

Genetic Testing for *FMR1* Mutations

Policy Number: AHS – M2028 – Genetic Testing for <i>FMR1</i> Mutations	Prior Policy Name and Number, as applicable:
Policy Revision Date: 12/08/2021	

I. Policy Description

Fragile X syndrome (FXS) is an X-linked disorder resulting from a loss of function mutation of the Fragile X Mental Retardation-1 (*FMR1*) gene (Saul & Tarleton, 1993); FXS is the most common cause of heritable intellectual disability (Coffee et al., 2009). *FMR1*-related disorders include FXS, fragile X-associated tremor/ataxia syndrome (FXTAS), and *FMR1*-related primary ovarian insufficiency (FRPOI). FXS results in a range of physical, cognitive, and behavioral effects of variable severity (Mila, Rodriguez-Reventa, & Matilla-Duenas, 2016), generally characterized by moderate intellectual disability and autistic characteristics in affected males and mild intellectual disability and emotional and/or psychiatric problems in affected females (Mila et al., 2016; Monaghan, Lyon, Spector, & American College of Medical, 2013).

II. Related Policies

Policy Number	Policy Title
AHS-G2035	Prenatal Screening
AHS-M2145	General Genetic Testing, Germline Disorders
AHS-M2146	General Genetic Testing, Somatic Disorders
AHS-M2167	Genetic Testing For Neurodegenerative Disorders
AHS-M2176	Testing for Autism Spectrum Disorders and Developmental Delay

III. Indications and/or Limitations of Coverage

Application of coverage criteria is dependent upon an individual’s benefit coverage at the time of the request. If there is a conflict between this Policy and any relevant, applicable government policy [e.g. Local Coverage Determinations (LCDs) or National Coverage Determinations (NCDs) for Medicare] for a particular member, then the government policy will be used to make the determination. For the most up-to-date Medicare policies and coverage, please visit their search website <http://www.cms.gov/medicare-coverage-database/overview-and-quick-search.aspx> or [the manual website](#)

1. Pre- and post-test genetic counseling **MEETS COVERAGE CRITERIA** for individuals undergoing fragile X mental retardation-1 (*FMR1*) gene testing.

2. Diagnostic genetic testing for *FMR1* gene CGG repeats and methylation status **MEETS COVERAGE CRITERIA** for:
 - a. Males and females with unexplained mental retardation, developmental delay, or autism spectrum disorder, *OR*
 - b. Symptomatic individuals with features of Fragile X syndrome or a family history of Fragile X syndrome, *OR*
 - c. Females with unexplained ovarian insufficiency, unexplained ovarian failure, or unexplained elevated follicle stimulating hormone (FSH) prior to age 40, *OR*
 - d. Individuals with unexplained late-onset tremor-ataxia, *OR*
 - e. Fetuses of known *FMR1* premutation or full mutation carriers
3. Carrier screening for *FMR1* gene CGG repeat length **MEETS COVERAGE CRITERIA** for individuals seeking pre-conception or prenatal care when:
 - a. There is a family history of Fragile X syndrome, *OR*
 - b. There is a family history of unexplained mental retardation, developmental delay, or autism spectrum disorder, *OR*
 - c. There is a family history of unexplained late-onset tremor ataxia, *OR*
 - d. There is a family history of unexplained premature ovarian insufficiency or failure.
4. Determination of *FMR1* gene point mutations **DOES NOT MEET COVERAGE CRITERIA.**
5. Determination of *FMR1* gene deletion **DOES NOT MEET COVERAGE CRITERIA.**
6. Population screening for Fragile X syndrome **DOES NOT MEET COVERAGE CRITERIA.**
7. Cytogenetic testing for Fragile X syndrome **DOES NOT MEET COVERAGE CRITERIA.**
8. Testing for the protein encoded by *FMR1* (FMRP) protein **DOES NOT MEET COVERAGE CRITERIA.**

