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PEARLS OF LABORATORY MEDICINE

Title: Duchenne Muscular Dystrophy

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Duchenne Muscular Dystrophy (DMD), Introduction

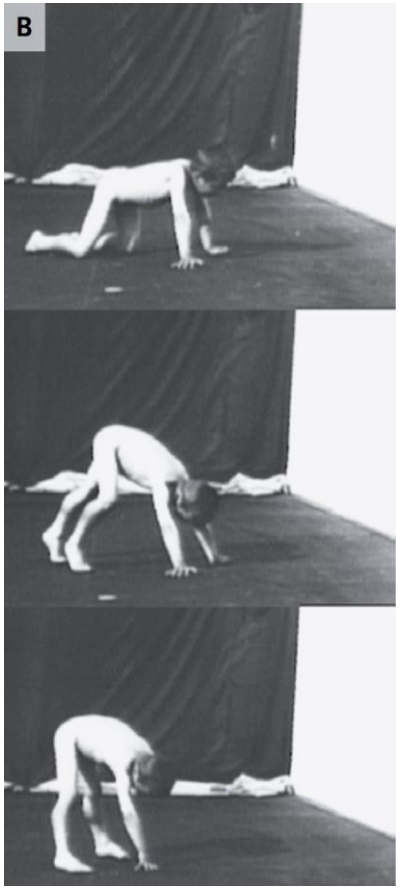
- X-linked, recessive muscular disorder affecting males
- Caused by mutations in the *DMD* gene that lead to deleterious effects on the structure or expression of the Dystrophin protein
- Disease prevalence ranges: 1:3500-1:6000
- Shares genetic etiology and clinical symptoms with Becker Muscular Dystrophy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

Clinical Features

- Typical disease progression:
  - Symptoms of muscle weakness before age five
    - Abnormal stride/toe walking
    - 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
  - Pseudohypertophy of the calf muscles

Gowers Sign



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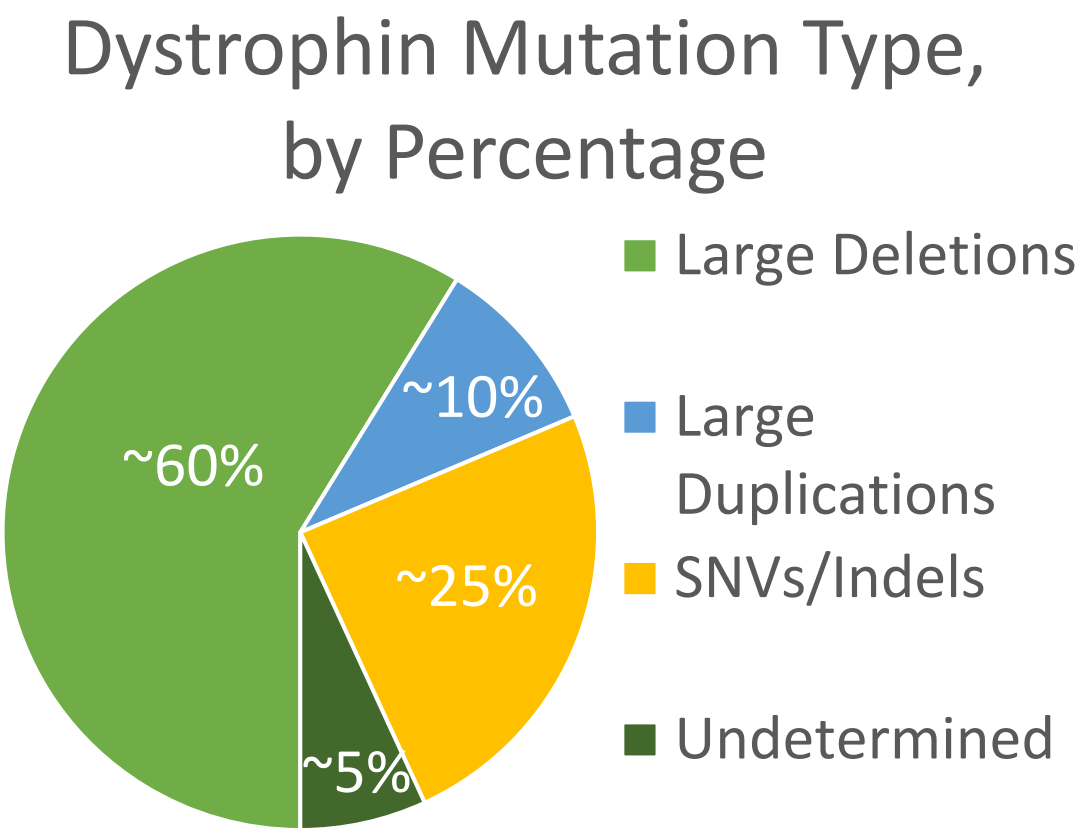
Clinical Features, Continued

