Population Screening for Fragile X Syndrome

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Learning Objectives

- After this presentation, you should be able to:
  - Describe what Fragile X syndrome is
  - Describe the molecular defect in Fragile X syndrome
  - Create a protocol for the molecular diagnostic testing
  - Describe the importance/problematic of a screening protocol
The Fragile X Gene

- Mutations involve trinucleotide (CGG) repeat expansions within a non-coding portion of the Fragile X Mental Retardation 1 (FMR1) gene

- The FMR1 protein (FMRP) participates in synapse function and plasticity
Fragile X Syndrome

- A neurodevelopmental disorder
- Most common heritable form of mental impairment
  - 20 - 30% of all X-linked mental retardation
  - 1 - 3% of all mental retardation
- Most common single gene associated with autism
  - 2 - 6% of all individuals with autism
- One-third of boys with fragile syndrome have autism (up to 60% with PDDNOS)
Fragile X Syndrome

- Broad spectrum of involvement
  - cognitive deficits (lowered IQ)
  - shyness/social anxiety → autism
  - mild to severe mood instability
- Mild physical features
  - large/prominent ears; macroorchidism
- Behavior is key
  - tactile defensiveness
  - poor eye contact
  - hand biting/hand flapping
  - ADHD
No change in the age of diagnosis of FXS

(Bailey et al., 2009, Pediatrics)
Fragile X Syndrome…

is caused by a large CGG-repeat expansion in a non-coding portion of the FMR1 gene.
Fragile X Syndrome
It is caused by a large CGG-repeat expansion in a non-coding portion of the FMR1 gene

- Typical (CGG) < 45
  - mRNA
  - FMRP
  - Clinical Normal

- Premutation (CGG) 55 - 200
  - mRNA
  - FMRP
  - Premutation-specific disorders
    - Primary Ovarian Insufficiency (POI)
    - Tremor Ataxia Syndrome (FXTAS)

- Full mutation (CGG) > 200
  - mRNA
  - FMRP
  - Fragile X Syndrome

1/130-250 females
1/250-810 males
1/2500-3600

Fragile site
Xq27.3

Gray/Intermediate
Alleles 45-54 CGG
The Fragile X Gene

A family affair

Four generations

89 yr: tremor/ataxia, cognitive decline, neuropathy
61 yr: tremor/ataxia, nl cognition, POI
38 yr: POI, anxiety, neuropathy, occ hand tremor

proband ↔ fragile X syndrome + autism (mild)
Screening for Fragile X

High risk screening
- Autism Spectrum Disorders
- Intellectual Disabilities
- Primary Ovarian Insufficiency
- Anxiety Disorders
- Movement Disorders

Population screening
- Newborn
- Prenatal
Prevalence of Fragile X Syndrome

- Full mutation alleles are approximately 1 in 4000 males and 1 in 2500 to 1 in 8,000 females (Turner et al., 1996); 1 in 2,500 female samples (Pesso et al., 2000)
- 1 in 800 males and 1 in 250 females have the premutation (Rousseau et al. 1995; 1996; Dombrowski et al 2002)
- In premutation females in Israel it is closer to 1 in 113 (Toledano-Ahladef et al., 2001); lower in Taiwan males with 1 per 1,674 (Tzeng et al. 2005)
- Using the known frequency for premutation females (1/126) the expected frequency for premutation males and for full mutation, both males and females, was determined to be 1/282 and 1/ 2,355 respectively (Hagerman 2008)
<table>
<thead>
<tr>
<th>Author</th>
<th>Location</th>
<th>No. Tested</th>
<th>Gender</th>
<th>Genotype</th>
<th>CGG Range</th>
<th>Prevalence</th>
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<td>Ashkenazi Jewish women</td>
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fhx= family history of FXS
### Prevalence from newborn screening studies

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* Need southern blot confirmation
** Racial data not collected for this study
**** Gender of the positive premutation is male
***** not confirmed
****** 45% Caucasian, 30% African American, 15% Hispanic, 2% Asian, 2% Multicultural, 1% American Indian, 5% Unknown