IV. Scientific Background

Fragile X Syndrome (FXS) and related disorders affects about 1 in 4,000 males and 1 in 6,000 to 8,000 females in the USA (NORD, 2017). Transmitted as an X-linked dominant trait with reduced penetrance, FXS is associated with a fragile site on the X chromosome (Yu et al., 1991) identified as the Fragile X Mental Retardation-1 (*FMR1*) gene (Santoro, Bray, & Warren, 2012). More than 99% of patients with FXS have a mutation in this gene with over 200 CGG repeats and atypical methylation (NORD, 2017). The protein encoded by *FMR1* (FMRP) is a multifunctional RNA-binding protein that regulates the translation of a subset of dendritic mRNAs and plays a central role in neuronal development and synaptic plasticity (Antar, Li, Zhang, Carroll, & Bassell, 2006; Ascano et al., 2012; Bechara et al., 2009; Castagnola et al., 2018; Didiot et al., 2008; Kenny et al., 2014; Parvin et al., 2018; Yang et al., 2018).

The absence of FMRP results in excessive and persistent mGluR-mediated protein synthesis in postsynaptic dendrites, dysregulation of ion homeostasis, and disruption of calcium ion homeostasis leading to abnormal synaptic signaling and dendritic development (Bear, Huber, & Warren, 2004; Castagnola et al., 2018; Finucane et al., 2012). The typical clinical phenotype includes intellectual disability, social impairment, autism spectrum disorder, speech and language delay, neurological dysfunction (seizures and abnormal sleep patterns), sensory hypersensitivity (Rais, Binder, Razak, & Ethell, 2018), and a characteristic physical appearance that typically develops in the second decade of life (Hersh & Saul, 2011). Autism disorders are seen in approximately one third of FXS patients, affecting males more frequently than females (Ormazabal, Solari, Espeche, Castro, & Buzzalino, 2019). 55-90% of patients with autism and FXS report gaze aversion, hand flapping, repetitive behaviors, reduced social interaction, anxiety, speech preservations, and aggressive behaviors (Reisinger, Shaffer, Tartaglia, Berry-Kravis, & Erickson, 2020).

Any genetic alteration that results in a lack of functional FMRP can cause FXS symptoms. The most common type of mutation of *FMR1* is the expansion of a CGG trinucleotide repeat in the 5' untranslated region of the gene (Jin & Warren, 2000). Normally, this ranges in size from 7 to ~60 repeats, with 30 being most common (Peprah, 2012). The full mutation consists of expansions of over 200 repeats which become abnormally hypermethylated, silencing the *FMR1* gene and expression of FMRP (Maurin, Zongaro, & Bardoni, 2014; Oberle et al., 1991). Molecular clinical correlations have shown that the resulting phenotype is related to the degree of methylation and mosaicism rather than the number of repeats (Hersh & Saul, 2011).

Alleles with 55 to 200 CGG repeats are generally unmethylated with normal transcript and FMRP level; however, they are extremely unstable during transmission to the next generation and are referred to as premutations (Zafarullah et al., 2020). Although premutation carriers produce normal levels of FMRP, mRNA levels are elevated, causing toxic effects such as protein sequestration and mitochondrial dysfunction (Garcia-Arocena & Hagerman, 2010; Tassone et al., 2000). As a consequence, RNA toxicity leads to neuronal toxicity and a spectrum of pre-mutation associated disorders such as primary ovarian insufficiency (FXPOI) (Rosario & Anderson, 2020) and tremor ataxia syndrome (FXTAS) (Zafarullah et al., 2020). An increased frequency of neurological, psychological, endocrine, and immune-related characteristics has been documented in premutation carriers (Hagerman & Hagerman, 2013; Raspa et al., 2018). Those with the premutation have higher rates of anxiety, depression, autistic traits, and physical health symptoms such as chronic fatigue and pain, fibromyalgia, and sleep disorders (Johnson, Herring, & Richstein, 2020).

