Genetic Testing for FMR1 Mutations (Including Fragile X Syndrome)

Policy # 00380
Original Effective Date: 08/21/2013
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Applies to all products administered or underwritten by Blue Cross and Blue Shield of Louisiana and its subsidiary, HMO Louisiana, Inc. (collectively referred to as the “Company”), unless otherwise provided in the applicable contract. Medical technology is constantly evolving, and we reserve the right to review and update Medical Policy periodically.

When Services May Be Eligible for Coverage
Coverage for eligible medical treatments or procedures, drugs, devices or biological products may be provided only if:

- Benefits are available in the member’s contract/certificate, and
- Medical necessity criteria and guidelines are met.

Based on review of available data, the Company may consider genetic testing for fragile X mental retardation 1 (FMR1) mutations to be eligible for coverage.

Patient Selection Criteria
Coverage eligibility will be considered for fragile X mental retardation 1 (FMR1) mutations the following patient populations:

- Individuals of either sex with intellectual disability, developmental delay, or autism spectrum disorder (see Policy Guidelines◊).
- Prenatal testing of fetuses of known carrier mothers (see Policy Guidelines◊).
- Affected individuals or relatives of affected individuals who have had a positive cytogenetic fragile X test result (see Policy Guidelines◊◊).
- Women with primary ovarian failure under the age of 40 in whom fragile X associated ovarian failure is suspected.
- Individuals with neurologic symptoms consistent with fragile X associated tremor/ataxia syndrome.

When Services Are Considered Investigational
Coverage is not available for investigational medical treatments or procedures, drugs, devices or biological products.

The use of genetic testing for fragile X mental retardation 1 (FMR1) mutations when patient selection criteria are not met or for all other uses is considered to be investigational.*

Policy Guidelines
According to the American College of Medical Genetics (ACMG), the following is the preferred approach to testing:

- Deoxyribonucleic acid (DNA) analysis is the method of choice if one is testing specifically for FXS and associated trinucleotide repeat expansion in the FMR1 gene.
- *For isolated cognitive impairment, DNA analysis for FXS should be performed as part of a comprehensive genetic evaluation that includes routine cytogenetic evaluation. Cytogenetic studies

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are critical, since constitutional chromosome abnormalities have been identified as frequently or more frequently than fragile X mutations in mentally retarded individuals referred for fragile X testing.

- Fragile X testing is not routinely warranted for children with isolated attention-deficit/hyperactivity disorder.
- For individuals who are at risk due to an established family history of FXS, DNA testing alone is sufficient. If the diagnosis of the affected relative was based on previous cytogenetic testing for FXS, at least one affected relative should have DNA testing.
- Prenatal testing of a fetus should be offered when the mother is a known carrier to determine whether the fetus inherited the normal or mutant \( FMR1 \) gene. Ideally DNA testing should be performed on cultured amniocytes obtained by amniocentesis after 15 weeks’ gestation. DNA testing can be performed on chorionic villi obtained by CVS [chorionic villus sampling] at 10 to 12 weeks gestation, but the results must be interpreted with caution because the methylation status of the \( FMR1 \) gene is often not yet established in chorionic villi at the time of sampling. A follow-up amniocentesis may be necessary to resolve an ambiguous result.
- If a woman has ovarian failure before the age of 40, DNA testing for premutation size alleles should be considered as part of an infertility evaluation and prior to in vitro fertilization.
- If a patient has cerebellar ataxia and intentional tremor, DNA testing for premutation size alleles, especially among men, should be considered as part of the diagnostic evaluation.

The ACMG Professional Practice and Guidelines Committee made recommendations regarding diagnostic and carrier testing for FXS to provide general guidelines to aid clinicians in making referrals for testing the repeat region of the \( FMR1 \) gene. These recommendations include testing of individuals of either sex who have intellectual disability, developmental delay, or autism spectrum disorder, especially if they have any physical or behavioral characteristics of fragile X syndrome.

Physical and behavioral characteristics of FXS include: typical facial features, such as an elongated face with 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.

