Trinucleotide repeat disorders: Huntington Disease
You MUST know material on course page objectives
Review pages 217-220 in Gelerhter/Collins/Ginsburg text

“We used to think our fate is in the stars.
Now we know, in large measure, our fate is in our genes.”
- James Watson
Types of Mutations

- Single base pair substitutions
  - missense, nonsense, splice site
- Deletions
- Duplications
- Inversions
- Insertions
- Repeat Expansions

Outline of Lecture

- Overview of types of trinucleotide repeat disorders
- Huntington disease
- Molecular testing for trinucleotide repeat disorders
- Ethical, legal, and social implications of predictive testing
- Pathophysiology of trinucleotide disorders
- Discussion with visiting patient and her family
Trinucleotide repeat:
a type of short tandem repeat

- The size of repeat region varies between individuals and is polymorphic in normal individuals
- For some trinucleotide repeats, when the number of repeats exceeds a certain threshold, a neurological disease results

Timeline of Gene Discoveries for Trinucleotide Repeat Disorders

- 1991: Fragile X MR syndrome, SBMA
- 1992: Myotonic dystrophy
- 1993: Huntington disease, SCA1, FRAXE, DRPLA/HR
- 1996: SCA2, Friedreich ataxia, SCA6
- 1997: SCA7
- 1999: SCA10, SCA5, SCA4
- 2000s: Other SCAs, Psychiatric disorders?
Major Features of Most Trinucleotide Repeat Disorders

- Neurological/cognitive symptoms
- Many are autosomal dominant with variable expression
  (exceptions: Friedreich ataxia (recessive); Spinobulbar muscular atrophy, Fragile X syndrome, FRAXE MR - X-linked recessive)
- Later age of onset
  (exceptions: congenital myotonic dystrophy, Fragile X syndrome, FRAXE)
- Meiotic and mitotic instability with some degree of anticipation in many of the conditions

Why Know About Trinucleotide Repeat Disorders?

- Over 15 genetic different disorders that cause a significant proportion of inherited neurological disease in adults and the most common cause of inherited mental retardation in males (Fragile X syndrome)
- Molecular diagnosis is available for diagnostic confirmation, predictive testing, prenatal testing, preconception testing, and preimplantation diagnosis.
- Genetic counseling issues are complex and important to understand as are related ethical issues
FMR1 in FRAXA Mental Retardation

- 17 exon FMR1 gene cloned in 1991
- Highest FMR1 expression in neurons and spermatogonia
- FMR1P associates with translating ribosomes and is involved in nucleocytoplasmic shuttling
- Approximate repeat ranges:
  - 6-45 CGGs (0-3 AGGs) Unmethylated, Stable, Normal
  - 46-60 CGGs (0-2 AGGs) Unmethylated, +/- Instability, Normal
  - 60-200 CGGs Unmethylated, Premutation - Unstable, Normal
  - > 200 CGGs - Methylated, no FMR1, Unstable, Affected

Myotonic Dystrophy

- Common adult-onset muscular dystrophy (1 in 8000 in Caucasians, 1 in 475 in Quebec
- Autosomal dominant - 19q13.2-3
- Expansion of 3’ UTR CTG repeat in 15 exon myotonic dystrophy protein kinase gene (DMPK)
  - 5 - 37 normal
  - 50 - 90 mild - cataract, balding, limited muscle involvement, > 50 years
  - 90 - 1000 classic muscle weakness, myotonia, cataracts, onset 20-30 years
  - > 1000 often congenital, hypotonia, developmental delays
- Instability of repeats - 10% expansion, 3% contraction.
- Congenital DM always due to maternal expansion
**Anticipation**

Increasing severity and/or decreasing age of onset of an inherited disease in successive generations within a family.

- **Friedreich Ataxia:**
  - Autosomal recessive **progressive neurological disorder**, onset < 25 years with ataxia due to expansion of GAA repeat in **intron** of FRATAXIN gene
- **Spinocerebellar Ataxia (many types):**
  - Autosomal dominant **progressive neurological disorder** characterized by ataxia usually in the 3rd or 4th decade due to expanded CAG repeat in **coding exons** of SCA genes
- **Myotonic Dystrophy:**
  - Autosomal dominant **progressive neurological disorder** with variable expression and anticipation due to expansion of 3’ **UTR** region of DMPK gene
- **Fragile X syndrome:**
  - X-linked recessive **mental retardation** syndrome due to expansion of CGG repeat in 5’ **UTR** region of FRAXA gene
Trinucleotide repeat disorders can involve expansions of various repeats in coding and non-coding regions of the gene.

