Genetic Testing for Duchenne and Becker Muscular Dystrophy

Effective: February 1, 2017

Next Review: January 2018
Last Review: January 2017

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

Mutations in the DMD gene, which encodes the protein dystrophin, may result in a spectrum of X-linked muscle diseases. The severe end of the spectrum includes the progressive muscle diseases Duchenne and Becker muscular dystrophy and dilated cardiomyopathy. Genetic testing can confirm a diagnosis of a dystrophinopathy and distinguish the less and more severe forms, as well as identify female carriers at risk.

MEDICAL POLICY CRITERIA

I Genetic testing for DMD gene mutations may be considered medically necessary if any of the following are met:

A In a male with signs and symptoms of a dystrophinopathy in order to confirm the diagnosis and direct treatment.

B For at-risk female relatives: (see Policy Guidelines)

1. To confirm or exclude the need for cardiac surveillance.
2. For preconception testing to determine the likelihood of an affected offspring in a woman considering a pregnancy.
II Genetic testing for DMD mutations is considered **not medically necessary** if the criteria above are not met.

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

**POLICY GUIDELINES**

- 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 mutation(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

- Heterozygous females are at increased risk for cardiomyopathy and need routine cardiac surveillance and treatment.

  At-risk females are defined as first- and second-degree female relatives and include the proband’s mother, female siblings of the proband, female offspring of the proband, the proband’s maternal grandmother, maternal aunts, and their female offspring.

**CROSS REFERENCES**

None

**BACKGROUND**

The dystrophinopathies include a spectrum of muscle diseases. The mild end of the spectrum includes asymptomatic increases in serum concentration of creatine phosphokinase and clinical symptoms such as muscle cramps with myoglobinuria and/or isolated quadriceps myopathy. The severe end of the spectrum includes progressive muscle diseases that lead to substantial morbidity and mortality. When skeletal muscle is primarily affected, they are classified as Duchenne or Becker muscular dystrophy and when the heart is primarily affected, as DMD-associated dilated cardiomyopathy (left ventricular dilation and heart failure).

**DUCHENNE MUSCULAR DYSTROPHY**
Duchenne muscular dystrophy (DMD), the most common muscular dystrophy, is a severe childhood X-linked recessive disorder that results in significant disability due to skeletal myopathy and cardiomyopathy. The disease is characterized by progressive, symmetric muscle weakness and gait disturbance resulting from a defective dystrophin gene.[1] The incidence of DMD is estimated to be 1 in 3,500 newborn male births,[2] and approximately one-third of DMD cases arise from new mutations and have no known family history.[1] Infant males with DMD are often asymptomatic. Manifestations may be present as early as the first year of life in some patients, but clinical manifestations most often appear during preschool from years 2 to 5. Affected children present with gait problems, calf hypertrophy, positive Gower’s sign, and difficulty climbing stairs. The affected child's motor status may plateau between 3 and 6 years of life with deterioration beginning at 6 to 8 years. The majority of patients will be wheelchair bound by ages 9 to 12 years but will retain preserved upper-limb function until a later period. Cardiomyopathy occurs after 18 years of age. Late complications are cardiorespiratory (e.g. decreased pulmonary function as a result of respiratory muscle weakness and cardiomyopathy). These severe complications commonly appear in the second decade of life and eventually lead to death.[1] Few individuals with DMD survive beyond the third decade.

BECKER MUSCULAR DYSTROPHY

Becker muscular dystrophy (BMD) is characterized by later-onset skeletal muscle weakness. Individuals remain ambulatory into their twenties. Despite the milder skeletal muscle involvement, heart failure from cardiomyopathy is a common cause of morbidity and the most common cause of death in these patients, with a mean age of death in the mid-forties.

FEMALE CARRIERS

Females heterozygous for a DMD mutation can manifest symptoms of the disease.[3] An estimated 2.5% to 7.8% of female carriers are manifesting carriers who develop symptoms ranging from a mild muscle weakness to a rapidly progressive DMD-like muscular dystrophy.[4] Female carriers are at increased risk for dilated cardiomyopathy. Most heterozygous women do not show severe myopathic features of DMD, possibly due to compensation by a normal X chromosome with inactivation of the mutated DMD gene in the affected X chromosome.[5] In some cases, this compensation can be reversed by a non-random or skewed inactivation of X chromosome resulting in greater expression of the affected X chromosome and some degree of myopathic features.[6] Other mechanisms of manifesting female carriers include X chromosome rearrangement involving the DMD gene and complete or partial absence of the X chromosome (Turner syndrome).[3]