*** No. Positive include both gender
• Children with FXS appear normal at birth
• Children with FXS are still not typically identified until after two years of age
• Families have to experience a “diagnostic odyssey” and children have little access to early intervention
• Many parents have additional children without knowing reproductive risk

To significantly change the current scenario, newborn screening for Fragile X is needed
Potential benefits of Newborn Screening for FXS

• Early identification provides the opportunity to optimize the infant’s environment

• Advantages for early intervention

• NBS provides access to family support services and information

• NBS offers other benefits: incidence, full range of GP expression

Potential concerns of Newborn Screening for FXS

• Ethical, legal, and social concerns

• Heightened anxiety about parenting, could affect parent-child bonding?

• NBS will identify some children who are or appear to be phenotypically normal or with other conditions not originally targeted for screening

• Risk in extended family members, raising ethical and legal issues
Fragile X Carrier Screening: Rationale

1 in 259 women in the general population is a carrier

Higher frequency reported in some studies (Israel)

Carrier status is essentially undetectable in reproductive years

Most women with premutations have no knowledge of their potential risk for delivering an affected child

Family history does not meet current criteria for screening

Most women have no knowledge of their potential risk for premature ovarian failure

More than 50% of families had more children after they had a fragile X child, but before that child was diagnosed (Bailey et al, 2003)

The chance to pursue assisted reproductive technology in order to avoid conception of an affected child

To consider termination of a pregnancy, or to prepare for the birth of a chronically ill or special needs child
Population Based Carrier Screening was NOT recommended because:

- Limited knowledge about intermediate expansions
- Could not predict phenotype in females
- Both community and physicians lacked knowledge about fragile X syndrome
- Limited counseling resources
- Lack of knowledge about community acceptance
- Costs of methods (PCR and Southern blot)
Fragile X is not currently included in newborn screening mainly because:

- a screening test is not available
- there is no medical treatment

however

- A laboratory test to screen for FXS is now available
- New targeted treatment are now available
- Although there is no cure for FX, children with the full mutation are likely to experience a range of impairments that could be reduced, delayed, or prevented through early intervention
Screening Test

- Lack of an effective screening tool for expanded *FMR1* alleles
- It must be able to reliably detect expanded alleles in both males and females
- It must be rapid
- It must be inexpensive
- It must use a small amount of DNA
- Major problem: apparent homozygosity in females
Development of a Rapid Screening Test

- A number of approaches have been developed and proposed to identify expanded FMR1 alleles
- Improved PCR method for the identification of FMR1 alleles
- They are applicable for screening both males and females, and for allele sizes throughout the premutation (55-200 CGG repeats) and full mutation ranges
- Capable of rapid detection of expanded alleles using a single dried blood spot (1-2mm)
- This methodology is suitable for screening large newborn or high-risk populations for expanded FMR1 alleles.
Criteria for Evaluating a Screening Test

**Validity**: provide a good indication of who does and does not have disease
- Sensitivity of the test
- Specificity of the test

**Reliability**: (precision): gives consistent results when given to the same person under the same conditions

**Yield**: Amount of disease detected in the population, relative to the effort
- Prevalence of disease/predictive value
Validity of Screening Test (Accuracy)

**Sensitivity**: Is the test detecting true cases of disease?  
(Ideal is 100%: 100% of cases are detected)

**Specificity**: Is the test excluding those without disease?  
(Ideal is 100%: 100% of non-cases are negative)
A great impediment to high-throughput screening of FMR1 expanded alleles

The betaine-based PCR approach is unable to distinguish between females who are homozygous for normal FMR1 alleles and full mutation females.
First and secondary PCR screening

1st PCR

(CGG)n

<table>
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<th>&gt; 200</th>
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<tr>
<td>55 - 200</td>
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<td>Intermediate</td>
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<td>Primer C</td>
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Primer F

2nd PCR

(CGG)n

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<td>Primer C</td>
<td>&lt; 44 Normal</td>
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Primer F
Sample: bloodspots/ DNA

