Fragile X (FMR1) With Reflex to Methylation Analysis

Last Literature Review: May 2024 Last Update: December 2025

Fragile X syndrome (FXS), the most common heritable form of intellectual disability (ID) and autism, is caused by full *FMR1* gene mutations.[1,2] Individuals with an *FMR1* gene premutation may develop fragile X-associated tremor ataxia syndrome (FXTAS), fragile X-associated primary ovarian insufficiency (FXPOI), or fragile X-associated neuropsychiatric disorders (FXAND).[1,2] These tests use 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 to distinguish between premutation and full mutation alleles.

For more information about the laboratory testing strategy for *FMR1*-related disorders, refer to the ARUP Consult Fragile X (FMR1)-Associated Disorders topic.

Featured ARUP Testing

Fragile X (FMR1) with Reflex to Methylation Analysis 2009033

Method: Capillary Electrophoresis / Polymerase Chain Reaction (PCR)

- Preferred test to diagnose FXS, FXTAS, or FXPOI
- May be used for carrier screening in individuals with a family history of FXS

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

Disease Overview

FXS in male individuals is associated with moderate ID, autism, perseverative speech, hyperactivity, social anxiety, hand flapping or biting, poor eye contact, connective tissue anomalies, and characteristic physical features.[1] Female individuals with FXS are usually less severely affected than males. FXTAS may present with late-onset progressive ataxia and intention tremor or include cognitive impairment and behavioral features.[1] Female individuals may develop FXPOI, which is characterized by hypergonadotropic hypogonadism before 40 years of age.[1] Symptoms of FXAND may include anxiety, depression, attention-deficit/hyperactivity disorder (ADHD), or addictive behavior.[1]

Prevalence

FXS[1]:

- Male individuals: approximately 1 in 4,000-7,000
- Female individuals: approximately 1 in 8,000-11,000

Premutation allele[1]:

- Female individuals: approximately 1 in 150-300
- Male individuals: approximately 1 in 300-800

Genetics

Gene

FMR1

Inheritance

X-linked[1,2]

Pattern of inheritance is subject to instability of CGG repeat sequence[1,2]

Penetrance

FXS[2]:

- Male individuals: complete
- Female individuals: 50% (depending on X-chromosome inactivation)

FXTAS[1,2]:

- Male individuals >50 years: approximately 40% (varies with age and CGG repeat length)
- Female individuals >50 years: approximately 16-20%

FXPOI[1,2]:

- Approximately 20% (varies by CGG repeat length)

Test Interpretation

Sensitivity/Specificity

- Clinical sensitivity/specificity: >99%[1,2]
- Analytic sensitivity/specificity: 99%[3,4]

Repeat sizing precision is +/- 1 for alleles with 5-70 CGG repeats, +/- 3 for alleles with 71-120 CGG repeats, and +/- 5 for alleles with 121-200 CGG repeats.

Results

Results Interpretation: Fragile X (FMR1) with Reflex to Methylation Analysis			
Allele Category	**Number of CGG Repeats**	**Methylation Pattern[a]**	**Interpretation**
Normal	<45	Not assessed	Not affected with FXS or other *FMR1*-associated disorders Not a carrier of FXS
Intermediate	45-54	Not assessed	Not affected with FXS or other *FMR1*-associated disorders Genetic counseling may be helpful
Premutation	55-200	Unmethylated	Not affected with FXS Carrier of premutation; thus, at increased risk for other *FMR1*-associated disorders Genetic counseling is recommended *FMR1* testing is recommended for at-risk family members
Full mutation	>200	Full	Consistent with a diagnosis of FXS in a male Increased risk for FXS in a female Genetic consultation is recommended *FMR1* testing is recommended for at-risk family members

[a]Methylation pattern is assessed when an expanded allele is detected to distinguish between premutations and full mutations. The methylation reflex is performed if ≥55 CGG repeats are detected in fetal samples, or when ≥100 CGG repeats are detected in blood samples.

Limitations

- AGG trinucleotide interruptions within the *FMR1* CGG repeat tract are not assessed.
- An estimated CGG repeat number is not provided for full mutations (alleles with >200 repeats).

- Rare *FMR1* variants unrelated to trinucleotide expansion will not be detected.
- Diagnostic errors can occur due to rare sequence variations.
- Methylation patterns are not fully established in early gestation; thus, prenatal diagnosis on chorionic villus sampling is not recommended.

References

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2. Spector E, Behlmann A, Kronquist K, et al. Laboratory testing for fragile X, 2021 revision: a technical standard of the American College of Medical Genetics and Genomics (ACMG). *Genet Med*. 2021;23(5):799-812.

3. 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.

4. 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.

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