CLINICAL DIAGNOSIS

DMD

The suspicion of DMD should be considered irrespective of family history, and is most commonly triggered by an observation of abnormal muscle function in a male child, the detection of an increase in serum creatine kinase tested for unrelated indications, or after the
discovery of increased serum transaminases (aspartate aminotransferase and alanine aminotransferases). Clinical examination by a neuromuscular specialist for DMD includes visual inspection of mechanical function such as running, jumping, climbing stairs and getting up from the floor. Common presenting symptoms include abnormal gait with frequent falls, difficulties in rising from the floor or in tip-toe walking, and pseudo hypertrophy of the calves. A clinical examination may reveal decreased or lost muscle reflexes and commonly a positive Gower sign. An elevation of serum creatine kinase, at least 10-20 times normal levels (between 5,000 and 150,000 IU/L), is non-specific to DMD but is always present in affected patients.[1] Electromyography and nerve-conduction were traditional parts of the assessment of neuromuscular disorders, but now these tests are no longer believed to be necessary for the specific assessment of DMD.[7] An open skeletal muscle biopsy is needed when a negative test for deletions or duplications to the DMD gene is negative. The biopsy will provide general signs of muscular dystrophy including muscle fiber degeneration, muscle regeneration, and increased content of connective tissue and fat. Dystrophin analysis on a muscle biopsy will always be abnormal in affected patients but is not specific to DMD.

BMD

Becker muscular dystrophy (BMD) has a clinical picture similar to DMD but is milder than DMD and has a later onset. BMD presents with progressive symmetric muscle weakness, often with calf hypertrophy, although weakness of quadriceps femoris may be the only sign. Activity-induced cramping may be present in some individuals, and flexion contractures of the elbows may be present late in the course. Neck flexor muscle strength is preserved, which differentiates BMD from DMD. Serum creatine kinase shows moderate-to-severe elevation (5-100 times the normal level).

Molecular Diagnosis

DMD is the only gene in which mutations are known to cause DMD, BMD and DMD-associated cardiomyopathy. Molecular genetic testing of DMD can establish the diagnosis of a dystrophinopathy without muscle biopsy in most patients with DMD and BMD.

The dystrophinopathies are X-linked recessive and penetrance is complete in males. DMD, the gene that codes for dystrophin is the largest known human gene[1] A molecular confirmation of DMD and BMD is achieved by confirming the presence of a pathogenic variant in this gene by a number of available assays. The large size of the dystrophin gene results in a complex mutational spectrum with over 5,000 different reported mutations, as well as a high spontaneous mutation rate.[8] A testing strategy is outlined in Table 1.

Treatment

There is no cure for Duchenne or Becker muscular dystrophy, and treatment is aimed at control of symptoms to improve quality of life. However, the natural history of the disease can be changed by several strategies such as corticosteroid therapy, proper nutrition or rehabilitative interventions. Glucocorticoids can slow the loss of muscle strength and may be started when a child is diagnosed or when muscle strength begins to decline.[7] The goal of this therapy is to preserve ambulation and minimize later respiratory, cardiac, and orthopedic
complications. Glucocorticoids work by decreasing inflammation, preventing fibrosis, improving muscle regeneration, improving mitochondrial function, decreasing oxidative radicals, and stopping abnormal apoptosis pathways.[1] Bone density measurement and immunization are prerequisites for corticosteroid therapy initiation, which typically begins at 2 to 5 years of age although there has been no demonstrated benefit of earlier therapy, before 5 years of age.[1]

New therapeutic trials require accurate diagnoses of these disorders, especially when the therapy is targeted toward specific mutations.[9] Several of these therapies are currently undergoing clinical trials with two of the most promising being anti-sense oligonucleotide induced exon-skipping and gene repair and replacement with an adeno-associated viral (AAV) vector.[10,11] Exon-skipping is a molecular therapy aimed at skipping the transcription of a targeted exon to restore a correct reading frame using antisense oligonucleotides. The result is a DMD protein that is formed without the mutated exon and a normal, non-shifted reading frame. Exon skipping may be able to restore DMD protein function so that the treated patient’s phenotypic expression more closely resembles BMD. Gene transfer using AAV vector therapy involves the transfer of a functional DMD gene to the patient using this nonpathogenic and low immune response vector.[12]

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.