Analytical Validity

While many fragile X testing methods have been developed, no single approach can characterize all aspects of *FMR1* mutations and expansions, especially when mosaicism is taken into consideration (Monaghan et al., 2013). In a diagnostic setting, it is important to not only detect presence of the CGG expansion, but to also determine its size and methylation status (Lim et al., 2017). Molecular diagnostic testing of *FMR1* currently relies on a combination of polymerase chain reaction (PCR) and Southern blot (the gold standard) for the CGG-repeat expansion and methylation analyses (Cai, Arif, Wan, Kornreich, & Edelmann, 2019; Rajan-Babu & Chong, 2016). Detection of rare point mutations and deletions requires sequence analysis (Sitzmann, Hagelstrom, Tassone, Hagerman, & Butler, 2018; Suhl & Warren, 2015). This has limited the ability to implement any type of population screening (Riley & Wheeler, 2017).

CGG repeat-primed PCR designed to detect the full range of fragile X expanded alleles followed by analysis via capillary electrophoresis (Chen et al., 2010; Lyon et al., 2010) and melt curve techniques (Rajan-Babu et al., 2015; Teo, Law, Lee, & Chong, 2012) minimizes the need for southern blot analysis. The FastFraX *FMR1* test was recently evaluated in 198 archived clinical samples, yielding results of 100% sensitivity (95% CI, 91.03% to 100%) and 100% specificity (95% CI, 97.64% to 100%) in categorizing patient samples into the respective normal, intermediate, premutation, and full mutation genotypes (Lim et al., 2017).

The triplet-primed PCR method (dTP-PCR) has been validated by comparison to southern blot analysis for use in determining mutations in the *FMR1* gene; clinical performance was confirmed with 40 samples resulting in 100% sensitivity and 90.48% specificity in the detection of CGG repeats greater than 30 (Skrlec, Barisic, & Wagner, 2018). This testing method may be utilized to screen a general population by quickly determining specific allelic changes in the *FMR1* gene (Skrlec et al., 2018).

Immunohistochemical detection of FMRP has been validated in lymphocytes and chorionic villi samples as an alternative prenatal diagnostic method for detection of full mutations in male fetuses; however, staining is more complex in female fetuses due to X-inactivation and is insufficient for diagnostic use (Oostra & Willemsen, 2001; Willemsen, Bontekoe, Severijnen, & Oostra, 2002). Clinical and analytical specificity and sensitivity of cytogenetic analysis for FXS are both insufficient (Monaghan et al., 2013).

In a retrospective design, Ramos, Ocampos, Barbato, Graça Bicalho, and Nisihara (2020) studied the performance of the commercial FragileEase PCR kit for FXS diagnosis and compared it to Southern blotting (SB), PCR, and AmpliX *FMR1* PCR. 90 DNA samples were analyzed using FragileEase from patients with a clinical suspicion of FXS or a family history and was compared with the results from the other methods. Overall, FragileEase PCR kit had high concordance with the results from PCR, SB, and AmpliX. FragileEase was able to detect normal, intermediate/gray zone, premutation, and full mutation alleles along with female homozygosity and mosaicism. The authors conclude that "FragileEase™ PCR, as well as other commercially available kits, efficiently detect *FMR1* mutations and simplify the workflow in laboratories that performing FXS diagnoses (Ramos et al., 2020)."

Clinical Validity and Utility

As the clinical phenotype of FMR-related diseases can be subtle, its detection, especially in the prepubertal period, can be difficult. Although phenotypic symptoms are not obvious at birth, both animal and neuroimaging studies suggest that the effects of FXS begin in the prenatal period (Riley & Wheeler, 2017). Families report significant delays in diagnosis of FXS with 24% of families reporting that they had seen a healthcare provider more than 10 times before testing. On average, caregivers or other individuals first report concern in regards to the child's development by 13 months; however, professional confirmation of a developmental delay did not occur until an average age of 21 months, and the FXS diagnosis did not occur until an average age of nearly 32 months. Meanwhile, many families had additional children with FXS before becoming aware of the reproductive risk (Bailey, Skinner, & Sparkman, 2003). Establishing a diagnosis of FXS allows for an understanding of the disorder and education on appropriate management strategies. Psychopharmacologic intervention to modify behavioral problems, such as attentional deficits, impulse control, anxiety and emotional lability in a child with FXS can be important in addition to speech therapy, occupational therapy, special educational services, and behavioral interventions (Hersh & Saul, 2011). A recent pilot of allopregnanolone in six males with FXTAS showed significant improvement in GABA metabolism, oxidative stress, and some of the mitochondria-related outcomes (Napoli et al., 2018).

