Excerpt from:


Dystrophinopathies

[Includes: Duchenne Muscular Dystrophy (DMD), Becker Muscular Dystrophy (BMD), DMD-Associated Dilated Cardiomyopathy]

Basil T Darras, MD
Bruce R Korf, MD, PhD,FACMG
David K Urion, MD
Last Update: March 21, 2008.

Summary

Disease characteristics. The dystrophinopathies include a spectrum of muscle disease caused by mutations in the DMD gene, which encodes the protein dystrophin. The mild end of the spectrum includes the phenotypes of asymptomatic increase in serum concentration of creatine phosphokinase (CK) and muscle cramps with myoglobinuria and isolated quadriceps myopathy. The severe end of the spectrum includes progressive muscle diseases that are classified as Duchenne/Becker muscular dystrophy when skeletal muscle is primarily affected and as DMD-associated dilated cardiomyopathy (DCM) when the heart is primarily affected.

Duchenne muscular dystrophy (DMD) usually presents in early childhood with delayed milestones, including delays in sitting and standing independently. Proximal weakness causes a waddling gait and difficulty climbing. DMD is rapidly progressive, with affected children being wheelchair bound by age 12 years. Cardiomyopathy occurs in all affected individuals after age 18 years. Few survive beyond the third decade, with respiratory complications and cardiomyopathy being common causes of death.

Becker muscular dystrophy (BMD) is characterized by later-onset skeletal muscle weakness; individuals remain ambulatory into their 20s. Despite the milder skeletal muscle involvement, heart failure from DCM is a common cause of morbidity and the most common cause of death. Mean age of death is in the mid-40s. DMD-associated DCM is characterized by left ventricular dilation and congestive heart failure.

Female carriers of DMD mutations are at increased risk for DCM.

Diagnosis/testing. DMD is the only gene associated with the dystrophinopathies. Molecular genetic testing of DMD can establish the diagnosis of a dystrophinopathy without muscle biopsy in most individuals with DMD and BMD. Virtually all males with DMD and at least 85% of males with BMD have identifiable DMD mutations. The number of individuals with DMD-associated DCM and identifiable DMD mutations is unknown. In
the remaining cases, a combination of clinical findings, family history, serum CK concentration, and muscle biopsy with dystrophin studies confirms the diagnosis.

**Management.**

*Treatment of manifestations:* aggressive management of DCM with anti-congestive medications in all persons and cardiac transplantation in severe cases; prednisone to improve the strength and motor function in children with DMD unless side effects are severe; deflazacort, a synthetic derivative of prednisolone used in Europe, may have fewer side effects than prednisone; physical therapy to promote mobility and prevent contractures.

*Prevention of secondary complications:* evaluation by pulmonologist and cardiologist before surgeries; pneumococcal and influenza immunizations annually; sunshine and a balanced diet rich in vitamin D and calcium to improve bone density and reduce the risk of fractures; weight control to avoid obesity.

*Surveillance:* for males with DMD or BMD: annual or biannual evaluation by a cardiologist beginning around age 10 years; monitoring for scoliosis; baseline pulmonary function testing before wheelchair dependence; frequent evaluations by a pediatric pulmonologist. For carriers: cardiac evaluation at least once after the teenage years.

*Testing of relatives at risk:* identification of female carriers because of the need for cardiac surveillance.

*Therapies under investigation:* oxandrolone, cyclosporine, aminoglycosides codons; PTC124, which promotes ribosomal read-through of nonsense (stop) mutations; stem cell therapy and gene therapy. Other: no benefit from immunosuppression with azathioprine, myoblast transfer, or creatine monohydrate.

*Genetic counseling.* The dystrophinopathies are inherited in an X-linked manner. The risk to the sibs of a proband depends on the carrier status of the mother. Carrier females have a 50% chance of transmitting the *DMD* mutation in each pregnancy. Sons who inherit the mutation will be affected; daughters who inherit the mutation are carriers and may or may not develop cardiomyopathy. Males with DMD do not reproduce. Males with BMD or *DMD*-associated DCM may reproduce. All of their daughters are carriers; none of the sons inherit their father's *DMD* mutation. Prenatal testing for pregnancies at increased risk is possible if the *DMD* disease-causing mutation in a family member is known or if informative linked markers have been identified.

