

Fragile X (*FMR1*) Mutation

TO DIAGNOSE FRAGILE X SYNDROME OR DETERMINE CARRIER STATUS

Disease Overview

- Fragile X syndrome, the most common heritable form of mental retardation, is characterized by moderate mental retardation in males and mild mental retardation in females, behavioral phenotype, connective tissue anomalies, and physical findings.
- Behavioral features may include: hyperactivity, perseverative speech, social anxiety, poor eye contact, hand flapping or biting, and autism spectrum disorders.
- Connective tissue anomalies include: hyperextensible finger and thumb joints, hand calluses, velvet-like skin, flat feet, and mitral valve prolapse.
- Characteristic appearance of adult males includes: macroorchidism, a long, narrow face, prominent ears and jaw, and single palmar crease.
- Older premutation carrier men may develop fragile X-associated tremor/ataxia syndrome (FXTAS) characterized by progressive cerebellar ataxia and intention tremor.
- Female premutation carriers may develop primary ovarian insufficiency (20 percent) or, rarely, FXTAS.
- Benefits derived from early diagnosis of fragile X syndrome include:
 - Speech, language, occupation and physical therapies, as well as educational interventions to help affected individuals maximize their potential.
 - Timely genetic counseling for families to aid in reproductive decision making and testing of at-risk relatives; approximately half of families do not learn of their child's fragile X syndrome diagnosis before having a subsequent pregnancy.
 - Identification of psychological and educational support resources from organizations such as the National Fragile X Foundation.

Epidemiology

- Disease prevalence: 1: 4,000 males; 1: 8,000 females.
- Premutation allele prevalence in Caucasians: 1: 1000 males; 1: 350 females.
- Although disease incidence data has typically been studied for Caucasian populations, fragile X syndrome also affects other racial/ethnic groups.

Genetics

- Fragile X syndrome is an X-linked dominant disorder with reduced penetrance. *FMR1* premutation disorders, FXTAS and POF, show age-related penetrance.

- The *FMR1* gene produces an RNA-binding protein, fragile X mental retardation protein (FMRP), which is expressed in multiple tissues.
- Fragile X syndrome is caused by an expanded trinucleotide CGG repeat in the 5' untranslated region of *FMR1*, which leads to hypermethylation and inhibition of gene transcription.
 - Full mutation: >200–230 CGG repeats (methylated)
 - Premutation: 55–200 CGG repeats (unmethylated)
 - Intermediate: 45–54 CGG repeats (unmethylated)
 - Common: 5–44 CGG repeats (unmethylated)
- Trinucleotide repeat expansion is the cause of FMRP deficiency in greater than 99 percent of affected individuals.
- Risk for CGG repeat expansion during parental transmission is dependant on the sex of the transmitting parent and size of the allele transmitted.
- Common alleles are stably transmitted.
- Unstable transmission of intermediate alleles is more likely in families of affected individuals than in the general population.
- Premutation alleles in females are unstable and may expand to full mutations in offspring; premutations of fewer than 59 repeats have not been reported to expand to a full mutation in a single generation. Premutation alleles in males may expand or contract by several repeats with transmission; however, expansion to full mutations has not been reported.
- Full mutations are typically maternally transmitted.
- It is not possible to predict disease severity resulting from a full mutation based on the size of the CGG repeat, degree of methylation, or pattern of X-inactivation (in females).
- Mosaicism, both for CGG repeat length and methylation status, has been reported in affected individuals and may reduce disease severity.

Indications for Ordering: Fragile X (*FMR1*) Mutation Diagnosis

- Individuals with unexplained mental retardation, developmental delay, autism, or late-onset cerebellar ataxia and intention tremor of unknown etiology.
- Females experiencing primary ovarian insufficiency or fertility problems associated with elevated follicle-stimulating hormone levels.
- Confirmation of a cytogenetic test result inconsistent with clinical phenotype.
- Prenatal testing for fetuses of carrier mothers.
- Individuals with a family history of fragile X syndrome or mental retardation of unknown etiology.