Huang et al. (2019) utilized a GC-rich PCR method to detect *FMR1* gene mutations in 30 pregnant woman who were known carriers of *FMR1* mutations or who contained *FMR1* gene deletion mosaicism; samples utilized chorionic villus, amniotic fluid, or umbilical blood samples. Southern blotting was used as a confirmatory measure. PCR results showed that 18 fetuses were normal, while others presented with full *FMR1* gene mutations, premutations, and/or mosaicism. Even with successful results, the authors state that the use of a single detection method may not be sufficient in determining *FMR1* genetic mutations (Huang et al., 2019).

Lee et al. (2020) utilized a customized PCR and software system to detect the *FMR1* gene expansions from dried blood spots (DBS) and performed analytical validity studies to determine its accuracy, specificity, sensitivity, and precision to be used for newborn screening. 963 newborn dried blood spots were studied by DNA extraction, *FMR1* PCR amplification, and capillary electrophoresis for automated CGG repeat analysis. While previous *FMR1* newborn screening assays were unsuitable for a routine laboratory setting, this fit-for-purpose *FMR1* screening method provides a reliable method for newborn screening that is both cost-effective and compatible with simple DBS elution methods already used in newborn screening laboratories. From the 963 DBS samples tested, 957 samples (99.4%) samples were classified as normal and 6 samples (0.6%) had premutation alleles with 55-76 CGG repeat expansions. Five out of the six premutation samples had one normal allele in addition to the premutation allele, while one out of the six had only one allele. Accuracy testing results were 100% concordance with reference genotypes with no false positive or false negative test results found. CGG expansions were consistently within six CGG repeats for larger expansions up to 200, within three CGG repeats for expansions up to 137, and within a single repeat for CGG expansions less than 80. However, the authors wrote that "further studies are required to identify if early screening of Fragile X syndrome would lead to better outcomes for the children, families, and society" (Lee et al., 2020).

Approximately twenty individuals have been reported with rare missense or nonsense mutations in the *FMR1* gene; also reported were other coding disturbances of the same gene resulting in physical, cognitive, and behavioral features similarly seen in FXS (Sitzmann et al., 2018). Recent studies of other *FMR1* mutations that can affect the level and function of the protein include analysis of SNPs showing that 31.66 % of the *FMR1* gene SNPs were disease-related and that 50 % of SNPs from online databases had a pathogenic effect (Tekcan, 2016). Screening of 508 males with clinical signs of mental retardation and developmental delay, but without CGG and GCC repeat expansions in the *FMR1* gene, revealed two missense mutations in the *FMR1* gene that would have not been diagnosed with standard molecular testing for FXS (Handt et al., 2014).

Cao et al. (2021) studied the clinical utility of screening *FMR1* gene mutations during early and middle pregnancy for those carrying high-risk CGG trinucleotide expansions. DNA samples from 316 pregnant women at 12-21 gestational weeks were collected and analyzed for CGG repeats using fluorescence PCR and capillary electrophoresis. The carrier rate of CGG repeats was 1 in 178 for the intermediate type and 1 in 772 for the premutation types. The highest frequency allele of CGG was 29 repeats, which accounted for 49.29%, followed by 30 repeats (28.56%) and 36 repeats (8.83%). In one case of a premutation type of CGG expansion, the couple chose to terminate the pregnancy. The authors conclude that "pregnant women should be screened for *FMR1* gene mutations during early and middle pregnancy, and those with high-risk CGG expansions should undergo prenatal diagnosis, genetic counseling and family study (Cao et al., 2021)."

