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

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). 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. 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.

### 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. The prevalence of carriers in the Caucasian population is an estimated 1 per 259 females and 1 per 813 males.

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. 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.

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

<table>
<thead>
<tr>
<th>Approximate # of CGG Repeats</th>
<th>Classification</th>
<th>Gene Function</th>
<th>Phenotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>5 to 44</td>
<td>Normal</td>
<td>Normal</td>
<td>Not affected</td>
</tr>
<tr>
<td>45 to 54</td>
<td>Indeterminate (&quot;gray zone&quot;)</td>
<td>Normal</td>
<td>Not affected</td>
</tr>
<tr>
<td>55 to 200</td>
<td>Premutation</td>
<td>Larger premutations may have decreased gene expression</td>
<td>Males: ~38% incidence of FXTAS after age 50 yrs, Females: ~20% incidence of premature ovarian failure</td>
</tr>
<tr>
<td>&gt;200</td>
<td>Full mutation</td>
<td>Loss of gene expression</td>
<td>Fragile X syndrome</td>
</tr>
</tbody>
</table>

FXTAS, fragile x-associated tremor/ataxia syndrome (i.e. progressive cerebellar ataxia and intention tremor).

Cut-offs are approximate and based on current research.
Table 2. Inheritance Pattern of \textit{FMR1} CGG Repeat Mutations

<table>
<thead>
<tr>
<th>Mutation in Parent</th>
<th>Classification</th>
</tr>
</thead>
<tbody>
<tr>
<td>Females with</td>
<td></td>
</tr>
<tr>
<td>Intermediate(^a) (&quot;gray zone&quot;)</td>
<td>Number of CGG repeats may increase to premutation size in offspring</td>
</tr>
<tr>
<td>Premutation</td>
<td>Premutation may expand during meiosis in oocytes; thus, mother may give birth to a child with a full mutation(^b)</td>
</tr>
<tr>
<td>Full mutation</td>
<td>Full mutation</td>
</tr>
<tr>
<td>Males(^c) with</td>
<td></td>
</tr>
<tr>
<td>Intermediate(^a) (&quot;gray zone&quot;)</td>
<td>Number of CGG repeats may increase to premutation size in daughters</td>
</tr>
<tr>
<td>Premutation</td>
<td>Premutation passed to daughters</td>
</tr>
<tr>
<td>Full mutation</td>
<td>Full mutation shrinks to premutation size in daughters</td>
</tr>
</tbody>
</table>

\(^a\) 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.

**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 \textit{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**


**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; 83908; 83909 (x2); 83912 (x2) (Reflex tests are performed at an additional charge and are associated with additional CPT codes.)

*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.

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