Molecular Test for Fragile X Intellectual Disability Syndrome

The UNC Hospitals Molecular Genetics Laboratory offers testing of the *FMR1* gene responsible for Fragile X syndrome of intellectual disability. The specific defect is an expansion of CGG trinucleotide repeats leading to inactivation of the gene.

Biology of the Disease: Fragile X Syndrome (FraX) is the most common form of inherited intellectual disability. It is characterized by varying degrees of intellectual disabilities, dysmorphic facial features, behavioral disturbances, developmental delay, autism, and macroorchidism in males. Females tend to be less severely affected. The *FMR-1* gene is localized to the long arm of the X chromosome (Xq27.3). A variable number of trinucleotide repeats, (CGG)n, is located 5' of the *FMR-1* coding sequence, and thenumber of these CGG repeats is increased in carriers of FraX and even more so in those affected with the disorder. While normal individuals have 5 to about 44 CGG repeats, carriers of the *FMR-1* "premutation" have about 59-200 repeats, and affectedpersons with the "full mutation" have more than 200 repeats. Expansion of the CGG sequence to greater than 200 repeats causes aberrant methylation of the CpG sites within the repeat as well as CpG sites within the *FMR-1* promotor. This results in transcriptional silencing of *FMR-1* and manifestations of FraX. Women whose genotype lies in the grey zone from 45 to 58 repeats are unlikely to have a child affected by FraX although variable repeat numbers and disease-causing expansions have sometimes been observed among family members. The number of AGG interruptions can improve expansion risk estimates for carriers of grey zone and premutation alleles.

Clinical Indications for Testing: Candidates for testing include individuals with unexplained intellectual disability, developmental delay, autism, post-pubertal macroorchidism in males, a family history of fragile X syndrome, or the fetus of a carrier mother. Genetic counseling is recommended.

Laboratory Testing for the Fragile X mutation: The preferred sample is ACD or EDTA anticoagulated blood (pale yellow top or lavender top, 7ml) which may be refrigerated up to 48 hours prior to testing. Triplet-primed PCR using gene specific primers that flank the *FMR1* CGG repeat region and a $(CGG)_5$ primer that anneals withinthe CGG repeat region are used to amplify CGG repeats of all sizes. The resulting PCR products are analyzed and sized by capillary gel electrophoresis. Methylation status for premutations greater than 100 CGG repeats, and full mutations (>200 CGG repeats) is determined by a *FMR1* mPCR assay. Carriers of grey zone and premutation sized alleles of 45-90 are reflexed to the Asuragen Xpansion Interpreter (XI) service via referral testing for determination of AGG interruptions (https://asuragen.com/portfolio/genetics/xpansion-interpreter/).

References:

1. Gene Reviews: https://www.ncbi.nlm.nih.gov/books/NBK1384/
2. The National Fragile X Foundation: http://www.fragilex.org

Questions?

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