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<thead>
<tr>
<th>5’ UTR</th>
<th>exon</th>
<th>intron</th>
<th>exon</th>
<th>intron</th>
<th>exon</th>
<th>3’ UTR</th>
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<tbody>
<tr>
<td>CGG</td>
<td>GAA</td>
<td>CAG</td>
<td>CTG</td>
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<td><strong>FRAXA</strong></td>
<td><strong>FA</strong></td>
<td><strong>HD</strong></td>
<td><strong>MD</strong></td>
<td><strong>SCAs</strong></td>
<td><strong>SMBa</strong></td>
<td><strong>DRLPA</strong></td>
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Dr. George Huntington (1850 -1916)
- Became interested in hereditary chorea in 1871
- Wrote his seminal paper on this disease when he was 22 in 1872
- Was a general practitioner - never on a medical faculty

Individual with Huntington Disease
Huntington Disease

- Average age of onset 40 years (range 2- >80 years); Progression over 10-25 years.
- Cognitive dysfunction: problem solving, cognitive flexibility, short term memory, visuospatial functioning; progression to a global subcortical dementia
- Personality changes: depression, apathy, irritability, impulsive behavior, affective disorders, rarely psychoses, increased alcohol use in early stages, increased suicide rate

Incidence of Huntington Disease per 100,000 People

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<tr>
<th>Region</th>
<th>Incidence</th>
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<tbody>
<tr>
<td>African Blacks</td>
<td>0.06</td>
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<tr>
<td>South African Whites</td>
<td>2.4</td>
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<tr>
<td>Japan</td>
<td>0.38</td>
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<tr>
<td>Finland</td>
<td>0.5</td>
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<tr>
<td>Hong Kong</td>
<td>2.5</td>
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<tr>
<td>American Blacks</td>
<td>3-7</td>
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<tr>
<td>Western Europeans</td>
<td>7</td>
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<tr>
<td>American Whites</td>
<td>7-10</td>
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<tr>
<td>North Sweeden</td>
<td>144</td>
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<tr>
<td>Tasmania, Australia</td>
<td>174</td>
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<tr>
<td>Moray Firth, Scotland*</td>
<td>560 (5 people in &lt;1000)</td>
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<tr>
<td>Zulia, Venezuela</td>
<td>700</td>
</tr>
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</table>
Woodrow Wilson Guthrie

Diagnosed in 1952, age 40 years
Died 15 years later at age 55 years

During 17 year career wrote more than 1,000 songs and left behind 2,500 lyrics.
Near the end of his life he could only use “yes” and “no” cards to communicate.
<table>
<thead>
<tr>
<th>Phase</th>
<th>Years</th>
<th>Symptoms</th>
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</thead>
<tbody>
<tr>
<td>Transitional</td>
<td>0-3</td>
<td>mood swings, behavioral disturbances, hyperreflexia, memory impairment, increased clumsiness, impairment of voluntary movements, eye movement abnormalities</td>
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<tr>
<td>Early</td>
<td>3-5</td>
<td>dysarthria, chorea, gait abnormalities</td>
</tr>
<tr>
<td>Middle</td>
<td>8-10</td>
<td>bradykinesia, rigidity, global dementia, dystonia, dysphagia</td>
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<tr>
<td>Late</td>
<td>15-25</td>
<td>incontinence, wasting, aspiration, bed ridden death</td>
</tr>
</tbody>
</table>
Pathology of Huntington Disease

- Brain atrophy involving caudate nucleus and putamen with loss of striatal neurons and secondary atrophy of globus pallidus
- Dilation of lateral and third ventricles
- Additional atrophy throughout cortex, especially frontal and parietal lobes
- Loss of small neurons precedes larger neurons with neurons utilizing GABA and enkephalin or substance P preferentially
- Fibrillar gliosis
Huntington Disease

- IT15/Huntingtin gene on 4p16.3 cloned in 1993
- Disease mutation - CAG expansion in exon 1
  - **Repeat number** | **Outcome**
    - 10-28: normal, no transmission of HD
    - 29-35: normal, maternal meiotic instability
    - 36-39: reduced penetrance (25%: 36 repeats, 90%: 39 repeats)
    - 40-100+: will develop HD if person lives long enough

- Increased meiotic instability in males - **Paternal transmission of expanded allele associated with over 3/4 of juvenile disease**
- Encodes 348 kD huntingtin protein which is a target for caspase 3, a protease associated with neuronal apoptosis
N N N HD N N

Mandy Wester examines a section of the Venn diagram, looking for clues.
So the HD disease was cloned . . . now what?