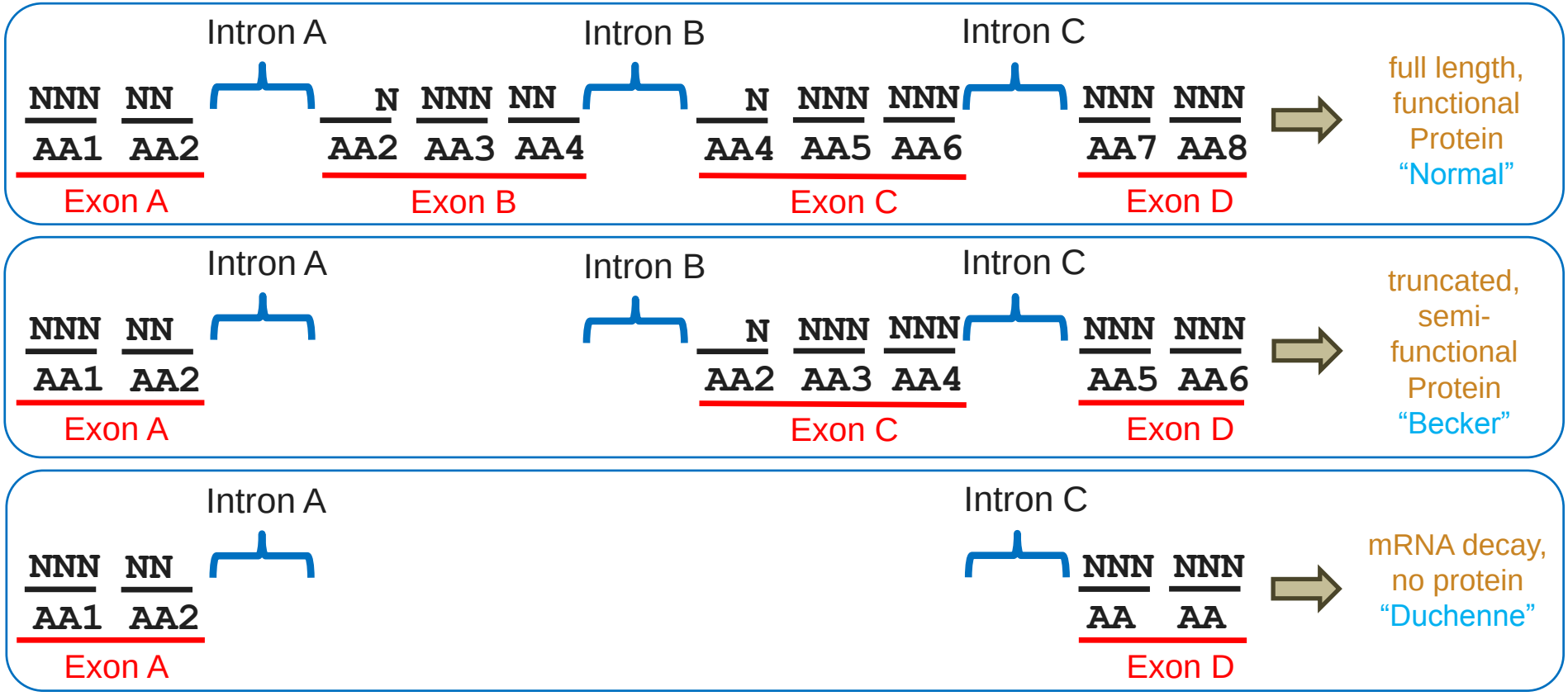
- Additional phenotypes observed in Duchenne cases:
  - Cognitive impairment
  - Gastrointestinal
  - Dilated Cardiomyopathy
  - Scoliosis
- Becker individuals present with a more varied and mild phenotype:
  - Later onset of phenotypes, including wheelchair dependency
  - Life expectancy is increased 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