V. Guidelines and Recommendations

American College of Medical Genetics and Genomics (ACMG) (Kronquist, Sherman, & Spector, 2008; Monaghan et al., 2013; Sherman, Pletcher, & Driscoll, 2005)

The American College of Medical Genetics and Genomics (ACMG) recommends FXS molecular genetic testing for:

“Fragile X syndrome:

- Individuals of either sex with mental retardation, developmental delay, or autism, especially if they have (a) any physical or behavioral characteristics of fragile X syndrome, (b) a family history of fragile X syndrome, or (c) male or female relatives with undiagnosed mental retardation.
- Individuals seeking reproductive counseling who have (a) a family history of fragile X syndrome or (b) a family history of undiagnosed mental retardation.
- Fetuses of known carrier mothers.
- Affected individuals or their relatives in the context of a positive cytogenetic fragile X test result who are seeking further counseling related to the risk of carrier status among themselves or their relatives. The cytogenetic test was used prior to the identification of the FMR1 gene and is significantly less accurate than the current DNA test. DNA testing on such individuals is warranted to accurately identify premutation carriers and to distinguish premutation from full mutation carrier women.

Ovarian dysfunction:

- Women who are experiencing reproductive or fertility problems associated with elevated follicle stimulating hormone (FSH) levels, especially if they have (a) a family history of premature ovarian failure, (b) a family history of fragile X syndrome, or (c) male or female relatives with undiagnosed mental retardation.

Tremor/ataxia syndrome:

- Men and women who are experiencing late onset intention tremor and cerebellar ataxia of unknown origin, especially if they have (a) a family history of movement disorders, (b) a family history of fragile X syndrome, or (c) male or female relatives with undiagnosed mental retardation” (Sherman et al., 2005).

ACMG does not recommend general population carrier screening.

The American College of Obstetricians and Gynecologists (ACOG, 2017)

The American College of Obstetricians and Gynecologists (ACOG) published committee opinion 691 (ACOG, 2017) which recommends Fragile X premutation carrier screening for women with a family history of fragile X-related disorders or intellectual disability suggestive of fragile X syndrome and who are considering pregnancy or are currently pregnant.

If a woman has unexplained ovarian insufficiency or failure or an elevated follicle-stimulating hormone level before age 40 years, fragile X carrier screening is recommended to determine whether she has an *FMR1* premutation.

All identified individuals with intermediate results and carriers of a fragile X premutation or full mutation should be provided follow-up genetic counseling to discuss the risk to their offspring of inheriting an expanded full-mutation fragile X allele and to discuss fragile X-associated disorders (premature ovarian insufficiency and fragile X tremor/ataxia syndrome).

Prenatal diagnostic testing for FXS should be offered to known carriers of the fragile X premutation or full mutation (ACOG, 2017).

This guideline was reaffirmed in 2020 (ACOG, 2017).

Society of Obstetricians and Gynecologists of Canada (SOGC) and Canadian College of Medical Geneticists (CCMG) Guidelines (Wilson et al., 2016)

Guidelines for FXS genetic testing were given in a joint statement from the SOGC and CCMG. It is stated that “Any woman with a personal or family history of Fragile X- or Fragile X mental retardation 1–related disorders; unexplained intellectual disability or developmental delay; autism; ovarian insufficiency with elevated follicle stimulating hormone at age < 40 years of unknown etiology; or any woman with a family history of male relatives with developmental delay, autism, or isolated cerebellar ataxia and tremor should be offered screening for this condition (II-2A) (GRADE moderate/moderate)” (Wilson et al., 2016). It is also stated that “Population carrier screening for Fragile X syndrome in all women of reproductive age cannot be recommended at this time (II-2D) (GRADE moderate/moderate)” and “Fragile X carrier testing must only occur after detailed genetic counselling and informed consent from the woman to be tested has been obtained (III-A) (GRADE low/moderate)” (Wilson et al., 2016).

