Fragile X Syndrome and *FMR1*-Associated Disorders

Background

Fragile X syndrome (OMIM#300624) is the most common inherited cause of intellectual disability, with an incidence of approximately 1 in 4,000 males and 1 in 8,000 females. The syndrome was first recognized in 1977 when Sutherland linked a fragile site on the X chromosome with the phenotype of X-linked mental retardation and macroorchidism.[1] The *FMR1* gene was isolated in 1991 and PCR-based testing has since been used to make or confirm diagnoses of fragile X syndrome.[2] In most cases, fragile X syndrome is caused by a trinucleotide (CGG) repeat expansion in the 5' untranslated (UTR) region of the gene. Normally, there are fewer than 45 CGG triplet repeats but affected individuals have greater than 200 CGG repeats. These full mutations of the CGG region result in hypermethylation of the promoter region, with subsequent silencing of gene expression and absence of the FMR1 protein (FMRP). Severity of symptoms of fragile X syndrome tend not to be affected by the number of trinucleotide repeats in the full mutation allele. However, individuals are less severely affected if they carry unmethylated full mutation allele or are mosaic for the full and premutation alleles. About 30-50% of females with full mutation are significantly affected.

Alleles with 55 to 200 CGG repeats are considered premutations; they do not cause fragile X syndrome but are prone to meiotic instability and may expand to full mutations in one generation. For reasons that are unclear, such expansions occur more often in female meiosis. Approximately 1 in 250 females is a carrier of an *FMR1* premutation and at risk of having a child with fragile X syndrome.[3] In addition, about 20% of women with premutations experience premature ovarian insufficiency (POI), with onset of menopause before the age of 40.[4] POI is not seen with increased frequency among women with full mutations.

Fragile X-associated tremor/ataxia syndrome (FXTAS), a late-onset neurodegenerative condition, has been identified as an *FMR1*-related disorder. FXTAS is seen predominantly in men, with onset after 50 years of age. The penetrance among male premutation carriers increases with age, with 75% showing symptoms by the ninth decade of life.[5] Women also have a risk of developing FXTAS but with lower frequency and milder phenotype, due to random X-inactivation of the expanded allele. In contrast to the gene silencing that occurs in alleles with full mutations, premutations are associated with an up-regulation of transcription resulting in a toxic accumulation of *FMR1* mRNA, usually in the presence of normal or slightly decreased protein levels. An intermediate, or "gray zone," also exists in the *FMR1* gene. Alleles containing 45 to 54 CGG repeats may also be unstable in meiosis but do not expand to full mutations in a single generation.

Clinical Indications

Fragile X Syndrome: The clinical phenotype associated with fragile X syndrome is variable and may be subtle in females. Therefore, the American College of Medical Genetics and Genomics (ACMG) suggests that testing be considered in all individuals with intellectual disability, developmental delay or autism, and especially in the presence of other fragile X characteristics or a family history of fragile X syndrome or undiagnosed mental retardation.[4]

Premature Ovarian Insufficiency: Further, ACMG and the American College of Obstetricians and Gynecologists (ACOG) recommend testing for women with evidence of premature ovarian insufficiency, i.e. infertility associated with elevated follicle stimulating hormone (FSH).[4,6] As above, *FMR1* testing should especially be considered when there is an accompanying family history of fragile X syndrome or undiagnosed mental retardation, or if there is family history of premature ovarian failure.

Fragile X Associated Tremor/Ataxia Syndrome: *FMR1* testing should also be considered in men and women with late onset cerebellar ataxia and intention tremor, especially in the presence of family history of movement disorders, fragile X syndrome or undiagnosed mental retardation.[4]

Carrier Testing: Reproductive carrier testing for fragile X syndrome is recommended for individuals with known family history of fragile X syndrome or with family history of undiagnosed intellectual disability. Population carrier screening for fragile X syndrome is not currently recommended due to the complex clinical implications of identifying expanded alleles. The approach to genetic counseling for the diverse phenotypes associated with premutations and full mutations has not yet been adequately addressed.[4]

Methodology

Determination of CGG expansion status is performed by PCR and capillary electrophoresis using the Amplidex *FMR1* kit from Asuragen. PCR is performed on DNA isolated from peripheral blood using labeled primers designed to amplify the *FMR1* repeat region by triplet repeat primed-PCR assay (TP-PCR). PCR products are run on the ABI 3730 Genetic Analyzer to determine the size(s) of the repeat expansion. This TP-PCR assay identified normal to full mutation *FMR1* alleles at 100% specificity and 97.4% sensitivity in a previous report.[7] Reflex methylation testing are performed separately for samples with premutation and/or full mutation.