**Cytogenetic Testing**

Cytogenetic testing was used before the identification of the \( FMR1 \) gene and is significantly less accurate than the current DNA test. The method is no longer considered an acceptable diagnostic method according to ACMG standards.
Genetic Counseling
Genetic counseling is primarily aimed at patients who are at risk for inherited disorders, and experts recommend formal genetic counseling in most cases when genetic testing for an inherited condition is considered. The interpretation of the results of genetic tests and the understanding of risk factors can be very difficult and complex. Therefore, genetic counseling will assist individuals in understanding the possible benefits and harms of genetic testing, including the possible impact of the information on the individual's family. Genetic counseling may alter the utilization of genetic testing substantially and may reduce inappropriate testing. Genetic counseling should be performed by an individual with experience and expertise in genetic medicine and genetic testing methods.

Background/Overview
Fragile X Syndrome
FXS is the most common cause of heritable intellectual disability, characterized by moderate intellectual disability in males and mild intellectual disability in females. FXS affects approximately 1 in 4000 males and 1 in 8000 females. In addition to intellectual impairment, patients present with typical facial features, such as an elongated face with 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.

Approximately 1% to 3% of children initially diagnosed with autism are shown to have FXS, with expansion of the CGG trinucleotide repeat in the FMR1 gene to full mutation size of 200 or more repeats. A considerable number of children evaluated for autism have been found to have FMR1 premutations (55-200 CGG repeats).

Treatment of FXS
Current approaches to therapy are supportive and symptom-based. Psychopharmacologic intervention to modify behavioral problems in a child with FXS may represent an important adjunctive therapy when combined with other supportive strategies including speech therapy, occupational therapy, and special education services. Medication management may be indicated to modify attention deficits, impaired 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.
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Genetics of FXS
FXS is associated with the expansion of the CGG trinucleotide repeat in the FMR1 gene on the X chromosome. Diagnosis of FXS may include using a genetic test that determines the number of CGG repeats in the fragile X gene. 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)
- Premutation: 55-200 CGG repeats (unmethylated)
- Intermediate: 45-54 CGG repeats (unmethylated)
- Normal: 5-44 CGG repeats (unmethylated)

Full mutations are associated with FXS, which is caused by expansion of the FMR1 gene CGG triplet repeat above 200 units in the 5’ untranslated region of FMR1, leading to hypermethylation of the promoter region followed by transcriptional inactivation of the gene. FXS is caused by a loss of the fragile X mental retardation protein.

Patients with a premutation are carriers and may develop an FMR1-related disorder, such as fragile X–associated tremor/ataxia syndrome (FXTAS) or, in women, fragile X–associated premature ovarian insufficiency. FXTAS is a late-onset syndrome, comprising 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.

Premutation alleles in females are unstable and may expand to full mutations in offspring. Premutations of fewer 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.

Premutation allele prevalence in whites is approximately 1 in 1000 males and 1 in 350 females. 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. Women with a premutation are at risk of premature ovarian insufficiency and at small risk of FXTAS; they carry a 50% risk of transmitting an abnormal gene, which contains either a premutation copy number (55-200) or a full mutation (>200) in each pregnancy.

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.

FDA or Other Governmental Regulatory Approval
U.S. Food and Drug Administration (FDA)
Clinical laboratories may develop and validate tests in-house and market them as a laboratory service; laboratory-developed tests (LDTs) must meet the general regulatory standards of the Clinical Laboratory Improvement Amendments (CLIA). The Xpansion Interpreter® test is available under the auspices of CLIA.
Laboratories that offer LDTs must be licensed by CLIA for high-complexity testing. To date, the U.S. FDA has chosen not to require any regulatory review of this test.

Asuragen offers the Xpansion Interpreter test, which analyzes AGG sequences that interrupt CGG repeats and may stabilize alleles, protecting against expansion in subsequent generations.

Centers for Medicare and Medicaid Services (CMS)
There is no national coverage determination (NCD). In the absence of an NCD, coverage decisions are left to the discretion of local Medicare carriers.

**Rationale/Source**
Validation of the clinical use of any genetic test focuses on 3 main principles: (1) analytic validity of the test, which refers to the technical accuracy of the test in detecting a variant that is present or in excluding a variant that is absent; (2) clinical validity of the test, which refers to the diagnostic performance of the test (sensitivity, specificity, positive and negative predictive values) in detecting clinical disease; and (3) clinical utility of the test, ie, how the results of the diagnostic test will be used to change management of the patient and whether these changes in management lead to clinically important improvements in health outcomes. Literature that describes the analytic validity, clinical validity, and clinical utility of genetic testing for FXS was sought.

