Fragile X Syndrome: Carrier Screening in the Prenatal Population

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Fragile X Syndrome

- Why screen for fragile X carriers?
- Who do we screen?
  - Current recommendations
  - Problems
- Population-based screening?
  - Rationale
  - Cost-effectiveness
  - Challenges
Fragile X Syndrome

- The most common cause of inherited mental retardation (MR).
- Second only to Down syndrome as an etiology for MR.
- Incidence of approximately 1 in 4000 males and 1 in 8000 females
- Found among all ethnic groups and occurs in families with no history of mental retardation
- 1 in 259 women are carriers of the fragile X premutation
  - Only the mother has to be a carrier for the fetus to be at risk for fragile X syndrome
Fragile X Syndrome

Males:
- Moderate to severe mental retardation, learning disabilities
- Long face, prominent ears, macro-orchidism
- Physical phenotype can be subtle, especially in young boys
- Hyperactivity, autism (approx. 1/3), hand flapping, hand biting, disordered speech and language
- Males are generally unable to live independently

http://www.nfxf.org
medgen.genetics.utah.edu
Fragile X Syndrome

- Females:
  - Less frequent and less severe in females
  - Mild to moderate mental retardation, learning disabilities
    - About 1/3 of females have significant intellectual disability.
  - Long face, prominent ears (more subtle in females than in males)
  - Poor eye contact, attention problems, shyness and social anxiety

http://www.nxfxf.org
A Spectrum of Clinical Involvement

Three Generations: The young man and woman on the right both carry the full mutation for fragile X syndrome. Their grandfather is now affected by FXTAS and is the fragile X syndrome carrier who passed on the carrier status to his daughter, their mother.

http://www.nfxf.org
FMR-1 gene: a triplet repeat disease

- **Stable allele**
  - $(\text{CGG})_n$
  - $n < 45$

- **Unstable allele**
  - $55 \geq n \leq 200$

- **Premutation**
  - $n \geq 200$

- **Expansion to full mutation**

- **Abnormal methylation of FMR-1 gene**

- **Absence of FMR-1 protein**
### Risk of Premutation Expansion: size of repeat and gender

<table>
<thead>
<tr>
<th>Maternal Repeat Size</th>
<th>% Of Offspring With a Full Mutation</th>
</tr>
</thead>
<tbody>
<tr>
<td>55-59</td>
<td>3.7%</td>
</tr>
<tr>
<td>60-69</td>
<td>5.3%</td>
</tr>
<tr>
<td>70-79</td>
<td>31.1%</td>
</tr>
<tr>
<td>80-89</td>
<td>57.8%</td>
</tr>
<tr>
<td>90-99</td>
<td>80.1%</td>
</tr>
<tr>
<td>&gt;100</td>
<td>94-100%</td>
</tr>
</tbody>
</table>

Source: Nolin et al., 2003
A Case for Prenatal Population-Based Carrier Screening?
ACOG / ACMG Recommendations: Fragile X Testing

- **Carrier Testing:** only individuals with:
  - a family history of fragile X or
  - undiagnosed mental retardation,
  - developmental delay or
  - autism

- **Prenatal diagnosis:** when the mother is a known carrier of fragile X (premutation or full mutation).

NSGC:  www.springerlink.com/media/c5v8ykryqj2rl6ugxuq/contributions/r/5/4/3/r54356r13r0740u7.pdf
Problems with Current Recommendations

- Risk factor based on screening alone: not effective in detecting carriers.
  
  - For example:
    
    - Maximal rate of detection of female PM carriers by active cascade screening (6%) is much lower than that by prenatal screening (60%). (Song et al, 2003).

- Largest proportion of fragile X syndrome births are in families without index cases.
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 silent in reproductive years.
  - except possible ovarian dysfunction
- 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.
FX Carrier Screening: rationale

- 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).
  - 222 children, 50% of these children had a full mutation
- Choice: couple can fulfill reproductive goals
  - 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.
Current Practices in Prenatal Screening

- Offer maternal serum screening (includes ultrasound) to all pregnant women to detect chromosomal abnormalities (eg. Down syndrome) and open neural tube defects.

- Offer Cystic Fibrosis carrier screening to all pregnant or preconception women of Caucasian or Ashkenazi Jewish ethnicity (make available to other ethnicities that have lower carrier frequencies).

- Hemoglobinopathy screening: almost universal.

- Targeted screening for diseases prevalent in specific ethnic groups (eg. Canavans, Tay-Sachs, FD).
Current Prenatal Screening: Frequency Comparison

- 1 in 625 couples are carriers for cystic fibrosis
- 1 in 270 risk of aneuploidy at age 35
  - 1 in 750 overall in North America
- 1 in 800 + risk of neural tube defect in gen pop.
- 1 in 259 women are carriers for fragile X.
Does Fragile X meet general principles of carrier screening?

1. The disorder should be considered a significant health problem or carry a burden of disease.
2. Diagnostic testing must be robust.
3. Screening should be accomplished in a simple manner.
4. Screening should be cost-effective.
5. There should be effective treatment or intervention for a positive result.


Are patients interested?
Screening principles:

1. Significant Health Problem

- Significant morbidity associated with fragile X syndrome.
- Significant cost of raising a child with fragile X syndrome of $615K (estimates range $500 to $1.1 million).

