



Diagnostic Genetic Testing for FMR1 and AFF2 Variants (Including Fragile X and Fragile XE Syndromes)

Effective: June 1, 2025

Next Review: February 2026

Last Review: April 2025

IMPORTANT REMINDER

Medical Policies are developed to provide guidance for members and providers regarding coverage in accordance with contract terms. Benefit determinations are based in all cases on the applicable contract language. To the extent there may be any conflict between the Medical Policy and contract language, the contract language takes precedence.

PLEASE NOTE: Contracts exclude from coverage, among other things, services or procedures that are considered investigational or cosmetic. Providers may bill members for services or procedures that are considered investigational or cosmetic. Providers are encouraged to inform members before rendering such services that the members are likely to be financially responsible for the cost of these services.

DESCRIPTION

Fragile X syndrome (FXS), caused by expansion of the *FMR1* gene, is characterized by intellectual disability. FXS is also associated with certain physical and behavioral characteristics, including typical facial features, connective tissue anomalies, autism spectrum disorder, and seizures. Fragile XE (FRAXE) syndrome is caused by expansion of the *AFF2* gene (also known as *FMR2*) and is associated with mild intellectual disability without consistent physical features.

MEDICAL POLICY CRITERIA

Note: This policy applies to diagnostic testing only. Reproductive carrier screening is addressed separately (see Cross References).

- I. Diagnostic genetic testing for *FMR1* variants may be considered **medically necessary** when one or more of the following criteria are met:
 - A. Individuals with intellectual disability, developmental delay, or autism spectrum disorder.
 - B. Individuals diagnosed with primary ovarian insufficiency before the age of 40.

- C. Prenatal testing of fetuses of known carrier mothers.
- D. Individuals with neurologic symptoms consistent with fragile X syndrome, including but not limited to ataxia and intention tremor.
- II. Diagnostic genetic testing for *FMR1* variants is considered **not medically necessary** in all other circumstances, including but not limited to children with isolated attention-deficit/hyperactivity.
- III. Genetic testing for *AFF2* (*FMR2*) variants is considered **investigational** for fragile XE (FRAXE) syndrome.

NOTE: A summary of the supporting rationale for the policy criteria is at the end of the policy.

LIST OF INFORMATION NEEDED FOR REVIEW

In order to determine the clinical utility of gene test(s), all of the following information must be submitted for review. If any of these items are not submitted, it could impact our review and decision outcome:

1. Name of the genetic test(s) or panel test
2. Name of the performing laboratory and/or genetic testing organization (more than one may be listed)
3. The exact gene(s) and/or variant(s) being tested
4. Relevant billing codes
5. Brief description of how the genetic test results will guide clinical decisions that would not otherwise be made in the absence testing
6. Medical records related to this genetic test:
 - History and physical exam including any relevant diagnoses related to the genetic testing
 - Conventional testing and outcomes
 - Conservative treatments, if any

CROSS REFERENCES

1. [Chromosomal Microarray Analysis \(CMA\) or Copy Number Analysis for the Genetic Evaluation of Patients with Developmental Delay, Intellectual Disability, Autism Spectrum Disorder, or Congenital Anomalies](#), Genetic Testing, Policy No. 58
2. [Reproductive Carrier Screening for Genetic Diseases](#), Genetic Testing, Policy No. 81

BACKGROUND

Human Genome Variation Society (HGVS) nomenclature^[1] is used to describe variants found in DNA and serves as an international standard. It is being implemented for genetic testing medical evidence review updates starting in 2017. According to this nomenclature, the term “variant” is used to describe a change in a DNA or protein sequence, replacing previously-used terms, such as “mutation.” Pathogenic variants are variants associated with disease, while benign variants are not. The majority of genetic changes have unknown effects on human health, and these are referred to as variants of uncertain significance.

Fragile X Syndrome

Fragile X syndrome (FXS) is the most common cause of heritable intellectual disability, characterized by mild to moderate intellectual disability. In addition to the intellectual impairment, patients present with typical facial characteristics such as an elongated face with a prominent forehead, protruding jaw, and large ears. Connective tissue anomalies include hyperextensible finger and thumb joints, hand calluses, velvet-like skin, flat feet, and mitral valve prolapse. The characteristic appearance of adult males includes macroorchidism. Patients may show behavioral problems including autism spectrum disorders, sleeping problems, social anxiety, poor eye contact, mood disorders and hand-flapping or biting. Another prominent feature of the disorder is neuronal hyperexcitability manifested by hyperactivity, increased sensitivity to sensory stimuli, and a high incidence of epileptic seizures.

