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, FXS, and SMA) with Reflex to Methylation 3000258**
- Screen for genetic variants that indicate carrier status for cystic fibrosis (CF), fragile X syndrome (FXS), 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

  o 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)
  
  o 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
  
  o Intermediate – 45-54 CGG repeats
    ▪ Unstably transmitted – more likely in families of affected individuals than general population
  
  o 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
  o Mental retardation in males
  o Variable expression in females

• Premutation (55-200 CGG repeats [unmethylated])
  o Male – at risk for FXTAS and will transmit premutation to all daughters
  o 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

  o 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**


