Fragile X Syndrome

Indications for Ordering

- Newborn screening
- Carrier screening for expectant women or those planning a pregnancy
- Individuals with unexplained
  - Intellectual disability
  - Developmental delay
  - Autism
  - Late onset cerebellar ataxia and intention tremor
- Females with
  - Primary ovarian insufficiency (POI)
  - Infertility associated with elevated follicle-stimulating hormone (FSH) levels
  - Family history of fragile X syndrome (FXS) or intellectual disability of unknown etiology
- Prenatal testing for women who carry a fragile X premutation or full mutation

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 analysis is performed for CGG repeat lengths of 55 or greater
    - Methylation analysis is used to distinguish between premutation and full mutation alleles
- For detailed descriptions of methodological considerations for fragile X testing, refer to ACMG Standards and Guidelines for Fragile X Testing (Monaghan, 2013)

Tests to Consider

Primary Tests

Fragile X (*FMR1*) with Reflex to Methylation Analysis 2009033

- Preferred test to diagnose fragile X syndrome and carrier screening in individuals with a positive family history

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

- Prenatal test for fetuses of mothers with fragile X premutations or full mutations
- Fetal testing for normal or intermediate maternal alleles is not recommended

Related Test

Genetic Carrier Screen (CF, FX, and SMA) 3000258

- Screen for genetic variants that indicate carrier status for cystic fibrosis (CF), fragile X syndrome (FX), and spinal muscular atrophy (SMA) in pregnant couples or those planning a pregnancy
- Do not use for diagnostic testing in patients with symptoms of CF, FXS, or SMA

Disease Overview

Prevalence

- Disease prevalence
  - 1/4,000 males
  - 1/8,000 females
- Premutation allele prevalence in U.S.
  - 1/1,000 males
  - 1/350 females

Symptoms

- Neurologic
  - Moderate, mild, or asymptomatic in females
  - Moderate to severe intellectual disability in males
- Behavioral
  - Hyperactivity
  - Perseverative speech
  - Social anxiety
  - Poor eye contact
  - Hand flapping
  - Autism spectrum disorder
- Characteristic appearance of adult males
  - Macroorchidism
  - Long, narrow face
  - Prominent ears and jaws
  - Single palmar crease
- Connective tissue anomalies
  - Hyperextensible finger and thumb joints
  - Hand calluses
  - Velvet-like skin
  - Flat feet
  - Mitral valve prolapse
- Fragile X-associated tremor/ataxia syndrome (FXTAS)
  - Older premutation males (less commonly premutation females)
  - Progressive cerebellar ataxia
  - Intention tremor
- Premutation females may develop POI
- Early diagnosis with early intervention likely maximizes outcomes

Genetics

Gene – *FMR1*

Inheritance – X-linked

Structure/function

Produces RNA-binding protein, fragile X mental retardation protein (FMRP)

- Expressed in many tissues

Mutations

- Trinucleotide CGG repeat expansion is cause of FMRP deficiency in most individuals
- Risk for repeat expansion is dependent on sex of transmitting parent and size of allele transmitted
  - Full mutation – >200-230 CGG repeats (methylated)
    - Males are affected
    - 1/3 of females are typically affected, 1/3 are mildly affected, and 1/3 are unaffected
    - Not possible to predict disease severity based on
      - Size of CGG repeat
      - Degree of methylation
      - Pattern of X-inactivation (in females)
  - Premutation – 55-200 CGG repeats (unmethylated)
    - Transmission unstable in females
    - May expand to full mutations in offspring
    - Premutations of >56 repeats have not been reported to expand to full mutations in a single generation
  - Intermediate – 45-54 CGG repeats
    - Unstably transmitted – more likely in families of affected individuals than general population
  - Normal – 5-44 CGG repeats
    - Stably transmitted
- Mosaicism may reduce disease severity

Test Interpretation

Sensitivity/specificity

- Clinical sensitivity/specificity – 99% (GeneReviews)
- Analytical sensitivity/specificity – 99% (Lyon, 2010; Grasso, 2014)

Results

- Full mutation (>200-230 CGG repeats [methylated]) – FXS
  - Mental retardation in males
  - Variable expression in females
- Premutation (55-200 CGG repeats [unmethylated])
  - Male – at risk for FXTAS and will transmit premutation to all daughters
  - Female – at risk for POI, FXTAS, and having offspring with full mutations due to allele expansion
    - Nearly 100% of maternal CGG repeats of >90 expand to full mutations in offspring
- Intermediate (45-54 CGG repeats) – offspring at increased risk for being a premutation carrier
- Negative (5-44 CGG repeats) – allele size in normal range and methylation pattern consistent with individual's sex

Limitations

- Estimated size is not provided for full mutations with >200 repeats
- Methylation patterns are not fully established at the time of chorionic villus sampling for fetal testing
  - Amniocyte analysis is recommended to distinguish a small, full mutation from a large premutation
- Rare mutations in *FMR1* unrelated to trinucleotide expansion will not be detected
- Diagnostic errors can occur due to rare sequence variations

References

- Saul RA, Tarleton JC. FMR1-Related Disorders. 1998 Jun 16 [Updated 2012 Apr 26]. In: Adam MP, Ardinger HH, Pagon RA, et al., editors. GeneReviews [Internet]. Seattle (WA): University of Washington, Seattle; 1993-2018. www.ncbi.nlm.nih.gov/books/NBK1384/
- Lyon E, Laver T, 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
- Grasso M, Boon EM, 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;16:23-31
- Monaghan KG, Lyon E, 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;15:575-586