XSense®

Fragile X with Reflex

Test Code 10300

Clinical Use

- Detect Fragile X Syndrome (FXS) carriers
- Determine an individual's risk of having a child with FXS
- Diagnose FXS postnatally

Clinical Background

FXS is the most common inherited cause of developmental delay and mental retardation, occurring in approximately 1 in 4000 males and 1 in 6000 to 8000 females.[1] The prevalence of carriers in the Caucasian population is an estimated 1 per 259 females and 1 per 813 males.[2,3]

Affected males usually have moderate to severe mental retardation, pervasive speech delay, and behavioral problems (e.g., attention deficit hyperactivity disorder [ADHD]). Autism-spectrum disorders are frequently diagnosed in the 2nd or 3rd year of life.[4] Affected females have a variable phenotype that can range from normal intelligence to severe mental retardation, with or without learning disabilities or personality disorders.

In more than 99% of cases, FXS is caused by an expansion of a polymorphic CGG trinucleotide repeat in the 5′ untranslated region of the *FMR1* gene, located on the X chromosome, resulting in hypermethylation of the FMR1 promoter.[5]

The extent of expansion and hypermethylation correlates negatively with the amount of a protein (absent in affected males and reduced in affected females) that plays a role in brain synaptic development. The severity of the phenotype is related to the extent of expansion (Table 1). Other rare mutations of *FMR1* associated with FXS include large deletions, point mutations, and missense mutations.

FMR1-related disorders are inherited in an X-linked dominant manner with variable penetrance, and inheritance is affected by the number of CGG repeats present (Table 2).[7] Individuals with CGG repeats in the intermediate and permutation range are carriers.

The molecular diagnosis of FXS is based on detecting the number of CGG repeats and methylation status of the *FMR1* gene. Polymerase chain reaction (PCR) can detect and accurately measure repeat numbers in the normal and small permutation ranges; Southern blot is required to quantify larger CGG repeats. Southern blot, however, is a time-consuming, laborious process, which has limited the potential of carrier screening. Therefore, a new method called triplet-primed PCR has been developed.[8] A unique amplicon containing stutter peaks is produced when the individual is at least a fragile X carrier. In these cases, a Southern blot will be performed to establish the exact size and methylation status of the expanded allele. The absence of stutter peaks indicated absence of an expanded allele.

Table 1. Number of CGG Repeats in *FMR1* and Associated Phenotype

Approximate # of CGG Repeats[a]	Classification	Gene Function	Phenotype
5 to 44	Normal	Normal	Not affected
45 to 54	Indeterminate ("gray zone")	Normal	Not affected
55 to 200	Premutation	Larger premutations may have decreased gene expression	**Males:** ~38% incidence of FXTAS after age 50 yrs **Females:** ~20% incidence of premature ovarian failure
>200	Full mutation	Loss of gene expression	Fragile X syndrome

FXTAS, fragile x-associated tremor/ataxia syndrome (i.e. progressive cerebellar ataxia and intention tremor).
[a]Cut-offs are approximate and based on current research.[6]

Table 2. Inheritance Pattern of *FMR1* CGG Repeat Mutations

Mutation in Parent	Classification
Females with	
Intermediate[a] ("gray zone")	Number of CGG repeats may increase to premutation size in offspring
Premutation	Premutation may expand during meiosis in oocytes; thus, mother may give birth to a child with a full mutation[b]
Full mutation	Full mutation
Males[c] with	
Intermediate[a] ("gray zone")	Number of CGG repeats may increase to premutation size in daughters
Premutation	Premutation passed to daughters
Full mutation	Full mutation shrinks to premutation size in daughters

[a] Individuals with an intermediate mutation status are considered carriers due to the potential of offspring inheriting the premutation.
[b] The greater the number of repeats, the greater chance of expansion to a full mutation.
[c] Sons are not affected because they only inherit the paternal Y chromosome. Males with full mutations are not likely to reproduce.

Individuals Suitable for Testing

- Individuals with a family history of FXS or undiagnosed mental retardation, including those seeking reproductive counseling
- Symptomatic children and adults

Methodology

- PCR and capillary electrophoresis to determine gender and number of CGG repeats
- Triplet-primed PCR and capillary electrophoresis to detect stutter peaks
- Southern blot confirmation of *FMR1* expansions performed as reflex if PCR indicates an expanded allele
- Results reported: Number of CGG repeats and methylation status of any expanded alleles

Reference Range

Negative (*FMR1* containing 5 to 44 CGG repeats)

Specimen Requirements

5.0 mL room temperature whole blood in an EDTA (lavender-top) tube, ACD (Solution A or B) yellow-top tube, or sodium heparin (green-top) tube (3.0 mL minimum).

CPT Codes*

83891; 83900; 83898; 83909 (x2); 83912 (x2) (Reflex tests are performed at an additional charge and are associated with additional CPT codes.)

Interpretive Information

A negative result indicates a normal gene. When >44 CGG repeats are identified, an individual's mutation status and phenotype are determined by the number of repeats present (see Table 1). The associated risk of having a child with FXS is explained in Table 2.

This assay does not detect other mutations (e.g., deletions, point mutations, missense mutations) that disrupt the function of the FMR1 gene and/or protein. Results should be interpreted in conjunction with other laboratory and clinical findings. Additional assistance in interpretation of results is available from our Genetic Counselors by calling 1.866.GENE.INFO (1.866.436.3463).

References

1. Pembrey MR, Barnicoat AJ, Carmichael B, et al. An assessment of screening strategies for fragile X syndrome in the UK. *Health Technol Assess*. 2001;5:1-95.
2. Rousseau F, Rouillard P, Morel ML, et al. Prevalence of carriers of permutation-size alleles of the *FMR1* gene—and implications for the population genetics of the fragile X syndrome. *Am J Hum Genet*. 1995;57:1006-1018.
3. Dombrowski C, Levesque S, Morel ML, et al. Premutation and intermediate-size *FMR1* alleles in 10572 males from the general population: loss of an AGG interruption is a late event in the generation of fragile X syndrome alleles. *Hum Mol Genet*. 2002;11:371-378.
4. Belmonte MK, Bourgeron T. Fragile X syndrome and autism at the intersection of genetic and neural networks. *Nat Neurosci*. 2006;9:1221-1225.
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6. Technical standards and guidelines for fragile X testing: a revision to the disease-specific supplements to the Standards and Guidelines for Clinical Genetic Laboratories of the American College of Medical Genetics. In: American College of Medical Genetics Standards and Guidelines for Clinical Genetics Laboratories. 2006 ed. American College of Medical Genetics Web site. http://www.acmg.net/Pages/ACMG_Activities/stds-2002/fx.htm. Accessed: January 19, 2010.
7. Hagerman PJ, Hagerman RJ. The fragile-X permutation: a maturing perspective. *Am J Hum Genet*. 2004;74:805-816.
8. Hantash FM, Goos DG, Tsao D, et al. Qualitative assessment of *FMR1* (CGG) triplet repeat status in normal, intermediate, permutation, full mutation and mosaic carriers in both sexes: implications for fragile X syndrome carrier and newborn screening. *Genet Med*. In press.

*The CPT codes provided are based on AMA guidelines and are for informational purposes only. CPT coding is the sole responsibility of the billing party. Please direct any questions regarding coding to the payor being billed.

This test was developed and its performance characteristics have been determined by Quest Diagnostics Nichols Institute. Performance characteristics refer to the analytical performance of the test.