Genetic Testing for *FMR1* Mutations (Including Fragile X Syndrome)

**Description**

Fragile X syndrome (FMS) is the most common inherited form of mental disability and known genetic cause of autism. The diagnosis includes use of a genetic test that determines the number of CGG repeats in the fragile X gene.

**Background**

**Fragile X syndrome**

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 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.

Approximately 1% to 3% of children initially diagnosed with autism diagnosis 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. (1) A considerable number of children being evaluated for autism have been found to have *FMR1* premutations (55-200 CGG repeats). (2) In one author’s experience, 2% of persons ascertained through a dedicated autism clinic had either an *FMR1* full mutation or premutation.

**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, 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
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Genetics of FXS

FXS is associated with the expansion of the CGG trinucleotide repeat in the fragile X mental retardation 1 (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. (3)

- 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 (FMRP).

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 (FXPOI). 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 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.

Premutation allele prevalence in whites is 1 in 1,000 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 either contains 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.
Regulatory Status

No U.S. Food and Drug Administration (FDA)-cleared genotyping tests were found. Thus, genotyping is offered as a laboratory-developed test. Clinical laboratories may develop and validate tests in-house (“home-brew”) 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 and may stabilize alleles and protect against expansion in subsequent generations. (6, 7)

Related Policy


Policy

*This policy statement applies to clinical review performed for pre-service (Prior Approval, Precertification, Advanced Benefit Determination, etc.) and/or post-service claims.

Genetic testing for FMR1 mutations may be considered medically necessary for the following patient populations:

- Individuals of either sex with intellectual disability, developmental delay, or autism spectrum disorder (see Policy Guidelines section*).
- Affected individuals who have had a positive cytogenetic fragile X test result (see Policy Guidelines**).

Genetic testing for FMR1 mutations is investigational for all other uses.

Policy Guidelines

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

- DNA analysis is the method of choice if one is testing specifically for fragile X syndrome and associated trinucleotide repeat expansion in the FMR1 gene.
- For isolated cognitive impairment, DNA analysis for fragile X syndrome should be performed as part of a comprehensive genetic evaluation that includes routine cytogenetic evaluation. Cytogenetic evaluation is important in these circumstances since constitutional chromosome abnormalities have been identified as frequently or more frequently than fragile X mutations in mentally retarded patients referred for fragile X testing.
- Fragile X testing is not routinely warranted for children with isolated attention-deficit/hyperactivity.
• For individuals who are at risk due to an established family history of fragile X syndrome, DNA testing alone is sufficient. If the diagnosis of the affected relative was based on previous cytogenetic testing for fragile X syndrome, 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 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.

** This is due to the fact that cytogenetic testing was used prior to the identification of the 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 ACMG Professional Practice and Guidelines Committee made recommendations regarding diagnostic and carrier testing for fragile X syndrome 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 with intellectual disability, developmental delay, or autism, especially if they have any physical or behavioral characteristics of fragile X syndrome.

Physical and behavioral characteristics of fragile X syndrome include: 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.

Rationale

Literature Review

Analytic Validity/Clinical Validity

Analytic validity refers to the technical accuracy of the test in detecting a mutation that is present or in excluding a mutation that is absent, and clinical validity refers to the diagnostic performance of the test (sensitivity, specificity, positive and negative predictive values) in detecting clinical disease.
For FXS, analytic and clinical validity are the same because the diagnosis of FXS is based upon the detection of an alteration in the FMR1 gene.

According to a large reference laboratory, the analytic sensitivity and specificity of FMR1 screen with reflex to FMR1 diagnostic, FMR1 diagnostic, and FMR1 fetal diagnostic, is 99%. (10, 11) Clinical sensitivity and specificity is 99% for premutation and full mutation 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 mutation can be determined by examining the size of the trinucleotide repeat segment and the 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 about 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 utilizes flanking primers to amplify a fragment of DNA spanning the repeat region. Thus, the sizes of the PCR products are indicative of the approximate number of repeats present in each allele of the individual being tested. The efficiency of the PCR reaction 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 the FMR1 methylation status, are limitations of the PCR approach. On the other hand, PCR analysis permits accurate sizing of alleles in the normal, the “gray zone,” and the premutation range on small amounts of DNA in a relatively short turnaround time. Also, the assay is not affected by skewed X-chromosome inactivation. (3, 9)

Unlike PCR, Southern blotting is time-consuming and requires large amounts of DNA. Alternatives to Southern blotting for determining FMR1 methylation status are in development. These include methylation-sensitive PCR and methylation-specific melting curve analysis. (12-15)

Quality assessment schemes have shown a wide disparity in allele sizing between laboratories. (16) Therefore, in 2011, a panel of genotyping reference materials for FXS syndrome was developed, which is expected to be stable over many years and available to all diagnostic laboratories. A panel of five 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; one donor was a normal female individual and the remainder had been identified after previous molecular genetic investigation. Classifications of these patients were: female premutation, male premutation, male full mutation and female full mutation. 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. Seventeen countries were represented among participating laboratories: 13 from Europe, 4 from North America, 3 from Australasia, and 1 from Asia. Collaborative validation study participants
were requested to test the 18 coded samples on 3 separate days using different lots of reagents or different operators if possible. A total of 18 non-consensus results were reported, giving an overall rate of nonconcordance of 4.9% (21 laboratories x 18 samples – 7 samples not tested), although these were clustered in 3 laboratories. There was no correlation between the non-concordant 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.