- Provide precise, rapid diagnosis including prenatal and predictive testing
- Improve molecular understanding of pathophysiology
- Increase ethical, psychosocial, and legal concerns
- Improve medical management
- Develop novel, targeted therapies

HD DNA testing:

- Diagnosis confirmation
- Prenatal diagnosis
- Predictive diagnosis
  - psychosocial impact
  - employment concerns
  - insurance issues
Molecular Detection of Trinucleotide Repeats:
Determine the size of the repeat

- ‘Short’ Repeats (eg. HD):
  - PCR based typing of alleles using primers directly flanking repeat region - With appropriate controls and size marker the size can be determined accurately
- Long repeats: determine size of the repeat
  - PCR and Southern blot analysis
- Methylation status (eg. Fragile X)
- Immunoassays
Predictive or Presymptomatic Testing

- How should cost and benefit be defined?
- Many psychosocial issues
- Who has a right to be tested or not to be tested? Who decides?
- Who has a right to the results?
- Probabilistic vs. Deterministic
  - susceptibility versus certainty of acquiring manifesting symptoms of a disease or disorder

Flunk the Gene Test and Lose Your Insurance
PUBLIC HEALTH CODE (EXCERPT) Act 368 of 1978
333.21072a

(1) A health maintenance organization shall not require an enrollee or his or her dependent or an asymptomatic applicant for coverage or his or her asymptomatic dependent to do either of the following:

(a) Undergo genetic testing before issuing, renewing, or continuing a health maintenance organization contract.

(b) Disclose whether genetic testing has been conducted or the results of genetic testing or genetic information.

THE INSURANCE CODE OF 1956 (EXCERPT) Act 218 of 1956
500.3407b

(1) An expense-incurred hospital, medical, or surgical policy or certificate delivered, issued for delivery, or renewed in this state shall not require an insured or his or her dependent or an asymptomatic applicant for insurance or his or her asymptomatic dependent to do either of the following:

(a) Undergo genetic testing before issuing, renewing, or continuing the policy or certificate in this state.

(b) Disclose whether genetic testing has been conducted or the results of genetic testing or genetic information.
Positive outcomes: Predictive DNA Tests

• Personal:
  • Positively influence life decisions and long term planning
  • Reduce morbidity and mortality by specific monitoring/surveillance, interventional risk reduction medical or surgical care, and/or lifestyle modifications

• Family:
  • Inform own reproductive choices
  • Enable informed health care choices of family members

• Society:
  • Improve medical care and health for populations at risk
  • Help prioritize use of medical resources
  • Facilitate development of molecular based therapies

HD Presymptomatic Testing:

• Recommended Protocol(HDSA):
  • genetic counseling
  • neurological evaluation
  • psychological/psychiatric evaluation
  • DNA test if no concerns
  • identification of local support person
  • test results given in person
  • follow-up visits
  • no testing of presymptomatic minors
Desirable Characteristics of Predictive Genetic Tests

- “Negative” in unaffected individuals and people who aren’t predisposed to the disease
  - (eg. specific for the disease, low false positives)
- “Positive” in affected individuals and those at increased risk of the disease
  - (eg. sensitive for the disease, low false negatives)
- “Positive” test reflects prognosis and/or directs clinical management
- Affordable, robust, reliable, reproducible!
Predictive tests are....

probabilistic

NOT
deterministic

Arlo is asymptomatic, what are his chances of getting Huntington Disease?
What are Abe’s chances?
The Guthrie Family Humanitarian Award honors a scientist, researcher or medical leader who has demonstrated compassion and concern for the care and support of people with Huntington's Disease.

Chance that an Asymptomatic Individual at 50% Risk for Inheriting Huntington Disease decreases with age

<table>
<thead>
<tr>
<th>Age</th>
<th>Risk(%)</th>
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<tbody>
<tr>
<td>25</td>
<td>49</td>
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<tr>
<td>30</td>
<td>48</td>
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<td>35</td>
<td>46</td>
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<td>19</td>
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<tr>
<td>65</td>
<td>13</td>
</tr>
<tr>
<td>70</td>
<td>6</td>
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</table>
UM MG Clinic Experience with Huntington Disease Testing

- Asymptomatic
- Prenatal
- Symptomatic

UM MG Clinic Experience with Huntington Disease Testing

- Positive
- Negative
- Intermediate
Again, genetic testing is a process, not just a laboratory procedure….

- Pre-testing evaluation, education, genetic counseling, and informed consent
- Laboratory analysis
- Accurate interpretation of results
- Follow-up must include psychosocial support, education, and management
Predictive testing of minors for adult-onset conditions where there is no preventative or curative treatment............

