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

**Fragile X (FMR1) with Reflex to Methylation Analysis 2009033**
- Preferred test to diagnose fragile X syndrome
- 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

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 <59 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**