National Society of Genetic Counselors (Finucane et al., 2012)

The National Society of Genetic Counselors published guidelines (Finucane et al., 2012) which recommend: “Centers offering population screening should ensure that they have the resources available to provide pre- and post-test genetic counseling that supports the psychosocial and clinical needs of the patient and family. In light of widespread *FMR1* testing among women without known risk factors, genetic counselors should anticipate seeing patients who did not receive any pre-test information, have no prior knowledge of *FMR1*-associated disorders, and are unprepared to learn that they have an *FMR1* mutation. Prenatal diagnosis should be offered to women with pre- or full mutations. Males with premutation alleles should receive genetic counseling about potential phenotypic risks to their daughters, all of whom will inherit premutations (Finucane et al., 2012).”

American Academy of Pediatrics Committee on Genetics (Hersh & Saul, 2011)

The American Academy of Pediatrics (Hersh & Saul, 2011) recommends testing for FXS in children with any of the following, particularly when associated with physical and behavioral characteristics of FXS or a relative with undiagnosed intellectual disability: developmental delay, borderline intellectual abilities or intellectual disability, or diagnosis of autism without a specific etiology.

European Molecular Genetics Quality Network (EMQN) (Biancalana, Glaeser, McQuaid, & Steinbach, 2015)

The EMQN published their best practice guidelines concerning FXS and fragile X-associated disorders in 2015 (Biancalana et al., 2015). They state, “Prenatal testing is not indicated for the pregnant partner of a male with a premutation,..” but they do recommend offering prenatal diagnosis to any woman with 55 or more CGG repeats; “Prenatal testing may be considered for a female fetus of a full mutation father as a cautionary measure (full mutation or MoMP [mosaic premutation and full mutation] or MoMe [methylation mosaic]).” Concerning molecular diagnostic analysis in FXS and fragile X-associated disorders, they state the following:

“It is best practice to use a method which detects the whole range of expansions when testing relatives (including prenatal diagnosis) in a family with any known fragile X disorder due to expansion. When testing the *FMR1* gene in population screening, the report must specify that rare cases of point mutation or deletion cannot be detected, nor rare cases of CGG expansion mosaicism (MoMN) if the method used cannot detect the whole range of expansions. It could be useful to confirm results by an independent method when detecting an expansion in an index case depending on specific pitfalls of each method (Biancalana et al., 2015).”

VI. State and Federal Regulations, as applicable

A. Food and Drug Administration (FDA)

A search of the FDA database on 10/19/2021 using the term “*FMR1*” yielded 0 results. Many labs have developed specific tests that they must validate and perform in house. These laboratory-developed tests (LDTs) are regulated by the Centers for Medicare and Medicaid (CMS) as high-complexity tests under the Clinical Laboratory Improvement Amendments of 1988 (CLIA '88). As an LDT, the U. S. Food and Drug Administration has not approved or cleared this test; however, FDA clearance or approval is not currently required for clinical use.

B. Centers for Medicare & Medicaid Services (CMS)

A53638 Billing and Coding: MoIDX: Fragile X - <https://www.cms.gov/medicare-coverage-database/view/article.aspx?articleid=53638&ver=19&bc=0>

VII. Applicable CPT/HCPCS Procedure Codes

CPT	Code Description
81243	FMR1 (fragile X mental retardation 1) (eg, fragile X mental retardation) gene analysis; evaluation to detect abnormal (eg, expanded) alleles
81244	FMR1 (fragile X mental retardation 1) (eg, fragile X mental retardation) gene analysis; characterization of alleles (eg, expanded size and promoter methylation status)
88248	Chromosome analysis for breakage syndromes; baseline breakage, score 50-100 cells, count 20 cells, 2 karyotypes (eg, for ataxia telangiectasia, Fanconi anemia, fragile X)
96040	Medical genetics and genetic counseling services, each 30 minutes face-to-face with patient/family
S0265	Genetic counseling, under physician supervision, each 15 minutes

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Procedure codes appearing in Medical Policy documents are included only as a general reference tool for each policy. They may not be all-inclusive.

VIII. Evidence-based Scientific References

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