- Only when there is an effective, curative, or preventive treatment that should be instituted early in life to achieve benefit
- At an age where children can understand implications and make informed decisions
Anticipation, Meiotic Expansion, and Parent of Origin Effects for Different Disorders

<table>
<thead>
<tr>
<th>Disorder</th>
<th>Paternal Effect</th>
<th>Maternal Effect</th>
<th>Repeat Count</th>
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</thead>
<tbody>
<tr>
<td>HD</td>
<td>paternal &gt; maternal - mild</td>
<td>C</td>
<td></td>
</tr>
<tr>
<td>SCA1</td>
<td>paternal &gt; maternal -mild</td>
<td>A</td>
<td></td>
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<tr>
<td>DRPLA</td>
<td>paternal &gt; maternal - mild</td>
<td>G</td>
<td></td>
</tr>
<tr>
<td>SMBA</td>
<td>paternal &gt; maternal - mild</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DM</td>
<td>maternal &gt;&gt;&gt; paternal - significant</td>
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</tr>
<tr>
<td>FRAXA</td>
<td>maternal &gt;&gt;&gt; paternal - significant</td>
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</tbody>
</table>

Most CAG Trinucleotide Repeat Diseases:

- Autosomal dominant progressive neurological disorders with variable expression and reduced penetrance
- Demonstrate mild meiotic instability that is paternal in origin
- Associated with normal alleles of 5-34 repeats and disease alleles of 40-100 repeats with an unstable intermediate repeat range
- Demonstrate that age of onset, rapidity of progression, and severity of disorder correlate with increasing repeat size
- CAG expansion leads to gain of function “neurotoxic” mutation
Pathophysiology of Expanded Polyglutamine Tracts

• Knock out mice lack the neurological phenotype
• Heterozygous transgenic mutant mice with expanded polyglutamine tracts exhibit neurological phenotype
• Cell loss is an apoptotic event
• The ‘toxic fragment’ hypothesis, where proteins are cleaved into a short toxic fragments with polyglutamine tracts that aggregate in the nucleus, has been raised
• Association of CAG expansions with GAPDH suggests further roles for regulation of cellular metabolism
• Key events regulating specificity of neuronal loss not understood

Potential Roles of Huntingtin

• A handful of huntingtin interacting proteins have been described and suggest additional roles for the protein:
  • HIP1 (homologous to yeast gene with cytoskeletal functions) with affinity to normal sized tracts
  • HIP2 which encodes an ubiquitin conjugating enzyme,
  • HAP1 has affinity to larger polyglutamine tracts
  • GAPDH has direct affinity for polyglutamine tracts and is involved in several key cellular functions
  • EGF receptor complex where huntingtin binds to SH3 domains of Grb2 and Ras-GAP suggesting some role in the EGF signaling pathway
Drug trials to prevent/slow HD symptom progression
### Assessing Functional Capacity

<table>
<thead>
<tr>
<th>Stage</th>
<th>Work Ability Score</th>
<th>Financial Affairs Score</th>
<th>Domestic Responsibility Score</th>
<th>Daily Living Skills Score</th>
<th>Where Care Provided</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 okay</td>
<td>full</td>
<td>full</td>
<td>full</td>
<td>full</td>
<td>home</td>
</tr>
<tr>
<td>2 lower</td>
<td>some help</td>
<td>full</td>
<td>full</td>
<td>full</td>
<td>home</td>
</tr>
<tr>
<td>3 marginal</td>
<td>major help</td>
<td>impaired</td>
<td>mild</td>
<td>mild</td>
<td>home</td>
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<td>4 poor</td>
<td>unable</td>
<td>unable</td>
<td>moderate</td>
<td>moderate</td>
<td>home/care facility</td>
</tr>
<tr>
<td>5 unable</td>
<td>unable</td>
<td>unable</td>
<td>severe</td>
<td>unable</td>
<td>total care required</td>
</tr>
</tbody>
</table>

- Coenzyme Q10 (dashed) vs no Coenzyme Q10 (solid)
- Remacemide (dashed) vs no Remacemide (solid)

**Total functional capacity (TFC)**

**Functional assessment**

**Independence scale**
A. A normal nerve cell newly-injected with mutant HD genes
B. The dying nerve cell with disappearance of the fingerlike processes
C. Dying cells rescued by adding functional protein.

Trinucleotide repeat disorders are neurological disorders involving expansions of various repeats in coding and non-coding regions of the gene

<table>
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<th>5’ UTR</th>
<th>exon</th>
<th>intron</th>
<th>exon</th>
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<th>exon</th>
<th>3’ UTR</th>
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<td>CGG</td>
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<td>DRLPA</td>
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Summary

- Trinucleotide repeat disorders account for a large proportion of inherited neurological and mental retardation conditions
- Sensitive and specific DNA based diagnosis may be used for diagnostic, predictive, and prenatal testing if desired
- Genetic counseling and education is useful for at-risk individuals to make informed choice about testing options
- Huntington Disease is an autosomal dominant later onset progressive neurodegenerative disorder due to expansion of a CAG repeat in coding region
- Guidelines for predictive and prenatal HD testing are well-established and serve as prototype for predictive testing for adult onset conditions