EVIDENCE SUMMARY

Validation of the clinical use of any genetic test focuses on 3 main principles:

1. The analytic validity of the test, which refers to the technical accuracy of the test in detecting a mutation that is present or in excluding a mutation that is absent;
2. The 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

The clinical utility of the test, i.e., 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.

Analytic Validity

Deletions of one or more exons account for 60-70% of mutations in individuals with DMD and BMD.[13,14] Duplications account for 5-10% of mutations in DMD and BMD.[13]

Multiplex PCR may be used to amplify exons known to be most frequently deleted in DMD-
affected patients. Results obtained from testing two polymerase chain reaction (PCR) multiplex sets suggest a detection rate of approximately 98% with this methodology.\cite{15,16} Multiplex PCR is the most widely available testing choice but is only able to detect deletions. In addition, this method does not cover the whole gene, so a deletion might not always be fully characterized.\cite{7} An alternative to multiplex PCR is the use of a quantitative assay (e.g. multiplex ligation-dependent probe amplification or comparative genomic hybridization [also called chromosomal microarray or CMA]) of all exons. These methods have the advantage of being able to detect whole exon deletions, as well as duplications.\cite{17,18}

Point mutations (small deletions or insertions, single-base changes, and splicing mutations) account for approximately 25-35% of mutations in males with DMD and about 10-20% of males with BMD. If deletion/duplication detection is negative, then dystrophin gene sequencing should be done to look for point mutations or small deletions/insertions.\cite{7}

Sequencing of the entire \textit{DMD} gene to detect point mutations can be performed by traditional PCR and Sanger sequencing, or by more automated methods such as universal long PCR combined with massive pyrosequencing. Wang et al. used next generation sequencing (NGS) of the entire \textit{DMD} gene to detect point mutations in ten males with DMD, five of whom were negative and five of whom were positive for deletions and duplications.\cite{19} In the five deletion/duplication-negative patients, all identified mutations considered pathogenic and were validated by Sanger sequencing, including four novel variants. In the five deletion/duplication-positive patients, NGS detected deletions and duplications by breakpoint analysis. Because NGS breakpoint analysis requires development of precise primers to identify and verify breakpoints, clinical use of NGS for this purpose is limited.

Certain types of assays may cause false-positive results if the method identifies an apparent single-exon deletion or duplication based on the absence or increased amplification, respectively, of a single PCR amplification, or hybridization; when this occurs, the result must be confirmed using an alternative assay. This different assay will verify whether the initial result could have been caused by a sequence variant preventing hybridization of a primer, probe, etc., or for duplications, if the result was an anomaly. Therefore, false positives are expected to be infrequent.

There is a lack of published studies in the peer-review literature that evaluate analytic validity. According to information from the website of a large reference laboratory, deletion/duplication analysis by CMA and point mutations by full gene sequencing detects 98-99% of mutations in both males and females.\cite{20,21}

**Clinical Validity**

Virtually all males with DMD/BMD have identifiable \textit{DMD} mutations, indicating a high clinical sensitivity for genetic testing. In males with DMD and BMD, phenotypes are best correlated with the degree of expression of dystrophin, largely determined by the reading frame of the spliced message obtained from the deleted allele.

A reading frame is the way in which a messenger RNA sequence of nucleotides can be read as a series of base triplets, and affects which protein is made. In DMD, the function of the
dystrophin protein is completely lost due to mutations that disrupt the reading frame. Therefore, prematurely truncated, unstable dystrophins are generated. In contrast, patients with BMD have low levels of full-length dystrophin or carry in-frame mutations that allow for the generation of partially functional proteins. This so-called reading frame rule explains the phenotypic differences between DMD and BMD patients. Since this rule was postulated in 1988,[22] thousands of mutations have been reported for DMD and BMD, of which an estimated 90% fit this rule.[23]

Recently, Manjunath et al. compared the sensitivity of multiplex ligation-dependant probe amplification (MLPA) and multiplex polymerase chain reaction (mPCR) in detecting deletions in 83 children with suspected DMD.[24] mPCR detected deletions in 60/83 (72.3%) of children, while MLPA in the same 83 samples detected deletions in 66/83 (79.5%) and duplications in 6/83 (6.5%), indicating that MLPA has the higher detection rate of the two techniques. Muscle biopsy and subsequent immunohistochemistry performed in the 11 MLPA-negative cases showed absent dystrophin staining in 4/83 (36.4%), indicating neither of these techniques are as sensitive as whole gene sequencing by NGS or deletion/duplication detection using a chromosomal microarray.

