FMR1 Carrier Screening: The how, the why, and the when

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FMR1-Related Disorders

Fragile X Syndrome (FXS)

Fragile X Associated-Primary Ovarian Insufficiency (FXPOI)

Fragile X-Associated Tremor/Ataxia Syndrome (FXTAS)
Fragile X syndrome is the most common cause of inherited mental retardation. The average IQ in adult males with the full mutation is ~40.

Full mutation carrier females can have a phenotype ranging from being affected with FXS, to be either mildly affected or unaffected.

Autistic-like features are common in individuals with FXS and include: hand flapping, hand biting, gaze avoidance and tactile defensiveness.

The physical characteristics can be subtle and include: a long narrow face, prominent ears, joint hypermobility and macroorchidism.
Fragile X-Associated
Primary Ovarian Insufficiency (FXPOI)

Decreased ovarian function in some female carriers of premutation alleles.

POI is defined as 4 months or more of disordered menses (amenorrhea, oligomenorrhea, polymenorrhea, or metrorrhagia) and menopausal FSH levels before the age of 40.

Approximately 20% of women who are premutation carriers will have FXPOI (1% general population risk).

Approximately 2% of women with ovarian insufficiency and approximately 12% of women with a personal and family history of ovarian failure are carriers of the premutation FMR1 allele.

A late onset progressive cerebellar ataxia in individuals with *FMR1* premutations

These individuals suffer from:
- short-term memory loss
- cognitive decline
- dementia
- parkinsonism
- peripheral neuropathy
- lower-limb weakness

<table>
<thead>
<tr>
<th>Age in Years</th>
<th>Risk of FXTAS</th>
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<tbody>
<tr>
<td>50-59</td>
<td>17%</td>
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<tr>
<td>60-69</td>
<td>38%</td>
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<tr>
<td>70-79</td>
<td>47%</td>
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<tr>
<td>≥80</td>
<td>75%</td>
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FXTAS occurs more commonly in male premutation carriers (40-45% of those over the age of 50) than in female premutation carriers.

2%-4% of men with adult-onset cerebellar ataxia carry an *FMR1* premutation

In the full mutation, *FMR1* becomes heterochromatinized (DNA methylation and histone modification changes) leading to transcriptional silencing.
The vast majority (>99%) of FXS is caused by expansion of the CGG repeat in the 5’ UTR of the gene.

There is a small percentage of cases of FXS (<1%) due to other mutations in $FMR1$ (namely deletions).

FXPOI and FXTAS are only associated with premutations.

Testing for FXS, FXPOI and FXTAS primarily consists of determining the length of the CGG repeat.

There are several methods that are used by laboratories to determine the length of the $FMR1$ CGG repeat.
Southern Blot (The Gold Standard)

EcoRI probe XhoI EcoRI

(CGG)n Exon 1

2.7kb 5.2kb

Neg male Pos male Neg female Pos female

>5.2 kb methylated (expanded CGG)

5.2 kb methylated (normal CGG)

2.7 kb unmethylated (normal CGG)
Southern Blot (The Gold Standard)

Labor intensive, time consuming and not amenable to high-throughput analysis
PCR Amplification and Sizing by Capillary Electrophoresis

# of repeats = (bp – 147) / 3

210bp-147/3=21 CGG repeats
429-147/3 = 94 CGG repeats
no amplification

Cannot distinguish between full mutation carrier females and homozygous females (~40%)
Quantitative DNA Methylation Analysis of FMR1

Figure 1. Workflow for amplification and detection of FMR1 amplicons using a three-primer FMR1 PCR. Input gDNA is amplified by two gene-specific primers (forward [Fwd] and reverse [Rev]) and a CGG repeat primer in a single tube. After amplification, the products, which include the full-length amplicon that completely encompasses the triplet repeat region and a multiplicity of CGG repeat primed products, are resolved by CE. The resulting electropherogram supports quantification of the number of CGG repeats, determination of the allele zygosity, and the sequence context of any AGG spacer elements.

31 CGG repeats

normal male

full mutation male
(~550 CGG repeats)
cannot size full mutations accurately by capillary electrophoresis due to limitation of resolution of the capillary
Premutation female

30 CGGs 95 CGGs
CGG Repeat Primed PCR

29 CGG

full mutation female
<table>
<thead>
<tr>
<th>CGG Repeat</th>
<th>Band Size</th>
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<tbody>
<tr>
<td>71 CGGs</td>
<td>1500 bp</td>
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<tr>
<td>638 CGGs</td>
<td>1000 bp</td>
</tr>
<tr>
<td>35 CGGs</td>
<td>750 bp</td>
</tr>
<tr>
<td>583 CGGs</td>
<td>500 bp</td>
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</table>

**CGG Repeat Primed PCR**

![Image of gel electrophoresis showing bands at 1500, 1000, 750, and 500 base pairs (bp).]
AGG Interruptions Modify The Risk For Expansion

Xpansion Interpreter™—The Next Step in Fragile X

Figure 1

Figure 2

Figure 1: Probability and magnitude of expansion in FXS mother-to-child transmissions based on the number of uninterrupted CCG repeats within the 5′ UTR of FMR1.

