Fragile X Syndrome

Disease Overview

Fragile X syndrome (FXS), the most common heritable form of intellectual disability (ID) and autism, is caused by full *FMR1* gene mutations. Individuals with an *FMR1* gene premutation may develop fragile X-associated tremor/ataxia syndrome (FXTAS), FX-associated neuropsychiatric disorder, or primary ovarian insufficiency (POI).

Diagnostic testing should be offered to individuals with unexplained intellectual disability (ID), developmental delay, autism spectrum disorder, late-onset cerebellar ataxia and intention tremor, POI, or infertility associated with elevated follicle-stimulating hormone (FSH) levels.

Screening for FXS should be offered to women with a positive family history of FXS, FXTAS, unexplained ID, or autism. Fetal testing should be offered to women who carry a fragile X premutation or full mutation.

Symptoms of FXS

- Moderate ID (median IQ of 40-45 but ranges from <10 to normal range)
- Autism in 50-70%
- Perseverative speech
- Behavioral issues: hand flapping/biting, attention deficit hyperactivity disorder (ADHD), social anxiety, poor eye contact, tactile defensiveness, aggressiveness, and irritability
- Large ears, long face, large jaw, prominent forehead, and large testes

Symptoms of FXTAS

- Cerebellar gait ataxia
- Intention tremor
- Parkinsonism; muscle rigidity, unbalanced shuffling gait, slowed movement and speech
- Moderate to severe short-term memory loss
- Executive function deficit
- Incontinence/ impotence

Prevalence

FXS:

- 1/4,000 males
- 1/8,000 females

Premutation allele in U.S.:

- 1/1,000 males
- 1/350 females

Featured ARUP Testing

Fragile X (FMR1) with Reflex to Methylation Analysis 2009033

Method: Polymerase Chain Reaction/Capillary Electrophoresis

- Preferred test to diagnose FXS, FXTAS, and for carrier screening in individuals with a positive family history
- Test Description
  - Triplet repeat-primed polymerase chain reaction (PCR) followed by size analysis using capillary electrophoresis to determine *FMR1* CGG repeat length
  - Methylation-specific PCR is performed for >100 CGG repeats to distinguish between premutation and full mutation alleles

Fragile X (FMR1) with Reflex to Methylation Analysis, Fetal 2009034

Method: Polymerase Chain Reaction (PCR)/Capillary Electrophoresis

- Prenatal test for women with fragile X premutations or full mutations.
- Contraindications
  - Prenatal testing for women with normal or intermediate allele sizes
  - Testing of chorionic villus (CVS) samples, because methylation patterns are not fully established in the first trimester of pregnancy

Genetics

Gene

FMR1

Inheritance

X-linked

Structure/Function

FMR1 codes for fragile X mental retardation protein (FMRP), an RNA-binding protein expressed in many tissues

Mutations

Caused by *FMR1* variants

- 99% caused by expansion of the *FMR1* gene CGG repeat
- Less than 1% caused by *FMR1* sequence variants or partial/full *FMR1* gene deletions
- Risk for CGG repeat expansion is dependent on sex of transmitting parent and size of repeat

CGG repeat sizes:

- Normal alleles: 5-44 CGG repeats (unmethylated)
  - Stably transmitted
- Intermediate alleles: 45-54 CGG repeats (unmethylated)
  - Unstable but will not expand to a full mutation in one generation
- Premutations: 55 to ~200 CGG repeats (unmethylated)
  - Females:
    - At risk for having offspring with FXS-transmission of CGG repeats to offspring is unstable
      - Premutations of <56 repeats have not expanded to full mutation in a single generation[1,2]
      - Stability of alleles <90 CGG repeats is influenced by the number of AGG interspersions within the CGG repeat sequence
      - Premutations >90 repeats nearly always expand to full mutation in offspring
    - 21% risk for POI (before age 40)
    - At risk for fragile X-associated neuropsychiatric disorders
    - 17% risk for FXTAS in women>50 years of age
  - Males:
    - Transmission of CGG repeats is stable
    - All of their daughters and none of their sons will inherit the premutation
    - 47% risk for FXTAS in men >50 years of age
- Full mutations: typically >200 CGG repeats (methylated)
  - Males are affected with FXS
  - 50% of females have moderate ID
  - Disease symptom severity cannot be predicted based on:
    - Size of CGG repeat
    - Degree of methylation
    - Pattern of X-inactivation (in females)

Test Interpretation

Sensitivity/Specificity

- Clinical sensitivity/specificity: 99%[3]
- Analytic sensitivity/specificity: 99%[4,5]

Results

Result	Number of CGG Repeats	Clinical Significance
Full mutation	>~200 (methylated)	Male: FXS with ID
		Female: Variable expression of FXS; 50% have ID
Premutation	55 to ~200 (unmethylated)	Male: 47% risk for FXTAS in men >50 years of age
		Female: At risk for POI At risk for FX-associated neuropsychiatric disorders 17% risk for FXTAS in women >50 years of age
Indeterminate	45 to ~54	Offspring at risk for inheriting premutation
Negative	5 to ~44	Normal; not affected with nor a carrier of FXS

Limitations

- Estimated CGG repeat number is not provided for full mutations (alleles with >200 repeats)
- Sizing precision of CGG premutation alleles is within two-three CGG repeats
- Methylation patterns are not fully established in the first trimester of pregnancy; thus, CVS is not recommended for prenatal diagnosis. A small, full mutation may be distinguished from a large premutation in amniocytes
- Rare *FMR1* variants unrelated to trinucleotide expansion will not be detected
- Diagnostic errors can occur due to rare sequence variations
- AGG trinucleotide interruptions within the *FMR1* CGG repeat tract are not assessed

References

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2. Nolin SL, Glicksman A, Tortora N, et al. Expansions and contractions of the FMR1 CGG repeat in 5,508 transmissions of normal, intermediate, and premutation alleles. *Am J Med Genet A*. 2019;179(7):1148-1156.

3. Hunter JE, Berry-Kravis E, Hipp H, et al. *FMR1* disorders. In: Adam MP, Ardinger HH, Pagon RA, et al, editors. GeneReviews, University of Washington; 1993-2020. [Last Update: Nov 2019; Accessed: Feb 2020]

4. Lyon E, Laver T, Yu P, et al. A simple, high-throughput assay for Fragile X expanded alleles using triple repeat primed PCR and capillary electrophoresis. *J Mol Diagn*. 2010;12(4):505-511.

5. Grasso M, Boon EMJ, Filipovic-Sadic S, et al. A novel methylation PCR that offers standardized determination of FMR1 methylation and CGG repeat length without southern blot analysis. *J Mol Diagn*. 2014;16(1):23-31.

Additional Resources

Monaghan KG, Lyon E, Spector EB, et al. ACMG Standards and Guidelines for fragile X testing: a revision to the disease-specific supplements to the Standards and Guidelines for Clinical Genetics Laboratories of the American College of Medical Genetics and Genomics. *Genet Med*. 2013;15(7):575-586.

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