**Molecular Genetics**

**Table A. Molecular Genetics of Dystrophinopathies**

<table>
<thead>
<tr>
<th>Gene Symbol</th>
<th>Chromosomal Locus</th>
<th>Protein Name</th>
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<tbody>
<tr>
<td><em>DMD</em></td>
<td>Xp21.2</td>
<td>Dystrophin</td>
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</table>
Normal allelic variants. The DMD gene spans 2.4 Mb of DNA and comprises 79 exons. It has at least four promoters. It is the largest known human gene. Innumerable intragenic variants have been described, many of which are useful as markers for genetic linkage analysis.

Pathologic allelic variants. Disease-causing alleles are highly variable, including deletion of the entire gene, deletion or duplication of one or more exons, and small deletions, insertions, or single-base changes. In both DMD and BMD, partial deletions and duplications cluster in two recombination hot spots, one proximal at the 5' end of the gene, comprising exons 2-20 (30%), and one more distal, comprising exons 44-53 (70%) [Den Dunnen et al 1989]. Duplications cluster near the 5' end of the gene, with duplication of exon 2 being the single most common duplication identified [White et al 2006]. More than 4,700 mutations have been identified [Aartsma-Rus et al 2006b].

Normal gene product. Dystrophin is a membrane-associated protein present in muscle cells and some neurons. The N-terminal domain binds to actin. A large rod domain includes 24 homologous repeats forming an α-helical structure, a cysteine-rich calcium-binding region near the C terminus, and a C-terminal domain that binds with other membrane proteins. Dystrophin is therefore part of a protein complex that links the cytoskeleton with membrane proteins that in turn bind with proteins in the extracellular matrix.

Abnormal gene product. Mutations that lead to lack of dystrophin expression tend to cause DMD, whereas those that lead to abnormal quality or quantity of dystrophin lead to BMD. In DMD-associated DCM, dystrophin expression is abnormal in the myocardium and may be normal or mildly abnormal in skeletal muscle [Ferlini et al 1999].

Diagnosis

Clinical Diagnosis

In addition to a positive family history compatible with X-linked inheritance, the following clinical findings support the diagnosis of Duchenne muscular dystrophy (DMD), Becker muscular dystrophy (BMD), and DMD-associated dilated cardiomyopathy (DCM) in males:

Duchenne muscular dystrophy (DMD)

- Progressive symmetrical muscular weakness, proximal greater than distal, often with calf hypertrophy
- Symptoms present before age five years
- Wheelchair dependency before age 13 years

Becker muscular dystrophy (BMD)

- Progressive symmetrical muscle weakness and atrophy, proximal greater than distal, often with calf hypertrophy (weakness of quadriceps femoris may be the only sign)
- Activity-induced cramping (present in some individuals)
- Flexion contractures of the elbows (if present, late in the course)
- Wheelchair dependency (if present, after age 16 years)
• Preservation of neck flexor muscle strength (differentiates BMD from DMD)

Note: The presence of fasciculations or loss of sensory modalities excludes the diagnosis of a dystrophinopathy. Individuals with an intermediate phenotype (outliers) have symptoms of intermediate severity and become wheelchair bound between ages 13 and 16 years.

Testing

Serum creatine phosphokinase (CK) concentration

[Note from GEMP presenter: Testing CK levels is an appropriate first line screening test in males suspected of being affected with a dystrophinopathy. Levels are usually 5 – 10 times normal. The use of CK levels for carrier testing of females is not recommended because the test lacks sensitivity and specificity.]

Electromyography (EMG) is useful in distinguishing a myopathic process from a neurogenic disorder. However, these findings are nonspecific, occurring in all myogenic disorders. In practice, EMG is used only rarely in the diagnosis of dystrophinopathies.

Skeletal muscle biopsy

Histology. Muscle histology early in the disease shows nonspecific dystrophic changes, including variation in fiber size, foci of necrosis and regeneration, hyalinization, and, later in the disease, deposition of fat and connective tissue.