Source: Musci and Caughey, 2005; Finucane et al., 1996
Screening principles:

3. Screening Should Be Simple

- Sample collection and patient education could be added to the existing prenatal genetic testing:
  - maternal serum screening: Down Syndrome, NTDs.
  - cystic fibrosis
Screening principles:

4. Is screening cost effective?

- A method for comparing the relative value of various clinical strategies:
  - Cost for a given health benefit.
  - How the individual values the health state or outcome.

\[ \text{cost effective} \neq \text{cost saving} \]
Cost Effectiveness Analysis

- Cost-utility analysis
  - Cost of $549K per fragile X diagnosis
  - Less than the cost of raising a child with this disorder

- Widespread fragile X carrier screening strategy
  - Identify 86% of the approximately 750 fragile X affected fetuses annually
  - 13% of patients who present too late to obtain prenatal diagnosis

- The program would be cost-effective yielding a cost-utility ratio of $14,858 per Quality Adjusted Life Year (QALY).
  - At a cost of $95 per test.
  - One child per patient.

Musci and Caughey, AJOG, 2005
Assumptions for baseline model:

- 80% of women could be screened with PCR alone, the remaining 20% would require Southern blot analysis.
- All women with a positive carrier test would undergo amniocentesis.
- Patient preferences based on published data for Down Syndrome.
- 87% of women with fragile X fetus would undergo pregnancy termination.

Musci and Caughey, AJOG, 2005
Attitudes toward prenatal carrier screening for Fragile X: a pilot study

- Pretest knowledge about Fragile X was limited:
  - 33% had heard of Fragile X syndrome before enrollment.
- Post-counseling: knowledge still limited.
- Participants were strongly in favor of being tested or screened.
- Participants did not experience undue anxiety with screening.
- Respondents hoped that knowledge of Fragile X in the general population would increase.
  - Recommended screening be offered during routine prenatal care.

Fanos, Spangner, and Musci: Genetics in Medicine 2006
Arguments against routine screening:

- The genetics is too complex.
  - Current education and counseling resources inadequate.
  - Genetic “manpower” shortage in United States.
- Cannot predict phenotype for female fetuses with full mutations.
- Lack of data regarding the preferences, attitudes and informational needs of patients.
- Burden on primary care obstetrician to provide informed consent: Time, effort, liability(?)
- Cost
Arguments for routine screening:

- Meets screening criteria: high incidence; associated with significant morbidity; a reliable test, cost-effective.
- Women who screen negative during one pregnancy do not need to be tested again in subsequent pregnancies.
- Women concerned about chromosome abnormalities, including Down syndrome, are interested in testing for other common causes of mental retardation.
- Systems for prenatal screening already in place.
- Only the mother needs to be offered carrier screening.
Challenges:

- To **reduce the cost** of DNA assay:
  - PCR: screening v. diagnostic testing
    - 10+ % would require Southern to resolve
    - Diagnostic testing for positives
  - High-throughput: sample number & rapid ‘turnaround’

- Obtain data regarding the **preferences, attitudes and informational needs** of patients for FX screening
  - Delivery of information & counseling

- **Education** of primary care physicians
Prospective study of fragile X screening program in order to:

- better understand patient attitudes, preferences, and behaviors.
- Determine best DNA test and logistics.
END
Fragile X Associated Conditions Among Carriers:

- Premature ovarian failure (POF)
  - 20% of premutation carriers have POF vs 1% in general population
  - Premutation alleles were found in 14% of women with a family history of POF and no known history of fragile X syndrome

- Fragile X Associated Tremor and Ataxia (FXTAS)
  - Neurological condition in some male adult carriers of the FMR1 premutation.
    - First described by Hagerman et al in 2001.
  - 30 - 40% of men 50+ years old with a premutation have FXTAS
    - estimated 13-fold increased risk of these symptoms compared with non-carriers
  - Has been reported in female premutation carriers (also >50 y.o.), though symptoms milder.
The fragile X mutation is an unstable CGG repeat that can expand dramatically (to a full mutation) when passed from mother to offspring.

- Full mutation alleles are associated with abnormal methylation, which turns off FMR-1 protein production.
- Loss of FMR-1 protein function interferes with normal brain development.
- FMR-1 protein is absent in full mutation males.
Fragile X Inheritance

- Both males and females can pass the mutation on the X chromosome
  - Women to either their sons or daughters and men to all their daughters but none of their sons
- Expansion of the CGG repeat is influenced by
  - The gender of the carrier:
    - Premutation females are at risk to have full mutation offspring while the premutation in males remains relatively stable when passed to daughters
  - The number of repeats:
    - In general, larger-sized repeats, in females, have a greater risk to expand to a full mutation in one generation. Alleles are less likely to expand when passed from males.
“…..the foremost purpose of prenatal screening is not to reduce the incidence of genetic disease but to fulfill a couple’s reproductive goals.”

Prenatal Screening/Testing:

- Provides individuals with
  - 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.
CEA: Decision Tree

Musci and Caughey, AJOG, 2005
FMR1: triplet repeat expansion

\[(CGG)_n\]

- \(n < 45\)
- \(55 \geq n \leq 200\)
- \(n \geq 200\)

No Transcription = No FMR Protein
The *FMR1* Gene: CGG repeat expansion

<table>
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<th>Normal</th>
<th>Premutation</th>
<th>Full mutation</th>
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<tbody>
<tr>
<td>gene</td>
<td>(CGG) (n &lt; 45)</td>
<td>(CGG) (55 \leq n \leq 200)</td>
<td>(CGG) (n \geq 200)</td>
</tr>
<tr>
<td>mRNA</td>
<td></td>
<td></td>
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<tr>
<td>FMRP</td>
<td>Normal</td>
<td>Premature ovarian failure</td>
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<tr>
<td>Clinical phenotype</td>
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Slide: courtesy of Randi Hagerman