Indications for Ordering: Fragile X (*FMR1*) Mutation Screen

- Newborn screening for infants without a family history of fragile X.
- Carrier screening for expectant women or those planning a pregnancy

Interpretation: Fragile X (*FMR1*) Mutation Diagnosis

- Full mutation (>200 CGG repeats): Fragile X syndrome; mental retardation in males and variable expression in females.
- Premutation (55–200 CGG repeats):
 - Male: at risk for FXTAS and will transmit premutation to all daughters.
 - Female: at risk for premature ovarian failure and having offspring with full mutations due to allele expansion (nearly 100 percent of maternal CGG repeats of >90 expand to full mutations in their offspring).
- Intermediate (45–54 CGG repeats): Patient's offspring may be at increased risk of being a premutation carrier.
- Negative: Allele repeat size in the normal range and methylation pattern is consistent with patient's gender.

Interpretation: Fragile X (*FMR1*) Mutation Screen

- Negative: Individuals without an allele in the premutation or full mutation range are considered to be at very low risk for being affected with fragile X syndrome or having affected offspring.
- Positive: Individuals with an allele in the premutation or full mutation range are considered to be at increased risk for having symptoms and/or affected offspring. Thus, reflex testing to Fragile X (*FMR1*) Mutation Diagnosis will be performed to determine precise CGG repeat sizes and methylation status for each allele.

Methodology for Fragile X (*FMR1*) Mutation Diagnosis

- PCR analysis to determine the *FMR1* CGG repeat length.
- Amplification of a sex-chromosome marker to confirm chromosomal sex.
- Methylation status of expanded alleles determined by Southern blot hybridization following methylation-specific restriction enzyme digest.
- Clinical and analytical sensitivity and specificity are 99 percent.

Methodology for Fragile X (*FMR1*) Mutation Screen

- PCR followed by size analysis using capillary electrophoresis; reflex to the Fragile X (*FMR1*) Mutation Diagnosis assay will be performed if a premutation or full mutation allele is suspected.
- Clinical and analytical sensitivity and specificity are 99 percent

Limitations for Fragile X (*FMR1*) Mutation Diagnosis

- Sizing of intermediate alleles (45–54 CGG repeats) is accurate to +/- two repeats.
- Methylation patterns may not be fully established at the time of chorionic villus sampling for fetal fragile X testing; amniocytes may be needed to distinguish a small full mutation from a large premutation.
- Rare mutations in *FMR1* unrelated to trinucleotide expansion may not be detected.
- Rare diagnostic errors may occur due to primer-site mutations.

Limitations for Fragile X (*FMR1*) Mutation Screen

- Precise sizing of normal alleles is not performed.
- Samples with expanded alleles will be reflexed to Southern blot to determine size and methylation status of premutation and full mutation alleles.
- Rare mutations in *FMR1* unrelated to trinucleotide expansion may not be detected.
- Rare diagnostic errors may occur due to primer-site mutations.
- Cannot be used for prenatal testing.

Related Test

Fragile X Syndrome, DNA Testing, Fetal (0050543)

References

1. American College of Medical Genetics. Technical standards and guidelines for fragile X: 2006 edition. http://www.acmg.net/Pages/ACMG_Activities/stds-2002/fx.htm (accessed on August 11, 2009).
2. Online GeneTests: *FMR1*-Related Disorders. <http://www.genetests.org> (accessed on August 11, 2009).
3. McConkie-Rosell A, et al. Genetic counseling for fragile X syndrome: updated recommendations of the NSGC. *J Genet Couns* 2005;14(4):249–70.
4. Sherman S, et al. Fragile X syndrome: diagnostic and carrier testing. *Genet Med* 2005;7(8):584–7.

Test Information

0040011
2001946

Fragile X (*FMR1*) Diagnostic
Fragile X (*FMR1*) Screen with Reflex to Fragile X (*FMR1*) Diagnostic

For specific collection, transport, and testing information, refer to the ARUP Web site at www.aruplab.com.
For information on test selection, ordering, and interpretation, refer to ARUP Consult® at www.arupconsult.com.