Current approaches to therapy are supportive and symptom-based. Psychopharmacologic intervention to modify behavioral problems in a child with fragile X syndrome may represent an important adjunctive therapy when combined with other supportive strategies including speech therapy, occupational therapy, special educational services, and behavioral interventions. Medication management may be indicated to modify attention deficits, problems with impulse control, and hyperactivity. Anxiety-related symptoms, including obsessive compulsive tendencies with perseverative behaviors, also may be present and require medical intervention. Emotional lability and episodes of aggression and self-injury may be a danger to the child and others around him or her; therefore, the use of medication(s) to modify these symptoms also may significantly improve an affected child's ability to participate more successfully in activities in home and school settings.

DNA studies are used to test for fragile X syndrome (FXS). Genotypes of individuals with symptoms of FXS and individuals at risk for carrying the pathogenic variant can be determined by examining the size of the CGG trinucleotide repeat segment and the methylation status of the *FMR1* gene on the X chromosome. There are no known forms of fragile X mental retardation protein (FMRP) deficiency that do not map to the *FMR1* gene. Two main testing approaches are used: polymerase chain reaction (PCR) and Southern blot analysis. In fragile X testing, the high fraction of GC bases in the repeat region makes it extremely difficult for standard PCR techniques to amplify beyond about 100-150 CGG. As a result, Southern blot analysis is commonly used to determine the number of triplet repeats in FXS and methylation status.

CGG-repeat expansion full mutations account for more than 99% of cases of fragile X syndrome (FXS). Therefore, tests that effectively detect and measure the CGG repeat region of the *FMR1* gene are more than 99% sensitive. Positive results are 100% specific. The patient is classified as normal, intermediate (or "gray zone"), premutation, or full mutation based on the number of CGG repeats.

- Full mutation: >200-230 CGG repeats (methylated)

Patients with a full mutation are associated with FXS, which is caused by expansion of the *FMR1* gene CGG triplet repeat above 200 units in the untranslated region of *FMR1*, leading to a hypermethylation of the promoter region followed by transcriptional inactivation of the gene. The FXS is caused by a loss of the fragile X mental retardation protein (FMRP). Approximately 1% to 3% of children ascertained on the basis of autism diagnosis are shown to have fragile X syndrome.

Full mutations are typically maternally transmitted. The mother of a child with an *FMR1* mutation is almost always a carrier of a premutation or full mutation. Men who are premutation carriers are referred to as transmitting males. All of their daughters will inherit a premutation, but their sons will not inherit the premutation. Males with a full mutation usually have intellectual disability and decreased fertility.

- Premutation: 55-200 CGG repeats (unmethylated)

Patients with a premutation are carriers and are at small risk for developing a *FMR1*-related disorder, fragile X-associated tremor/ataxia syndrome (FXTAS). This disorder is a late onset, progressive development of intention tremor and ataxia often accompanied by progressive cognitive and behavioral difficulties including memory loss, anxiety, reclusive behavior, deficits of executive function and dementia, or premature ovarian insufficiency (FXPOI).

Premutation alleles in females are unstable and may expand to full mutations in offspring. Premutations of less than 59 repeats have not been reported to expand to a full mutation in a single generation. Premutation alleles in males may expand or contract by several repeats with transmission; however, expansion to full mutations has not been reported. A considerable number of children being evaluated for autism have been found to have *FMR1* premutations (55-200 CGG repeats).^[2]

- Intermediate: 45-54 CGG repeats (unmethylated)
- Normal: 5-44 CGG repeats (unmethylated)

Fragile XE Syndrome

Fragile XE syndrome (FRAXE) is much rarer than FXS, and affects an estimated 1 on 25,000 to 100,000 males.^[3] This disorder is characterized by mild intellectual disability, though some affected individuals may have borderline cognitive function that is not severe enough to be classified as a disability.