CGG-repeat expansion full mutations account for more than 99% of cases of 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. There are no known forms of fragile X mental retardation protein (FMRP) deficiency that do not map to the FMR1 gene.

Clinical utility:

Refers to how the results of the diagnostic test will be used to change patient management and whether these changes in management lead to clinically important improvements in health outcomes.

Evidence on the clinical benefit of testing for FXS is largely anecdotal. Clinical utility of genetic testing can be considered in the following clinical situations: 1) individuals with a clinical diagnosis of intellectual disability, developmental delay, or autism, especially if they have any physical or behavioral characteristics of FXS, a family history of FXS, or male or female relatives with undiagnosed intellectual disability, and (2) individuals seeking reproductive counseling.

Clinical utility for these patients depends on the ability of genetic testing to make a definitive diagnosis and for that diagnosis to lead to management changes that improve outcomes. No studies were identified that described how a molecular diagnosis of FXS changed patient management. Therefore there is no direct evidence for the clinical utility of genetic testing in these patients.

Because there is no specific treatment for FXS, making a definitive diagnosis will not lead to treatment that alters the natural history of the disorder. There are several potential ways in which adjunctive management might be changed following genetic testing after confirmation of the diagnosis. The American Academy of Pediatrics (AAP) (5) and the American Academy of Neurology (AAN) (17) recommend cytogenetic evaluation to look for certain kinds of chromosomal abnormalities that may be causally related to their condition. AAN guidelines note that only in occasional cases will an etiologic diagnosis lead to specific therapy that improves outcomes but suggest the more immediate and general clinical benefits of achieving a specific genetic diagnosis from the clinical viewpoint, as follows:

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

Hersh et al (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 mutation.

Testing the repeat region of the FMR1 gene in the context of reproductive decision making may include testing individuals with either a family history of FXS or a family history of undiagnosed intellectual disability, fetuses of known carrier mothers, or in 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 (because 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 would accurately identify premutation carriers and distinguish premutation from full mutation carrier women.)

Practice Guidelines and Position Statements

American College of Medical Genetics (ACMG) Professional Practice and Guidelines Committee make the following recommendations regarding diagnostic and carrier testing for FXS: The purpose of these recommendations is to provide general guidelines to aid 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 mental retardation.
- 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. (1)
American Academy of Pediatrics (AAP)

AAP recommends 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. (5)

American Congress of Obstetricians and Gynecologists (ACOG)

ACOG (Committee Opinion, 2010) recommends that prenatal testing for FXS should be offered to known carriers of the fragile X premutation or full mutation, and to women with a family history of fragile X-related disorders, unexplained intellectual disability or developmental delay, autism, or premature ovarian insufficiency. (18)

Summary

Fragile X syndrome (FXS) is the most common inherited cause of intellectual disabilities and the most common genetic cause of autism. The genetics of FXS are complex, and there is a broad spectrum of clinical involvement across generations in families affected by the fragile X mutations. A thorough family history, patient assessment and genetic counseling should guide testing for individuals affected by the many manifestations of these mutations. Analytic sensitivity and specificity for diagnosing these disorders has been demonstrated to be sufficiently high.

There are a variety of ways management may change as a result of genetic testing. Evidence on the impact on health outcomes of documenting FMR1 gene mutations is largely anecdotal but may end the need for additional testing in the etiologic workup of an intellectual disability, aid in management of psychopharmacologic interventions, and assist in reproductive decision making. Therefore, genetic testing for FMR1 mutations may be considered medically necessary in individuals of either sex with intellectual disability, developmental delay, or autism spectrum disorder.

References


Policy History

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<th>Date</th>
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<tr>
<td>September 2013</td>
<td>New Policy</td>
<td>Policy updated with literature review; references 3-4, 6-8, 10-15, and 17-18, added. Policy statements and entire policy updated to reflect current DSM-V diagnostic categories, ie, “intellectual disability” replaces “mental retardation” No change to policy statements except the addition of Genetic testing for FMR1 is investigational for all other uses.</td>
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<tr>
<td>December 2014</td>
<td>Update Policy</td>
<td>Policy updated with literature review; references 3-4, 6-8, 10-15, and 17-18, added. Policy statements and entire policy updated to reflect current DSM-V diagnostic categories, ie, “intellectual disability” replaces “mental retardation” No change to policy statements except the addition of Genetic testing for FMR1 is investigational for all other uses.</td>
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Keywords

FMR1 Genetic Testing

This policy was approved by the FEP® Pharmacy and Medical Policy Committee on December 5, 2014 and is effective January 15, 2015.

Signature on File

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