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SPECIAL ARTICLE

Fragile X Syndrome

Scientific Background and Screening Technologies

Justine I. Lyons, Gregory R. Kerr, and Patricia W. Mueller

From the Molecular Risk Assessment Laboratory, Newborn Screening and Molecular Biology Branch, Centers for Disease Control and Prevention, Atlanta, Georgia

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Address correspondence to Patricia W. Mueller, Ph.D., Centers for Disease Control and Prevention, Mailstop F24, 4770 Buford Hwy. NE, Atlanta, GA 30341. E-mail: pwm2@cdc.gov.

Fragile X is the most common inherited cause of mental retardation with a prevalence of 1 in 4000 for males and 1 in 5000 to 8000 for females. The American College of Medical Genetics and Genomics has recommended diagnostic testing for fragile X in symptomatic persons, women with ovarian dysfunction, and persons with tremor/ataxia syndrome. Although medical and scientific professionals do not currently recommend screening nonsymptomatic populations, improvements in current treatment approaches and ongoing clinical trials have generated growing interest in screening for fragile X. Here, we briefly review the relevant molecular basis of fragile X and fragile X testing and compare three different molecular technologies available for fragile X screening in both males and females. These technologic approaches include destabilizing the CGG-repeat region with betaine and using chimeric CGG-targeted PCR primers, using heat pulses to destabilize C-G bonds in the PCR extension step, and using melting curve analysis to differentiate expanded CGG repeats from normals. The first two-step method performed with high sensitivity and specificity. The second method provided agarose gel images that allow identification of males with expanded CGG repeats and females with expanded CGG-repeat bands which are sometimes faint. The third melting curve analysis method would require controls in each run to correct for shifting optimal cutoff values. *(J Mol Diagn 2015, 17: 463—471; http://dx.doi.org/10.1016/j.jmoldx.2015.04.006)*

Reduction of the protein coded for by the fragile X mental retardation gene (*FMR1*) causes fragile X syndrome, a genetic condition that causes a range of developmental problems, including learning disabilities, cognitive impairment, and behavioral abnormalities.[1] Expansion of CGG repeats in the 5′ untranslated region (UTR) of the *FMR1* gene is associated with hypermethylation and inactivation of gene expression (Figure 1).

Function of the *FMR1* Gene

Evidence suggests that the protein coded for by the *FMR1* gene on the long arm of chromosome X (Xq27.3) binds mRNA and associates with polyribosomes in neurons.[2] The protein likely shuttles mRNA from the nucleus through the cytoplasm and localizes dendritic mRNA where it represses synaptic protein synthesis. After the group 1 metabotropic glutamate receptor is stimulated, regulatory *FMR1* protein (FMRP) production is believed to repress mRNA translation and protein synthesis and to control permanent physical changes that alter synaptic connections linked with the process of learning and memory.[3—6]

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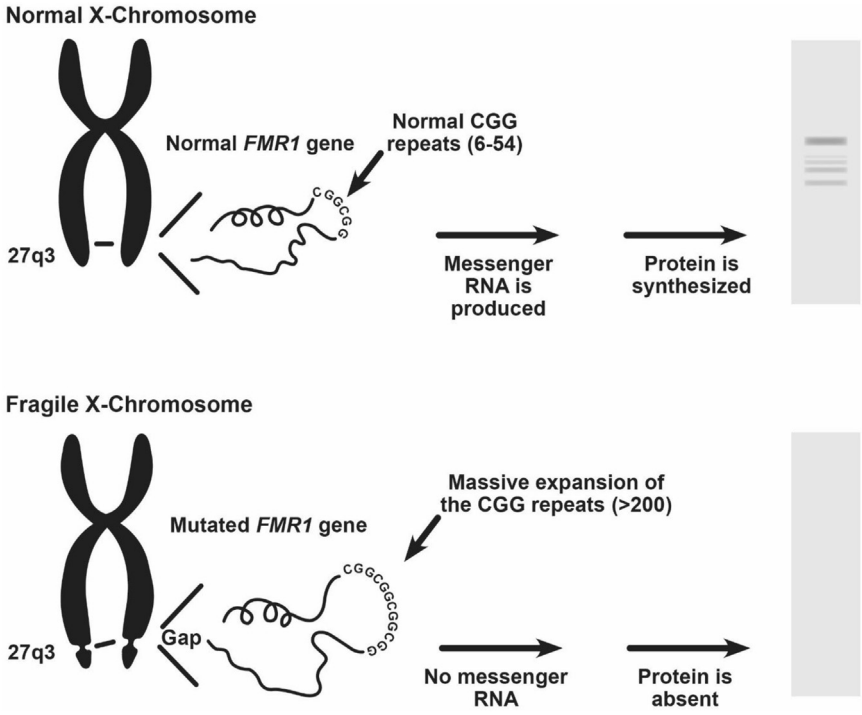

Figure 1 Expansion of the CGG repeats in the 5′ untranslated region of the *FMR1* gene on the X chromosome can result in decreased mRNA and FMRP production, causing fragile X. FMRP, *FMR1* protein.

Mutations and Associated Conditions

Normal Range

Greater than 99% of fragile X cases are caused by expansions of CGG repeats in the *FMR1* 5′ UTR.[7] The other 1% is caused by a variety of other mutations, primarily including gross deletions and duplications, regulatory mutations, and missense and nonsense mutations. The CGG-repeat numbers of individual genes have been categorized, and the borders of these categories are approximate. The normal range of CGG repeats is considered generally to be as high as 44 CGG repeats, and these repeats are interrupted typically every 9 or 10 repeats by an AGG triplet. These AGG triplets likely anchor the region and prevent slippage during DNA replication. The number and spacing of AGG triplets within CGG-repeat regions may help predict risk of expansion of $<$100 repeats.[8]

Gray Zone (Intermediate Range)

The range of 45 to 54 CGG repeats is referred to as the gray zone or intermediate range; for alleles of this size, neither disease associations nor the rate of expansion are fully understood. However, this range is not associated with fragile X syndrome, and gray zone alleles expanding to a full mutation in one generation have not been observed.[7]

Premutation Range

Alleles with approximately 55 to 199 CGG repeats are considered premutations. These alleles are transmitted unstably from parent to child, and expansions from this range to the full-mutation range typically occur during maternal transmission. Because mutations of this size possibly can have somatic mosaicism that includes a full mutation, careful examination of the range of allele sizes is warranted.[7] The smallest *FMR1* premutation that was reported to expand to a full mutation (to approximately 538 CGG repeats) in a single generation is 56 CGG repeats. In addition in this case, two AGG interruptions in the grandfather's gray zone allele of 52 CGG repeats were absent when transmitted to his daughter.[9]

Expansion of an allele into the premutation range perturbs gene expression,[7] and two conditions are associated with this range of expansions. Reductions in FMRP occur in this range and are associated with increased *FMR1* mRNA. Premutation alleles may shift transcription of the *FMR1* mRNA to an upstream site, and this use of an alternative start site may correlate with increased transcription levels. This RNA-mediated toxicity is associated with fragile X-associated tremor/ataxia syndrome,[10,11] a late-onset, progressive development of intention tremor and ataxia frequently accompanied by cognitive and behavioral difficulties. Although most persons with premutations do not show fragile X-related features, females with premutations generally $>$80 CGG repeats are at approximately 20% risk of fragile X-associated premature ovarian insufficiency.[7] Older males and females with premutations are at risk of fragile X-associated tremor/ataxia syndrome, with higher risk in males. The penetrance of fragile X-associated tremor/ataxia syndrome increases with age and CGG repeat length.

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