Genetic testing of the FMR1 Gene in Fragile X Syndrome

INCLUDES
Fragile X tremor/ataxia syndrome (FXTAS) and fragile X-associated primary ovarian insufficiency (POI)

CLINICAL FEATURES
Fragile X syndrome type A (FXS or FRAXA) is the most common inherited cause of intellectual disability, with an estimated prevalence of 1/4000 to 1/6000.[1,2] 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 45CGG 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.[1,3,10] 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.[1]

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.[1] 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).[3,4] FXTAS is a neurodegenerative disorder characterized by cerebellar ataxia, intention tremor, parkinsonism, progressive cognitive decline, and psychiatric disorders.[4,5] Additionally, approximately 21% of female premutation carriers develop primary ovarian insufficiency (POI) leading to early menopause andinfertility.[3,6,7] 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.[1,6,8]

GENETICS
Greater than 98% of individuals with FXS harbor a CGG repeat expansion in the 5' untranslated region of the FMR1gene, which results in hypermethylation of the FMR1promoter region with consequent gene silencing and absence of FMRP protein expression. Point mutations or whole/partial deletions of FMR1are very rare. The categories of CGG repeat sizes include:

Normal: <45
Intermediate ('grey zone'):45-54
Premutation:55-200
Full mutation:>200

Approximately 12% of individuals with FXS exhibit somatic mosaicism for full mutations, premutations, and/or even normal CGG repeat sizes.[1,10] Additionally, 6% of males with FXS have both methylated and unmethylated alleles and are considered methylation mosaics.[1,10] Individuals with size or methylation mosaicism may have a milder phenotype than individuals with fully methylated alleles.[1,10]

Variantsin the FMR1 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.9The 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.1In rare cases, contraction of a premutation to a normal repeat size has been reported in mother-to-daughter transmissions.[1] 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.[1,10] 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 FMR1-related disorders in future generations.[1,10]

TEST METHODS

Using genomic DNA obtained from the submitted specimen, repeat analysis was performed via the Asuragen AmplideX PCR/CE FMR1 kit. The sample was 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 greater than 54 are reported with an accuracy of +/-5 repeats. Internal standards were analyzed along with clinical samples to evaluate assay performance. The Asuragen AmplideX mPCR FMR1 kit was used to determine the methylation pattern for alleles with greater than 54 repeats.

TEST SENSITIVITY

Full variants in the FMR1 gene are estimated to account for approximately 0.2-3.0% of individuals with intellectual disability, autism spectrum disorders, and/or nonspecific special needs.[1,11] Premutations in the FMR1 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.[1,3,5,10]

REFERENCES:

1. McConkie-Rosell A et al. Genetic counseling for fragile x syndrome: updated recommendations of the national society of genetic counselors. Journal Of Genetic Counseling. 2005 14(4):249-70.16047089.
2.Sherman S et al. Fragile X syndrome:diagnostic and carrier testing. Genetics In Medicine : Official Journal Of The American College Of Medical Genetics. 2005 7(8):584-7.16247297.
3.Pirozzi F et al. The FRAXopathies: definition, overview, and update. American Journal Of Medical Genetics. Part A. 2011 155A(8):1803-16.21739597.
4.Jacquemont S et al. Penetrance of the fragile X-associated tremor/ataxia syndrome in a premutation carrier population. Jama : The Journal Of The American Medical Association. 2004 291(4):460-9.14747503.
5.Berry-Kravis E et al. Fragile X-associated tremor/ataxia syndrome: clinical features, genetics, and testing guidelines. Movement Disorders : Official Journal Of The Movement Disorder Society. 2007 22(14):2018-30, quiz 2140.17618523.
6.Willemsen R et al. CGG repeat in the FMR1 gene: size matters. Clinical Genetics. 2011 80(3):214-25.21651511.
7.ACOG Committee Opinion No. 469: Carrier screening for fragile X syndrome. Obstetrics And Gynecology. 2010 116(4):1008-10.20859177
8.Hunter JE et al. Is there evidence for neuropsychological and neurobehavioral phenotypes among adults without FXTAS who carry the FMR1 premutation? A review of current literature. Genetics In Medicine : Official Journal Of The American College Of Medical Genetics. 2009 11(2):79-89.19265746.
9.Fernandez-Carvajal Iet al. Expansion of an FMR1 grey-zone allele to a full mutation in two generations. The Journal Of Molecular Diagnostics : Jmd. 2009 11(4):306-10.19525339. (PMID: 19525339).Spector and Kronquist (2006) Technical Standards and Guidelines for Fragile X Testing: Supplement to Standards and Guidelines for Clinical Genetics Laboratories of the American College of Medical Genetics. Online: http://www.acmg.net/Pages/ACMG_Activities/stds-2002/stdsmenu-n.htm Accessed July 2011.
10.Crawford DC et al. FMR1 and the fragile X syndrome: human genome epidemiology review. Genetics In Medicine : Official Journal Of The American College Of Medical Genetics. 3(5):359-71.11545690.
11.Jacquemont S et al. Clinical utility gene card for: fragile X mental retardation syndrome, fragile X-associated tremor/ataxia syndrome and fragile X-associated primary ovarian insufficiency. European Journal Of Human Genetics : Ejhg. 2011 19