Interpretation

FMR1 CGG repeat size*	
Normal	<45
Intermediate	45–54
Premutation	55–200
Full mutation	>200

**Based on recommendations of the ACMG Quality Assurance Committee and the Professional Practice and Guidelines Committee*[8,9]

Thorough interpretation of results is dependent on the indication for testing and relies on good communication of clinical information from the ordering provider. CGG repeats less than 45 are considered normal and are not consistent with a diagnosis of or risk for fragile X syndrome or any of the *FMR1*-related disorders. Individuals with alleles in the intermediate range of 45-54 CGG repeats would be expected to have a normal phenotype but future generations may be at risk for expansion of the repeat into the premutation or full mutation ranges. Individuals with premutations of 55-200 repeats generally do not have fragile X syndrome but are at risk of other *FMR1*-associated disorders, POI or FXTAS. Premutation carrier females may have children with fragile X syndrome. Full mutations of greater than 200 CGG repeats are consistent with a diagnosis of fragile X syndrome but are not associated with increased risk of POI or FXTAS. Methylation studies are ordered as a reflex test for samples with premutations and/or full mutations. In cases where the test results are not consistent with the clinical phenotype, additional testing may be required to assess other mutations in *FMR1*.

Due to the complex issues surrounding fragile X syndrome and *FMR1*-related disorders, documented informed consent and genetic counseling is recommended for all families undergoing testing.

References

1. Sutherland GR. Fragile sites on human chromosomes: demonstration of their dependence on the type of tissue culture medium. *Science*. 1977;197(4300):265-6.
2. Verkerk AJ, Pieretti M, Sutcliffe JS, Fu YH, Kuhl DP, Pizzuti A, Reiner O, Richards S, Victoria MF, Zhang FP, *et al*. Identification of a gene *(FMR-1)* containing a CGG repeat coincident with a breakpoint cluster region exhibiting length variation in fragile X syndrome. *Cell*. 1991 May 31;65(5):905-14.
3. Rousseau F, Rouillard P, Morel ML, Khandjian EW, Morgan K. Prevalence of carriers of premutation-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.
4. Sherman S, Pletcher BA, Driscoll DA. American College of Medical Genetics Practice Guideline: Fragile X syndrome: diagnostic and carrier testing. *Genet Med*. 2005; 7(8):584-87.
5. Jacquemont S, Hagerman RJ, Leehey MA, Hall DA, Levine RA, Brunberg JA, Zhang L, Jardini T, Gane LW, Harris SW, *et al*. Penetrance of the fragile X-associated tremor/ataxia syndrome in a premutation carrier population. *JAMA.* 2004; 291(4):460-69.
6. Carrier screening for fragile X syndrome. Committee Opinion No. 469. American College of Obstetricians and Gynecologists. *Obstet Gynecol*. 2010; 116:1008-10.
7. Seneca S, Lissens W, Endels K, Caljon B, Bonduelle M, Keymolen K, De Rademaeker M, Ullmann U, Haentjens P, Van Berkel K, Van Dooren S. Reliable and Sensitive Detection of Fragile X (Expanded) Alleles in Clinical Prenatal DNA Samples with a Fast Turnaround Time. *J Mol Diagn* 2012; 14:560-68.
8. Kronquist KE, Sherman SL, and Spector EB. Clinical significance of tri-nucleotide repeats in Fragile X testing: A clarification of American College of Medical Genetics guidelines. *Genet Med* 2008;10(11):845-47.
9. Spector EB, Kronquist KE. Fragile X: technical standards and guidelines. ACMG standards and guidelines for clinical genetics laboratories. 2005. Available at: http://www.acmg.net/pages/acmg_activities/stds-2002/fx.htm.

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Test Overview

Test Name	Fragile X Syndrome and *FMR1*-Associated Disorders
Methodology	PCR performed on DNA isolated from peripheral blood using triplet repeat primer PCR assay (Asuragen) designed to amplify the *FMR1* repeat region.
Specimen Requirements	Peripheral Blood: 5 ml in an EDTA tube (purple top).
Billing Code	82365
CPT Code	81243

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