**ANALYTIC VALIDITY AND CLINICAL VALIDITY**
Analytic validity refers to the technical accuracy of a test in detecting a variant that is present or in excluding a variant that is absent. Clinical validity refers to the diagnostic performance of a test in detecting clinical disease. According to a large reference laboratory, the analytic sensitivity and specificity of FMR1 testing is 99%. Clinical sensitivity and specificity are 99% for premutation and full variant alleles. Diagnostic errors can occur due to rare sequence variations.

DNA studies are used to test for FXS. Genotypes of individuals with symptoms of FXS and individuals at risk for carrying the variant can be determined by examining the size of the trinucleotide repeat segment and methylation status of the FMR1 gene. Two main approaches are used: polymerase chain reaction (PCR) and Southern blot analysis.

The difficulty in fragile X testing is that the high fraction of GC bases in the repeat region makes it extremely difficult for standard PCR techniques to amplify beyond 100 to 150 CGG repeats. Consequently, Southern blot analysis is commonly used to determine the number of triplet repeats in FXS and methylation status.

PCR analysis uses flanking primers to amplify a fragment of DNA spanning the repeat region. Thus, the sizes of PCR products are indicative of the approximate number of repeats present in each allele of the individual being tested. The efficiency of PCR is inversely related to the number of CGG repeats, so large mutations are more difficult to amplify and may fail to yield a detectable product in the PCR assay. This, and the fact that no information is obtained about FMR1 methylation status, are limitations of the PCR approach.
On the other hand, PCR analysis permits accurate sizing of alleles in the normal zone, the “gray zone,” and premutation range on small amounts of DNA in a relatively short turnaround time. Also, the assay is not affected by skewed X-chromosome inactivation.

Unlike PCR, Southern blotting is time-consuming and requires large amounts of DNA. Alternatives to Southern blotting for determining \textit{FMR1} methylation status have been developed. They include methylation-sensitive PCR and methylation-specific melting curve analysis. One test currently available in Europe (FastFraX; TNR Diagnostics, Singapore) combines a direct triplet repeat-primed PCR with melting curve analysis for detecting CGG expansions. For detecting expansions of more than 55 CGG repeats in \textit{FMR1}, the sensitivity and specificity were 100% (95% confidence interval [CI], 91% to 100%) and 100% (95% CI, 99% to 100%), respectively. A review article by Rajan-Babu and Chong assessed several PCR-based techniques for characterization of \textit{FMR1} variants.

Quality assessment schemes have shown wide disparity in allele sizing between laboratories. Therefore, in 2011, a panel of genotyping reference materials for FXS was developed and is expected to be stable over many years and available to all diagnostic laboratories. A panel of 5 genomic DNA samples was endorsed by the European Society of Human Genetics and approved as an International Standard by the Expert Committee on Biological Standardization at the World Health Organization. Patient blood samples were collected from 6 consenting donors: 1 donor was a normal female and the remainder had been identified after previous molecular genetic investigation. Classifications of these patients were: female premutation, male premutation, male full variant, and female full variant. In all, 38 laboratories were invited to take part in the study, 23 laboratories agreed to participate, and results were returned by 21 laboratories. The participating 21 laboratories evaluated the samples (blinded, in triplicate) using their routine methods alongside in-house and commercial controls. A total of 18 nonconcordant results were reported, giving an overall rate of nonconcordance of 4.9% (21 laboratories \( \times \) 18 samples \( \times \) 7 samples not tested = 371 samples), although the results were clustered in 3 laboratories. There was no correlation between nonconcordant results and any particular sample or a specific method. One laboratory reported 12 of the 18 nonconcordant results. This laboratory was contacted, and their testing protocol was changed.

\textbf{Section Summary: Analytic Validity and Clinical Validity}

CGG-repeat expansion full variants account for more than 99% of cases of FXS. Therefore, tests that are analytically valid, that detect and measure the CGG repeat region of the \textit{FMR1} gene, are also clinically valid. Tests have been shown to be more than 99% sensitive. Positive results are 100% specific. There are no known forms of fragile X mental retardation protein deficiency that do not map to the \textit{FMR1} gene.

\textbf{CLINICAL UTILITY}

\textit{FXS, FXTAS, and FXPOI}

Clinical utility refers to how results of a diagnostic test will be used to change patient management and whether these changes in management lead to clinically important improvements in health outcomes. The conditions caused by abnormal CGG repeats in the \textit{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 \textit{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.