First PCR Screening with C & F primers

Analysis: PCR Result
Agarose gel/ QIAxcel/ capillary electrophoresis

Female = Single band
Male = No band

Second Screening PCR with C & chimeric primers

Analysis: PCR Result
Agarose gel/ capillary electrophoresis

Smear/ serial peaks

Expanded allele (male/ female)
Follow up with Southern Blot Analysis

Finished Female double band/ Male single band

No

No

Yes

Female = Single band
Male = No band

Yes

Normal female homozygote

Finished
Newborn proband
29, 106 CGG
NF = Normal Female
NM = Normal Male
PF = Premutation Female
PM = Premutation Male
FF = Full mutation Female
FM = Full mutation Male
Southern Blot analysis
Within the CGG repeat tract AGG interruptions are found, generally 1–3 present in normal/intermediate alleles (6–54 CGG repeats) and usually 0–1 in premutation alleles (55–200 CGG repeats). They are present at specific locations, generally occurring after 9 or 10 uninterrupted CGG repeats [(CGG)\(_9\) AGG (CGG)\(_9\) AGG (CGG)\(_n\)].
Premutation female, CGG = 30, 55

Yrigollen et al., 2011
Influence of AGG interruptions on transmission

(Yrigollen et al., 2012)

\[(CGG)_{10} \text{ AGG } (CGG)_{19} = 30\]
\[(CGG)_{9} \text{ AGG } (CGG)_{9} \text{ AGG } (CGG)_{53} = 73\]
\[(CGG)_{9} \text{ AGG } (CGG)_{9} \text{ AGG } (CGG)_{61} = 81\]
\[(CGG)_{12} \text{ AGG } (CGG)_{10} = 23\]
\[(CGG)_{9} \text{ AGG } (CGG)_{9} \text{ AGG } (CGG)_{63} = 83\]
\[(CGG)_{12} \text{ AGG } (CGG)_{10} = 23\]
\[(CGG)_{9} \text{ AGG } (CGG)_{9} \text{ AGG } (CGG)_{82} = 102\]
Influence of AGG interruptions on transmission

\[(\text{CGG})_{10} \text{ AGG (CGG)}_9 \text{ AGG (CGG)}_9 = 30 \]
\[(\text{CGG})_{71} = 71\]

Yrigollen et al., 2012
Risk of Premutation Expansion: size of repeats and gender

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<th>No. Premutation</th>
<th>No. Full Mutation</th>
<th>% Full Mutation</th>
<th>Total</th>
<th>No. 0 AGG</th>
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<td>80-89</td>
<td>7</td>
<td>78</td>
<td>93%</td>
<td>85</td>
<td>43</td>
<td>30</td>
<td>12</td>
</tr>
<tr>
<td>90-99</td>
<td>2</td>
<td>56</td>
<td>97%</td>
<td>58</td>
<td>22</td>
<td>22</td>
<td>14</td>
</tr>
<tr>
<td>100-109</td>
<td>0</td>
<td>54</td>
<td>100%</td>
<td>54</td>
<td>41</td>
<td>6</td>
<td>7</td>
</tr>
<tr>
<td>110-119</td>
<td>0</td>
<td>20</td>
<td>100%</td>
<td>20</td>
<td>15</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>120-129</td>
<td>0</td>
<td>30</td>
<td>100%</td>
<td>30</td>
<td>28</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>130-139</td>
<td>0</td>
<td>8</td>
<td>100%</td>
<td>8</td>
<td>8</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>140-149</td>
<td>0</td>
<td>1</td>
<td>100%</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

* %Full mutation was calculated for each mother then averaged across mothers

Table 1a: Transmission Results by Total CGG Length

Table 1: Transmission Results by Pure CGG Length

- Reduced risk of transmission of a full mutation for all maternal (premutation) repeat lengths below ~100 CGG repeats, with a differential risk (0 versus 2 AGG) exceeding 60% for alleles in the 70-to 80-CGG repeat range.

Yrigollen et al., 2012
Summary

- A reliable DNA test, PCR based, capable to detect expanded alleles for the normal through the full mutation range in both genders is now available.

- The test works on blood spot cards making suitable for large population screening.

- Pilot studies to validate the methodology

- An inventory of benefits and risks in necessary to make a policy decision