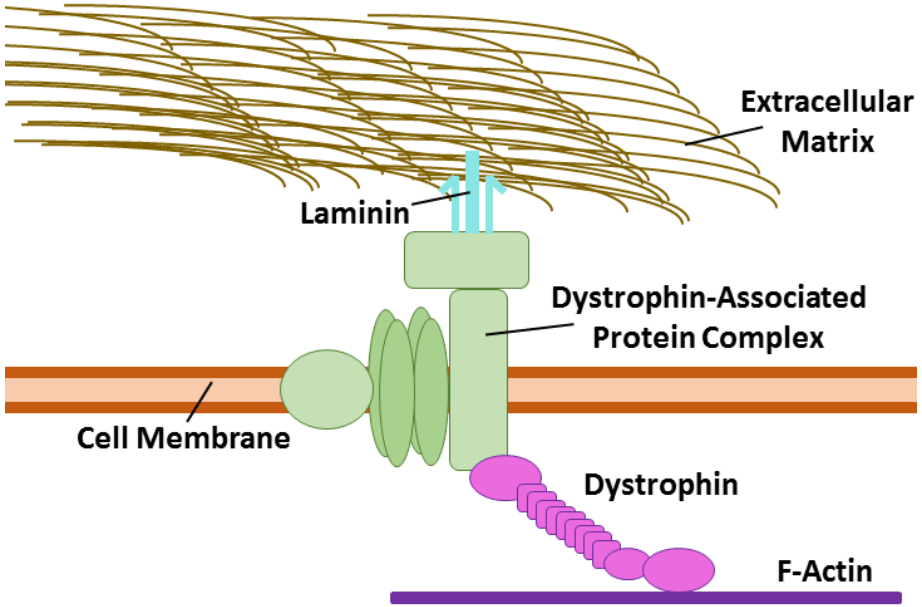


Genotype/Phenotype Correlations: Reading Frame Rule



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
    - Commonly affects elbows, ankles and neck
    - Progressive muscle weakness, cardiac disease and arrhythmias are observed
    - Associated genes include: *LMNA, FHL1, EMD*
  - Limb-Girdle Muscular Dystrophy
    - Wide array of muscular phenotypes, more diverse genetic etiology
    - 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

Creatine phosphate + ADP → Creatine + ATP

ATP + Glucose → Glucose-6-phosphate + ADP

Glucose-6-phosphate + $NADP^+$ → 6-phosphogluconate + $NADPH^+$ + H^+

Associated enzyme:

CK

Hexokinase

G6DPH

- 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

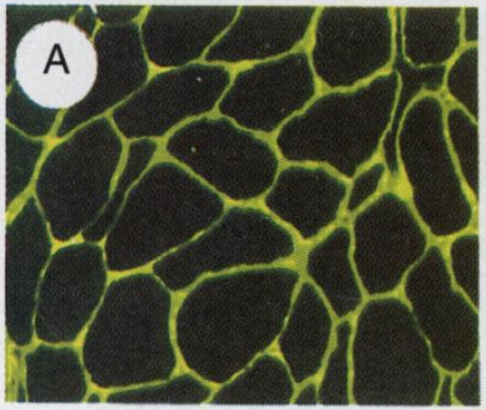
Confounding results may be caused by:

- Mosaicism
- Intronic/noncoding variants
- Other genetic complexities

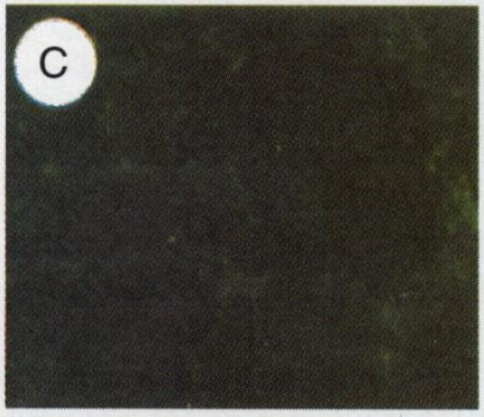
Muscle Biopsy and Protein Evaluation

- 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

Dystrophin localization in normal tissue



Dystrophin localization in Duchenne tissue



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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.

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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
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