Similar to FXS, FRAXE is caused by a trinucleotide repeat expansion – nearly all cases are due to the presence of more than 200 repeats of CCG in the *AFF2* gene (sometimes referred to as *FMR2*). Individuals with 50 to 200 CCG repeats are said to have a premutation, which is not associated with impaired cognition.

Regulatory Status

No FDA-cleared genotyping tests were found. Thus, genotyping is offered as a laboratory-developed test. Clinical laboratories may develop and validate tests in-house and market them as a laboratory service. Such tests must meet the general regulatory standards of the Clinical Laboratory Improvement Act (CLIA). The laboratory offering the service must be licensed by CLIA for high-complexity testing.

Asuragen offers the Xpansion Interpreter™ test which analyzes AGG sequences that interrupt the CGG repeats which have been suggested to stabilize alleles and protect against expansion in subsequent generations.

Note: An additional test for developmental delays, Lineagen FirstStepDxPLUS, offers sequencing of *FMR1* in combination with a chromosomal microarray genetic test. When *FMR1*

analysis is bundled with CMA analysis or any other genetic test, additional plan medical policies may apply. For the plan's medical policy on CMA analysis, see Cross References in the section above.

EVIDENCE SUMMARY

The focus of this review is on evidence related to the clinical utility of the testing, which is the ability of test results to:

- Guide decisions in the clinical setting related to either treatment, management, or prevention, and
- Improve health outcomes as a result of those decisions.

FMR1

The conditions caused by abnormal CGG repeats in the *FMR1* gene, FXS, FXTAS, and FXPOI, do not have specific treatments that alter the natural history of the disorders. However, because they represent relatively common causes of conditions that are often difficult to diagnose and involve numerous diagnostic tests, the capability of *FMR1* testing to obtain an accurate definitive diagnosis and avoid additional diagnostic testing supports its clinical utility. Knowledge that the condition is caused by fragile X provides important knowledge to offspring and the risk of disease in subsequent generations.

Since there is no specific treatment for FXS, a definitive diagnosis will not lead to treatment that alters the natural history of the disorder. However, there are several potential ways in which adjunctive management might be changed following genetic testing after confirmation of the diagnosis.^[4 5] Although not related specifically to *FMR1* testing, the American Academy of Pediatrics (AAP) and the American Academy of Neurology (AAN)/Child Neurology Society (CNS) guidelines, described in more detail below, noted the following more immediate and general clinical benefits of achieving a specific genetic diagnosis:

- limit additional diagnostic testing;
- anticipate and manage associated medical and behavioral comorbidities;
- improve understanding of treatment and prognosis;
- allow counseling regarding risk of recurrence in future offspring and help with reproductive planning;
- early diagnosis and intervention in an attempt to ameliorate or improve behavioral and cognitive outcomes over time.

In a 2012 review by Abrams, the importance of early diagnostic and management issues, in conjunction with the identification of family members at risk for or affected by *FMR1* variants is discussed.^[6] The expanded CGG repeat in the *FMR1* gene, once thought to have clinical significance limited to fragile X syndrome, is now well established as the cause for other fragile X-associated disorders including fragile X-associated primary ovarian insufficiency and fragile X-associated tremor ataxia syndrome in individuals with the premutation (carriers).

Also, FXS is associated with a number of medical and behavioral comorbidities.^[7] Behavioral comorbidities may include attention problems, hyperactivity, anxiety, aggression, poor sleep, and self-injury. Individuals with FXS are also prone to seizures, recurrent otitis media, strabismus, gastrointestinal disturbances, and connective tissue problems. A correct diagnosis can lead to the appropriate identification and treatment of these comorbidities.

Hersh (2011) reported on families with an affected male and whether an early diagnosis would have influenced their reproductive decision making.^[4] After a diagnosis in the affected male was made, 73% of families reported that the diagnosis of FXS affected their decision to have another child, and 43% of the families surveyed had had a second child with a full mutation.