Although not related specifically to \textit{FMR1} testing, 2011 American Academy of Neurology (AAN) guidelines on evaluation of individuals with developmental delay noted several benefits of a definitive genetic diagnosis, even though specific therapy is not often available. Such benefits include:

- Limit additional diagnostic testing;
- Anticipate and manage associated medical and behavioral comorbidities;
- Improve understanding of treatment and prognosis; and
- Allow counseling regarding risk of recurrence in future offspring and help with reproductive planning.

AAN guidelines also emphasized the importance of early diagnosis and intervention in an attempt to ameliorate or improve behavioral and cognitive outcomes over time.

\textbf{Reproductive Decision Making}

Testing the repeat region of the \textit{FMR1} gene in the context of reproductive decision making may include individuals with either a family history of FXS or a family history of undiagnosed intellectual disability, fetuses of known carrier mothers, or affected individuals or their relatives who have had a positive cytogenetic fragile X test result who are seeking further counseling related to the risk of carrier status among themselves or their relatives. (Cytogenetic testing was used before identification of the \textit{FMR1} gene and is significantly less accurate than the current DNA test. DNA testing would accurately identify premutation carriers and distinguish premutation from full mutation carrier women.) The inheritance patterns of the \textit{FMR1} gene have been well characterized, and the penetrance of the fragile X–associated syndromes is very high. Thus the clinical utility of knowing the presence and type of \textit{FMR1} gene variant is self-evident.

Hersh and Saul (2011) reported on families with an affected male and whether an early diagnosis would have influenced their reproductive decision making. 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 variant.

\textbf{SUMMARY OF EVIDENCE}

For individuals who have intellectual disability, developmental delay, or autism spectrum disorder, who are asymptomatic with a family history of FXS or intellectual disability seeking reproductive counseling, with known \textit{FMR1} variant carrier status and current pregnancy seeking prenatal testing, who are asymptomatic with a positive cytogenetic fragile X test result seeking further counseling on carrier status risk, with ovarian failure before age 40 with clinical suspicion of fragile X–associated ovarian failure, or with neurologic symptoms consistent with fragile X–associated tremor or ataxia syndrome who receive \textit{FMR1} variant testing, the evidence includes studies evaluating the analytic and clinical validity of \textit{FMR1} variant testing. Relevant outcomes are test accuracy and validity, and/or resource utilization, and/or changes in
reproductive decision making. The analytic sensitivity and specificity for diagnosing these disorders have been demonstrated to be sufficiently high. The evidence demonstrates that FMR1 variant testing can establish a definitive diagnosis of FXS and fragile X–related syndromes when the test is positive for a pathogenic variant. Following a definitive diagnosis, management may change in various ways. At minimum, providing a diagnosis eliminates the need for further diagnostic workup. Results may aid in management of psychopharmacologic interventions, assist in informed reproductive decision making, or both. A chain of evidence supports improved outcomes following FMR1 variant testing. The evidence is sufficient to determine that the technology results in a meaningful improvement in the net health outcome.

References

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08/01/2013 Medical Policy Committee review
08/21/2013 Medical Policy Implementation Committee approval. New policy.
08/07/2014 Medical Policy Committee review
08/20/2014 Medical Policy Implementation Committee approval. No change to coverage.
08/06/2015 Medical Policy Committee review
08/19/2015 Medical Policy Implementation Committee approval. No change to coverage.
08/04/2016 Medical Policy Committee review
08/17/2016 Medical Policy Implementation Committee approval. No change to coverage.
01/01/2017 Coding update: Removing ICD-9 Diagnosis Codes
08/03/2017 Medical Policy Committee review
08/23/2017 Medical Policy Implementation Committee approval. Added fragile X associated tremor/ataxia syndrome and FMR1-related primary ovarian failure to medically necessary indications.

Next Scheduled Review Date: 08/2018

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A. In accordance with nationally accepted standards of medical practice;

B. Clinically appropriate, in terms of type, frequency, extent, level of care, site and duration, and considered effective for the patient's illness, injury or disease; and

C. Not primarily for the personal comfort or convenience of the patient, physician or other health care provider, and not more costly than an alternative service or sequence of services at least as likely to produce equivalent therapeutic or diagnostic results as to the diagnosis or treatment of that patient's illness, injury or disease.

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