreams, and her mother’s beloved books and letters to find the personality of the woman Huntington's stole away. Despite such painful circumstances, Wexler writes with clarity and depth about mothers and sisters, about the nature of living at risk, and how her family was alternately driven apart and flung together by this destiny they could not escape.

In later chapters, she explores how her father, Milton, and sister, Nancy, developed innovative methods to stir up science. Nancy, like Alice, living at risk, helped organize the effort that led to the stunning discovery in 1983 of a genetic marker for Huntington’s, decades before most scientists thought possible. She then
When Phebe Hedges, a woman in East Hampton, New York, walked into the sea in 1806, she made visible the historical experience of a family affected by the dreaded hereditary disorder of movement, mind, and mood her neighbors called St. Vitus’s dance. Although East Hampton is known more for its celebrities than its contributions to medical science, this book shows how local families and a community helped to shape new medical knowledge and define the clinical entity known initially as Huntington’s chorea—today called Huntington’s disease—after the East Hampton physician George Huntington who described it in 1872.

Starting with the life of Phebe Hedges, Alice Wexler uses Huntington’s as a lens to explore heredity, disability, and medical knowledge among lay people as well as scientists and physicians. She addresses these themes through three overlapping stories: the lives of nineteenth century families who, despite “that disorder,” were integrated and sometimes prominent in their community; the emergence of Huntington’s chorea as a new paradigm of heredity; and the legal and scientific controversies that followed in its diagnosis and treatment.