Figure 2: Modification of expansion risk based on number of AGG interruptions.

Data from Figures 1 and 2 were presented at the 2010 AMF and 2011 ACMG conferences.

Complete references available at www.agoxygen.com/ClinicalLab
1. Newborn/infant screening for FXS

2. Carrier screening for premutations in women of reproductive age
An overarching concept is utility—that is, an approach that delivers the greatest good to the greatest number of people”

“Newborn screening policy development should be primarily driven by what is in the best interest of the affected newborn, with secondary consideration given to the interests of unaffected newborns, families, health professionals, and the public.”

“To be included as a primary target condition in a newborn screening program, a condition should meet the following minimum criteria:

a. It can be identified at a period of time (24 to 48 hours after birth) at which it would not ordinarily be clinically detected.
b. A test with appropriate sensitivity and specificity is available.
c. There are demonstrated benefits of early detection, timely intervention, and efficacious treatment.”
Newborn Screening

Recommended Uniform Screening Panel of the Secretary’s Advisory Committee on Heritable Disorders in Newborns and Children (31 conditions)

<table>
<thead>
<tr>
<th>ACMG Code</th>
<th>Core Condition</th>
<th>Metabolic Disorder</th>
<th>Endocrine Disorder</th>
<th>Hemoglobin Disorder</th>
<th>Other Disorder</th>
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<tbody>
<tr>
<td>PROP</td>
<td>Propionic acidemia</td>
<td>Organic acid</td>
<td>Fatty acid oxidation disorders</td>
<td>Amino acid disorders</td>
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<tr>
<td>MTH</td>
<td>Methylmalonic acidemia (methylmalonyl-CoA mutase)</td>
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<td>CM A, B</td>
<td>Methylmalonic acidemia (cobalamin disorders)</td>
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<td>IVA</td>
<td>Isovaleric acidemia</td>
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<td>3-MCC</td>
<td>3-Methylcrotonyl-CoA carboxylase deficiency</td>
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<tr>
<td>MBA</td>
<td>3-Methylcrotonyl-CoA carboxylase deficiency</td>
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<tr>
<td>MCD</td>
<td>Holocarboxylase synthase deficiency</td>
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<tr>
<td>BKT</td>
<td>β-Ketothiolase deficiency</td>
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<tr>
<td>GA1</td>
<td>Glutaric acidemia type I</td>
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<tr>
<td>CUD</td>
<td>Carbohydrate uptake defect/carbohydrate transport defect</td>
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<td>MCAD</td>
<td>Medium-chain acyl-CoA dehydrogenase deficiency</td>
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<td>VLCAD</td>
<td>Very long-chain acyl-CoA dehydrogenase deficiency</td>
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<td>LCHAD</td>
<td>Long-chain L-3 hydroxyacyl-CoA dehydrogenase deficiency</td>
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<td>TFP</td>
<td>Trinucleotide protein deficiency</td>
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<tr>
<td>ASA</td>
<td>Argininosuccinic acidemia</td>
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<tr>
<td>CIT</td>
<td>Citrullinemia, type I</td>
<td></td>
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<tr>
<td>MSUD</td>
<td>Maple syrup urine disease</td>
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<tr>
<td>HCY</td>
<td>Homocysteuria</td>
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<tr>
<td>PKU</td>
<td>Classic phenylketonuria</td>
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<tr>
<td>TYR I</td>
<td>Tyrosinemia, type I</td>
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<tr>
<td>CH</td>
<td>Primary congenital hypothyroidism</td>
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<tr>
<td>CAR</td>
<td>Congenital adrenal hyperplasia</td>
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<tr>
<td>HB S</td>
<td>S, S disease (Sickle cell anemia)</td>
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<tr>
<td>HB S/TH</td>
<td>S, S, Thalassemia</td>
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<tr>
<td>HB S/C</td>
<td>S, C disease</td>
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<tr>
<td>BMTOT</td>
<td>Bilirubinemia deficiency</td>
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<tr>
<td>CCMD</td>
<td>Critical congenital heart disease</td>
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<tr>
<td>CF</td>
<td>Cystic fibrosis</td>
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<tr>
<td>GALT</td>
<td>Classic galactosemia</td>
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<tr>
<td>HEAR</td>
<td>Hearing loss</td>
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<tr>
<td>SCID</td>
<td>Severe combined immunodeficiencies</td>
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Newborn Screening for FXS

**Benefits:**
1. early intervention therapies such as speech and occupational therapy (drugs in clinical trials)
2. prevention of diagnostic odyssey
   avg age of diagnosis -~36 months in males and ~42 months in females
3. identify at-risk families before having second child with FXS
   ~25% of families have a second child with FXS before knowing there first child is affected