The feasibility of newborn screening is being investigated.^[8] However, there is currently no treatment for FXS that would reduce mortality or morbidity if given in infancy. Also, there are a number of ethical concerns with newborn screening for FXS, including the need for informed consent from both parents, the need for genetic counseling for both full mutation and premutation status, and the detection of carriers in infants.^[9]

AFF2

As with FXS, there are no specific treatments available for people diagnosed with FRAXE. In addition, FRAXE is a far less common disorder with a variable presentation ranging from relatively normal cognition to mild intellectual disability. There is limited evidence regarding the clinical utility of testing for *AFF2*. Several studies have screened for FRAXE in populations with intellectual disability^[10-13], but only one identified a patient with this disorder.^[14]

PRACTICE GUIDELINE SUMMARY

THE AMERICAN COLLEGE OF MEDICAL GENETICS

The purpose of the following American College of Medical Genetics (ACMG) guideline^[15] recommendations is to provide aid to clinicians in making referrals for testing the repeat region of the *FMR1* gene:

- Individuals of either sex with intellectual disability, 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 intellectual disability
- Individuals seeking reproductive counseling who have (a) a family history of fragile X syndrome or (b) a family history of undiagnosed intellectual disability
- 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.

In the clinical genetics evaluation in identifying the etiology of autism spectrum disorders, the ACMG recommends testing for FXS as part of first tier testing.^[16]

In 2021, the ACMG released a revised technical standard on laboratory testing for fragile X.^[17] The authors noted that the new laboratory standards "are in general agreement" with the 2005 ACMG policy statement summarized above.

THE AMERICAN ACADEMY OF PEDIATRICS

In 2011, the American Academy of Pediatrics (AAP) published consensus guidelines which suggested that, because children with FXS may not have apparent physical features, any child who presents with developmental delay, borderline intellectual abilities or intellectual disability, or has a diagnosis of autism without a specific etiology should undergo molecular testing for FXS to determine the number of CGG repeats.^[4]

In 2014, the AAP updated their consensus guidelines which recommend Fragile X testing in patients with global developmental delay (GDD) or intellectual disability (ID).^[18] Specifically, the AAP guideline recommended, “fragile X testing should be performed in all boys and girls with GDD/ID of unknown cause. Of boys with GDD/ID of uncertain cause, 2% to 3% will have fragile X syndrome (full mutation of *FMR1*, >200 CGG repeats), as will 1% to 2% of girls (full mutation).”

THE AMERICAN COLLEGE OF OBSTETRICIANS AND GYNECOLOGISTS

The 2017 (reaffirmed in 2023) American College of Obstetricians and Gynecologists (ACOG) committee opinion recommended prenatal testing for fragile X syndrome for known carriers of the fragile X premutation or full mutation and for women with a family history of fragile X-related disorders or intellectual disability suggestive of fragile X syndrome.^[19] They additionally recommended *FMR1* premutation testing for women younger than 40 with unexplained ovarian insufficiency or failure, or an elevated follicle-stimulating hormone level.

SUMMARY

There is enough research to show that testing the *FMR1* gene can improve the diagnostic process for individuals with fragile X-related symptoms and help in informed reproductive decision making. Also, clinical guidelines based on research from several U.S. professional associations recommend this testing for certain people. Therefore, genetic testing for *FMR1* may be considered medically necessary for patients when criteria are met.

For all other situations, *FRM1* gene testing provides no benefit in directing medical management and is therefore considered not medically necessary.

There is not enough research to show that testing for *AFF2* (*FMR2*) variants can help improve health outcomes for patients or inform reproductive decision making. In addition, there are no clinical guidelines based on research that recommend *AFF2* testing. Therefore, genetic testing for *AFF2* is considered investigational.

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CODES

Codes	Number	Description
CPT	81171	AFF2 (ALF transcription elongation factor 2 [FMR2]) (eg, fragile X intellectual disability 2 [FRAXE]) gene analysis; evaluation to detect abnormal (eg, expanded) alleles
	81172	AFF2 (ALF transcription elongation factor 2 [FMR2]) (eg, fragile X intellectual disability 2 [FRAXE]) gene analysis; characterization of alleles (eg, expanded size and methylation status)
	81243	FMR1 (fragile X messenger ribonucleoprotein 1) (eg, fragile X syndrome, X-linked intellectual disability [XLID]) gene analysis; evaluation to detect abnormal (eg, expanded) alleles
	81244	FMR1 (fragile X messenger ribonucleoprotein 1) (eg, fragile X syndrome, X-linked intellectual disability [XLID]) gene analysis; characterization of alleles (eg, expanded size and promoter methylation status)
HCPCS	None	

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