Title: Duchenne Muscular Dystrophy
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Duchenne Muscular Dystrophy (DMD), Introduction

- X-linked, recessive muscular disorder affecting males
- Caused by mutations in the *DMD* gene that lead to deleterious effects on the structure or expression of the Dystrophin protein
- Disease prevalence ranges: 1:3500-1:6000
- Shares genetic etiology and clinical symptoms with Becker Muscular Dystrophy (BMD)
Clinical Features

• Typical disease progression:
  • Symptoms of muscle weakness before age five
    o Abnormal stride/toe walking
    o Difficulty running, jumping, climbing stairs
  • Progressive muscle wasting in late childhood-teenage years
  • Death in late teens to twenties (unassisted)
  • Life into thirties with medical assistance

• Characteristic clinical phenotypes:
  • Gower’s Sign
  • Pseudohypertrophy of the calf muscles

Clinical Features, Continued

• Additional phenotypes observed in Duchenne cases:
  • Cognitive impairment
  • Gastrointestinal
  • Dilated Cardiomyopathy
  • Scoliosis

• Becker individuals present with a more varied and mild phenotype:
  • Later onset of phenotypes, including wheelchair dependency
  • Life expectancy is increased relative to Duchenne cases

• A carrier phenotype is observed in a minority of cases
  • Cardiac abnormalities, such as dilated cardiomyopathy
  • Intellectual impairment
  • Scoliosis/lordosis
Dystrophin Mutational Spectrum

- **DMD** is one of the largest genes in the human genome
- Large deletions are the most common type of mutation observed in Duchenne patients
- Also observed:
  - Large duplications
  - SNVs+small indels
- Mosaic cases should be carefully considered during case evaluation

Dystrophin Mutation Type, by Percentage

- Large Deletions: ~60%
- Large Duplications: ~10%
- SNVs/Indels: ~25%
- Undetermined: ~5%
Genotype/Phenotype Correlations: Reading Frame Rule

- **Intron A**
  - Exon A: NNN/AA1 AA2
  - Exon B: N/NNN NN/AA2 AA3
  - Exon C: N/AA4 AA5 AA6
  - Exon D: NNN/AA7 AA8

- **Intron B**
  - Exon A: N/NNN NN/AA2 AA3
  - Exon B: N/AA4 AA5 AA6

- **Intron C**
  - Exon A: NNN/AA1 AA2
  - Exon B: NNN/AA5 AA6

- **Protein “Normal”**
  - Full length, functional

- **Protein “Becker”**
  - Truncated, semi-functional

- **Protein “Duchenne”**
  - mRNA decay, no protein

- **Exon A**
  - Exon B
  - Exon C
  - Exon D
Dystrophin Cellular Biology and Pathogenesis

- The Dystrophin protein serves as a link between intracellular actin and the Dystrophin-Associated Protein Complex
- Loss of Dystrophin function or expression leads to:
  - Myofiber membrane destabilization
  - Tissue inflammation
  - Accumulation of fibrotic/fatty tissue within muscle
  - Loss of muscle function
Related Muscular Dystrophies

• Other muscular dystrophies can be caused by mutations in genes whose proteins serve to mediate cell/environment interaction
  • Emery-Dreifuss Muscular Dystrophy
    o Commonly affects elbows, ankles and neck
    o Progressive muscle weakness, cardiac disease and arrhythmias are observed
    o Associated genes include: *LMNA*, *FHL1*, *EMD*
  • Limb-Girdle Muscular Dystrophy
    o Wide array of muscular phenotypes, more diverse genetic etiology
    o Associated genes include: Sarcoglycans A,B,D,G, *DYSF*, *CAPN3*
  • Screening and diagnosis of these muscular dystrophies share similarities with Duchenne/Becker muscular dystrophy.
Diagnosis of Duchenne/Becker Muscular Dystrophy

• Only mutations in the *DMD* gene can lead to Duchenne or Becker muscular dystrophy
• The DMD Care and Considerations Working Group has established that Duchenne should be suspected when any of the following is observed:
  • Not walking by ~17 months (no family history)
  • Any suspicion of abnormal muscle function (positive family history)
  • Patient has unexplained increase in transaminases
• Prior to more detailed testing, a doctor may test for elevated levels of creatine phosphokinase
• Diagnosis must be made through genetic testing if available, or muscle biopsy
Screening/Creatine Phosphokinase Measurement

- Disruption of the myofiber membrane leads to an increase in creatine phosphokinase (CK) in patient serum
- Different assays have been developed to quantitatively measure CK

Chemistry leading to a measurable increase in NADPH allows measurement using a spectrophotometer.
- Affected individuals typically have a CK concentration above 1000U/L
- CK elevations can be detected within the first week of life
Molecular Genetic Testing – Copy Number Variation Testing

- Testing for large copy number variation can occur prior to sequence level testing
- Thorough investigation of DMD copy number variation requires investigation of all exons within the DMD gene

Methods for analysis of copy number include:

- Multiplex Ligation-Dependent Probe amplification (MLPA)
- Array Comparative Genomic Hybridization (aCGH)
- SNP array
- Quantitative Real-Time PCR

Confounding results may be caused by:

- Mosaicism
- Translocations
- Other genetic complexities
Molecular Genetic Testing – Sequence Level Testing

- Analysis of *DMD* at the nucleotide level can pick up causal mutations that are missed by copy number analysis
- Sanger sequencing + multiplexing are common

**Interpretation of sequence level variation should follow ACMG guidelines and consider:**
- Variant allele frequency in control populations
- Previous publications supporting pathogenic/benign nature of variant
- Predicted impact on amino acid/protein structure
- Allele segregation within family, if appropriate
- Other factors outlined by ACMG guidelines

**Confounding results may be caused by:**
- Mosaicism
- Intronic/noncoding variants
- Other genetic complexities
Muscle Biopsy and Protein Evaluation

- Muscle biopsy can be used when genetic testing is inconclusive or not available.
- Antibodies that bind to the c-terminal, n-terminal, and central domains of the protein should be used to account for potentially altered protein structure.
- Some protein expression is observed in affected individuals.

Genetic Counseling and Considerations

- ACMG has stated that genetic testing should be considered for:
  - Males with muscular dystrophy symptoms
  - Females with a family history of disease
  - Females with a previously affected child
- Carrier females have a 50% risk of having an affected son, 50% risk of having a carrier daughter
- Genetic testing/counseling is not expected to identify/prevent all cases. As many as 1/3 of Duchenne patients are thought to be caused by de novo mutations.
Points to Remember

- Duchenne muscular dystrophy is an X-linked, recessive disorder that causes muscle weakness, cardiomyopathy and premature death. Becker muscular dystrophy is a related but milder muscular dystrophy.

- Loss or reduction of Dystrophin expression or structure leads to destabilization of the myofiber membrane, which leads to tissue damage and muscle weakness.

- Duchenne and Becker muscular dystrophies are caused by mutations within the DMD gene. Diagnosis of the disease is accomplished through identification of a causal mutation or by muscle biopsy.

- The most common causal mutation type within Duchenne/Becker cases are exonic deletions. Exonic duplications, SNVs, and indels can also cause disease.
References

Disclosures/Potential Conflicts of Interest

Upon Pearl submission, the presenter completed the Clinical Chemistry disclosure form. Disclosures and/or potential conflicts of interest:

- **Employment or Leadership:** Natera
- **Consultant or Advisory Role:** No disclosures
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