In 2016, Li et al. used MLPA, PCR, and NGS to perform mutation analyses in 81 unrelated patients with Duchenne/Becker muscular dystrophy from the Henan Province in China.[25] MLPA identified DMD gene deletion/duplications in 67 cases, and these results were validated with PCR and Sanger sequencing. An additional 13 mutations were found using NGS and validated with Sanger sequencing, including six novel mutations.

**Clinical Utility**

No studies were identified that reported on clinical utility. However, the clinical utility of testing for DMD gene mutations for the index case includes:

- Establishing the diagnosis and initiating/directing treatment of the disease, such as glucocorticoids, evaluation by a cardiologist, avoidance of certain agents (e.g. botulinum toxin injections), and prevention of secondary complications (immunizations, reducing risk of fractures).
- Distinguishing between DMD and BMD.
- Avoidance of a muscle biopsy in the majority of cases.

The clinical utility of testing for DMD gene mutations for at-risk female relatives includes:

- Testing to identify heterozygous females to confirm or exclude the need for cardiac surveillance.
- Preconception testing in a woman considering offspring who would alter reproductive decision-making based on test results.

**PRACTICE GUIDELINE SUMMARY**

An international consortium of scientists conferred and developed the consensus-based, "Best Practice Guidelines on Molecular Diagnosis in DMD/BMD Muscular Dystrophies." The
guidelines recommend genetic testing when there is a clinical suspicion of a dystrophinopathy. In addition, the guidelines recommend to first screen for deletions and duplications. If no deletion or duplication is detected, but the clinical diagnosis is verified, the guidelines recommend screening for point mutations.[9]

The American Academy of Neurology and American Association of Neuromuscular and Electrodiagnostic Medicine 2015 guidelines on evaluation, diagnosis and management of congenital muscular dystrophy (CMD) recommendations state that, “when available and feasibly, physicians might order targeted genetic testing for specific CMD subtypes that have well-characterized molecular causes.”[26] This is a level C recommendation, the lowest allowable recommendation level.

SUMMARY

There is enough research to show that genetic testing can improve health outcomes for males suspected of having a dystrophinopathy and at-risk female relatives. Clinical guidelines based on research recommend testing of the DMD gene in patients that have signs and symptoms of Duchenne and/or Becker muscular dystrophy. Therefore, genetic testing for DMD gene mutations may be considered medically necessary to establish a diagnosis in a male with clinical signs and symptoms suggestive of a dystrophinopathy and in at-risk female relatives. Genetic testing for DMD gene mutations is considered not medically necessary when the policy criteria are not met.

REFERENCES

17. Sansovic, I, Barisic, I, Domic, K. Improved detection of deletions and duplications in the DMD gene using the multiplex ligation-dependent probe amplification (MLPA) method. *Biochemical genetics*. 2013 Apr;51(3-4):189-201. PMID: 23224783


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To establish the diagnosis of a proband with DMD or BMD in a male with clinical findings that suggest a dystrophinopathy:

- Perform DMD genetic testing for deletion/duplication analysis first.
- If a mutation is not identified, perform sequence analysis for a point mutation.
- If a disease-causing DMD mutation is identified, the diagnosis of a dystrophinopathy is established.
- In cases where a distinction between DMD and BMD is difficult, the reading frame “rule” states that the type of deletion/duplication (those that alter the reading frame [out-of-frame], which correlates with the more severe phenotype of DMD versus those that do not alter the reading frame [in-frame] which correlate with the milder BMD phenotype) can distinguish the DMD and BMD phenotypes with 91-92% accuracy.
Table 1. Testing Strategy

- If no disease-causing DMD mutation is identified, skeletal muscle biopsy is warranted for western blot and immunohistochemistry studies of dystrophin.

For carrier testing in at-risk female relatives:

- When the proband’s DMD mutation is known, test for that deletion/duplication or point mutation using appropriate testing method.
- When an affected male is not available for testing, perform testing by deletion/duplication first and if no mutation is identified, by sequence analysis.

The evaluation of relatives at risk includes females who are the sisters or maternal female relatives of an affected male and females who are a first-degree relative of a known or possible carrier female.

*Date of Origin: January 2014*