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

Tests to Consider

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/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

Related Tests

Genetic Carrier Screen, (CF, FXS, and SMA) with Reflex to Methylation 3000258

Method: Polymerase Chain Reaction/Fluorescence Monitoring, Polymerase Chain Reaction/Capillary Electrophoresis, Multiplex Ligation-dependent Probe Amplification

- Carrier testing for cystic fibrosis (CF), fragile X syndrome (FXS), and spinal muscular atrophy (SMA) in expectant couples or those planning a pregnancy
- Not recommended for diagnostic testing in patients with symptoms of CF, FXS, or SMA

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]
- Analytical 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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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. PubMed

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. PubMed

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. PubMed

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