

CGG Repeat Analysis of the FMR1 Gene in Fragile X Syndrome

INCLUDES

Fragile X syndrome (FXS), fragile X-associated tremor/ataxia syndrome (FXTAS), and fragile X-associated primary ovarian insufficiency (FXPOI)

CLINICAL FEATURES

Fragile X syndrome (FXS) is the most common inherited cause of intellectual disability, with an estimated prevalence of 1/4000 to 1/6000 (PMID: 16047089; 16247297). Nearly all cases of FXS are caused by the expansion of an unstable CGG repeat in the 5' untranslated region of the *FMR1* gene. Most individuals in the general population have less than 45 CGG repeats, while individuals with a full variant causing FXS have more than 200 CGG repeats. Males with FXS exhibit mild to severe intellectual disability, often associated with autism-spectrum disorders, ADHD, speech and language delay, anxiety, and other characteristic behaviors. Physical features include macrocephaly and a distinctive facial appearance including large ears, a long face with a prominent forehead, prognathism, and a high arched or cleft palate. Macroorchidism maybe observed in postpubertal males. Females with full variants exhibit a broad spectrum of clinical presentations depending in part on the pattern of X inactivation. Approximately 50% of females with a full variant have borderline or mild intellectual disability, and they also may exhibit shyness and anxiety (PMID: 16047089; 21739597; 23765048). Some females with full variants have more significant cognitive, behavioral, and physical features of FXS similar to those observed in males, while others have normal intelligence with only subtle learning disabilities (PMID: 16047089). An estimated 1 in 259 females and 1 in 755 males in the general population have an *FMR1* premutation with 55 to 200 CGG repeats (PMID: 16047089). While they do not have FXS, premutation carriers have an increased risk for neurological, psychiatric, and physical disorders. Approximately 40% of male and 8–17% of female premutation carriers over the age of 50 report experiencing symptoms of fragile X-associated tremor/ataxia syndrome (FXTAS) (PMID: 21739597; 14747503). FXTAS is a neurodegenerative disorder characterized by cerebellar ataxia, intention tremor, parkinsonism, progressive cognitive decline, and psychiatric disorders (PMID: 14747503; 17618523). Additionally, approximately 21% of female premutation carriers develop primary ovarian insufficiency (POI) leading to early menopause and infertility (PMID: 21739597; 21651511; 20859177). Premutation carriers may also have an increased frequency of learning difficulties, depression, social anxiety, and other medical issues, although additional research is necessary to clarify the association between premutation alleles and these disorders (PMID: 16047089; 21651511; 19265746).

GENETICS

Greater than 98% of individuals with FXS harbor a CGG repeat expansion in the 5' untranslated region of the *FMR1* gene, which results in hypermethylation of the *FMR1* promoter region with consequent gene silencing and absence of FMRP protein expression. Point mutations or whole/partial deletions of *FMR1* are very rare. The categories of CGG repeat sizes include:

Normal: <45

Intermediate ('grey zone'): 45–54

Premutation: 55–200

Full mutation: >200

Approximately 20–40% of individuals with FXS exhibit somatic mosaicism for full mutations, premutations, and/or even normal CGG repeat sizes (size mosaicism) or have both methylated and unmethylated alleles and are considered methylation mosaics (PMID: 23560306). Individuals with size or methylation mosaicism may have a milder phenotype than individuals with fully methylated alleles (PMID: 23560306). Variants in the *FMRI* gene are inherited in an X-linked manner. The CGG repeat size is unstable when transmitted from a female premutation carrier to her children. The risk of expansion depends in part on the number of maternal CGG repeats and the presence of interspersed AGG repeats. The smallest premutation that has been reported to expand to a full variant in one generation is 56 repeats (PMID: 19525339). The risk for expansion to a full variant ranges from ~3% for maternal alleles with 59 repeats to almost 100% for maternal alleles with 100 or more repeats (PMID: 16047089; 23560306). In rare cases, contraction of a premutation to a normal repeat size has been reported in mother-to-daughter transmissions (PMID: 16047089). Male premutation carriers typically transmit a premutation to their daughters, and ~1/3 of father-to-daughter transmissions actually result in contraction to a smaller premutation allele (PMID: 16047089; 23765048). Intermediate alleles of 45–54 CGG repeats have not been reported to expand to a full variant in one generation but may increase the risk for *FMRI*-related disorders in future generations (PMID: 16047089; 23765048).

TEST METHODS

Using genomic DNA obtained from the submitted specimen, repeat analysis is performed via the Asuragen AmpliEx PCR/CE *FMRI* kit. The sample is evaluated by repeat-primed PCR to identify expanded alleles, as well as determine the number of repeats in alleles with fewer than 200 repeats. Nucleotide repeat numbers up to 54 are reported with an accuracy of ± 2 repeats and repeat numbers from 54–200 are reported with an accuracy of ± 5 repeats. The exact number of repeats cannot be determined for alleles with greater than 200 repeats. Internal standards are analyzed along with clinical samples to evaluate assay performance. The Asuragen AmpliEx mPCR *FMRI* kit is used to determine the methylation pattern for alleles with greater than or equal to 150 repeats.

TEST SENSITIVITY

Full variants in the *FMRI* gene are estimated to account for approximately 0.2–3.0% of individuals with intellectual disability, autism spectrum disorders, and/or nonspecific special needs (PMID: 16047089; 11545690). Premutations in the *FMRI* gene are estimated to account for approximately 2% of sporadic cases and 14% of familial cases of POI, while 1–2% of adult males with ataxia harbor premutations causing FXTAS (PMID: 16047089; 21739597; 17618523; 23765048).