**Risks:**
1. stigmatize clinically unaffected or mildly affected full mutation carrier females (vulnerable child syndrome)
2. identify infants with adult onset disorders (FXTAS and FXPOI)
3. secondary findings
   (47,XXY, 45,X and 46,XX males and 46,XY females)
4. impairment of parental bonding (ameliorated by infant screening)
Newborn Screening

Approximately 4 million births each year in the United States

Full mutation prevalence in the general population is approximately 1 in 4000-5000

Approximately 400-500 males with full mutations (FXS)
Approximately 400-500 females with full mutations (variable phenotype)

Sex chromosome abnormalities
- Klinefelter syndrome 47,XXY (~1 in 600) ~3300/year
- Turner syndrome 45,X (~1 in 2000) ~1000/year
- Sex reversal-46,XX males and 46,XY females (~1 in 20,000) ~200/year

FMR1 premutations (if you size the CGG repeat)
- Males with premutation at risk for FXTAS (1 in 800) ~2500/year
- Females with premutation at risk for FXPOI and FXTAS (1 in 178) ~11,000

~ 18,000 infants with a sex chromosome abnormality (~4500) or FMR1 premutation (13,500) will be identified along with the 800-1000 infants with the full mutation
Benefits:
1. early identification of child with FXS
2. prevention of diagnostic odyssey
3. identification at-risk families
4. inform women of the risk of FXPOI
5. Minimize identification of individuals with sex chromosome abnormalities

Risks:
1. stigmatize clinically unaffected full mutation carrier females (vulnerable child syndrome)
2. identify fetuses with adult onset disorders (FXTAS and FXPOI)
3. impairment of parental bonding

Studies investigating women’s attitudes toward carrier screening found that they generally viewed it favorably.

However, studies found that women were unprepared for positive results indicating that these women need targeted counseling and education about *FMR1* related disorders.

Premutation carrier testing is both a carrier test assess reproductive risk and a diagnostic test for ovarian insufficiency.

ACOG guidelines recommend carrier screening for FMR1 premutations if a woman requests it or has one of the following:

1. family history of FXS, FXPOI or FXTAS
2. family history of unexplained MR or developmental delay
3. family history of unexplained autism
4. personal or family history of ovarian insufficiency

All identified carriers of FMR1 premutations should be referred for genetic counseling.

If a woman carries a premutation, prenatal testing by CVS or amniocentesis should be offered.

In the United States, there are approximately 4 million pregnancies per year. (Hamilton et al. 2011. National Vital Statistics Report 60: 2)

Approximately 2-3% of adult women of reproductive age have a relative with MR or developmental delay, 1% with a relative with autism, 1% with POI or a family history of POI.

~4%-5% of all pregnant women are candidates for \textit{FMR1} premutation screening. Equates to ~160,000 - 200,000 pregnancies/year in the United States.

\textit{FMR1} pre- and full mutations cause <5% of MR, DD, autism and POF, so the vast majority of these women will not carry a premutation.

In addition, the vast majority of women who carry a premutation do not have one of the risk factors (FH of FXS, MR, DD, or POF) that would trigger testing.

The majority of women who are at risk of having a child with FXS will not be identified by following the ACOG guidelines.
In 2010, ~4 million pregnancies in United States

Estimates of premutation carrier rates varies widely among different groups
  1 in 158 in Israel  (Berkenstadt et al. 2007 Prenat. Diagn. 27:991-994)

In the US, ~22,500 pregnancies each year carried by women with premutation

~800-1000 full mutation carriers (male and female) are born each year

Only 3-5% of all women who screen positive for premutation will have a child with full mutation.
Of those 22,500 pregnancies, approximately one half, ~11,250, will inherit an expanded allele.

Out those 11,250, approximately 800-1000 will be full mutations, the remaining ~10,300 will be premutations.

Thus, for every fetus with full mutation you will identify ~10-12 fetuses with premutations.

Half will be male of which 45% will develop FXTAS after the age of 50
Half will be female of which ~20% will develop FXPOI and ~16% will develop FXTAS.

Because of these issues, general population screening is not recommended at this time except as part of clinical research protocol.
When? Before or during pregnancy?

Studies have shown that preconception carrier screening is preferred over screening during pregnancy. Preconception screening offers more choices if a woman wants to avoid having a child with FXS:

1. adoption
2. egg donation
3. preimplantation diagnostics

Other advantages to preconception screening:

1. more time to absorb the results and make decisions
2. less stressful
3. know ahead of time about risk of FXPOI

However, logistically speaking it may be easier to offer during pregnancy (similar to CF screening that is currently being offered to women during pregnancy).

Future Studies Needed Before Implementation of FXS Population Screening

- Development and assessment of counseling and educational strategies that facilitate informed decision-making and specifically support the needs of the general population.
- Studies exploring approaches to offering screening in preconception settings to enable screening to be widely accessible.
- Longitudinal studies to look at pretest and posttest outcomes and to allow examination of long-term consequences and satisfaction with or regrets about decisions to have or not have testing.
- The inclusion of health economic measures in studies in which screening is offered.
- Clinical trials to establish the benefit of early interventions in FXS to guide policy decisions on whether to introduce newborn screening.