Western blot and immunohistochemistry are summarized in Table 2.

Table 2. Findings in the Dystrophin Protein from Skeletal Muscle Biopsy

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>Western Blot</th>
<th>Immuno-</th>
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|            | Dystrophin Molecular Weight ² | Dystrophin Quantity ³ | histochemistry ¹  
| Males      |              |         |  
| DMD        | Nondetectable | 0%-5%   | Complete/almost complete absence  
| Intermediate | Normal/abnormal | 5%-20% |  
| BMD        | Normal Abnormal | 20%-50% | Normal appearing or reduced intensity ± patchy staining  
|            |              | 20%-100% |  


<table>
<thead>
<tr>
<th>Female Carriers</th>
<th>DMD random XCI</th>
<th>Normal/abnormal</th>
<th>&gt;60% (^5) (70±9%, Pegoraro et al [1995])</th>
<th>Mosaic pattern</th>
</tr>
</thead>
<tbody>
<tr>
<td>DMD skewed XCI</td>
<td>Normal/abnormal</td>
<td>&lt;30% on average (29±25%, Pegoraro et al [1995]) (^6)</td>
<td>Mosaic pattern</td>
<td></td>
</tr>
</tbody>
</table>

1. Uses monoclonal antibodies to the C terminus, N terminus, and rod domain of dystrophin [Hoffman et al 1988]

2. Normal molecular mass is 427 kb.

3. The quantity of dystrophin is expressed in percent of control values. The reference ranges shown in this table are the ones currently used by clinical laboratories and reflect approximate and reconciled data from the literature.

4. XCI = X-chromosome inactivation

5. Quantitative analysis of dystrophin in female carriers is not useful in clinical practice because of the wide range of values and the significant overlap with normal values.

6. Intermediate, severe cases

**Molecular Genetic Testing**

*Gene.* DMD is the only gene known to be associated with DMD, BMD, and DMD-associated DCM.

**Clinical testing**

- **Diagnostic testing**
  - **Deletion/duplication analysis**
    - *Multiplex PCR* can be used to detect deletions, which account for approximately 65% of mutations in individuals with DMD and 85% in those with BMD. Approximately 98% of deletions are detectable by these methodologies.
    - *Southern blotting and quantitative PCR analysis* can be used to detect duplications. Duplications may lead to in-frame or out-of-frame transcripts and account for the disease-causing mutations in approximately 6%-10% of males with DMD or BMD.
    - *Multiple ligation probe amplification* (MLPA) has been developed for deletion/duplication analysis of the *DMD* gene in probands and carrier females
Mutation scanning or sequence analysis detect the small deletions or insertions, single-base changes, or splicing mutations that account for approximately 30%-35% of mutations in DMD

• Carrier testing
  o When the proband's DMD mutation is known
    - Carrier testing for deletions and duplications may be performed using quantitative analysis for gene dosage.
  o When the proband's DMD mutation is not known. Linkage analysis can be offered to at-risk females to determine carrier status in families with more than one affected male with the unequivocal diagnosis of DMD/BMD/DMD-associated DCM. Linkage studies are based on accurate clinical diagnosis of DMD/BMD/DMD-associated DCM in the affected family members and accurate understanding of the genetic relationships in the family. Linkage analysis relies on the availability and willingness of family members to be tested.
    - Note: The large size of the DMD gene leads to an appreciable risk of recombination. It has been estimated that the gene itself spans a genetic distance of 12 centimorgans [Abbs et al 1990]; thus, multiple recombination events among different members of a family may complicate the interpretation of a linkage study. Linkage testing is not available to families in which there is a single affected male.

• Prenatal diagnosis and preimplantation diagnosis for at-risk pregnancies require prior identification of the disease-causing mutation in the family. The usual procedure is to determine fetal sex by karyotype or specialized studies to identify the sex chromosomes from cells obtained by chorionic villus sampling (CVS) at approximately ten to 12 weeks' gestation or by amniocentesis usually performed at approximately 15-18 weeks' gestation. If the karyotype is 46,XY, DNA extracted from fetal cells can be analyzed for the known disease-causing mutation or using the linkage previously established.