

PEARLS OF LABORATORY MEDICINE

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TITLE: Duchenne Muscular Dystrophy

PRESENTER: Kevin Dumas, Ph.D.

Slide 1:

Hello, my name is Kevin Dumas. I work in variant curation and analysis at Natera. Welcome to this Pearl of Laboratory Medicine on “Duchenne Muscular Dystrophy”

Slide 2: Duchenne Muscular Dystrophy, Introduction

Duchenne muscular dystrophy (DMD) is an X-linked, recessive muscular disorder caused by mutations in the *DMD* gene, which codes for the Dystrophin protein. Duchenne muscular dystrophy is phenotypically identified by muscle weakness observed in early childhood, which progresses to wheelchair dependence by early teenage years. Duchenne is fatal, with untreated individuals living into their late teens or early twenties. Incidence rates identified for Duchenne muscular dystrophy range from 1:3500 to 1:6000. Due to its X-linked inheritance pattern, affected Duchenne individuals are male. Duchenne is the more severe of the two muscular dystrophies associated with the *DMD* gene, the other being Becker muscular dystrophy (BMD) which has an incidence rate about one third of Duchenne muscular dystrophy.

Slide 3: Clinical Features

Individuals with Duchenne muscular dystrophy are observed to have muscle weakness beginning before age five. In addition to difficulty jumping, climbing stairs and an abnormal gait, affected patients typically display pseudohypertrophy of the leg muscles, which occurs when adipose and fibrotic tissue replace deteriorating muscle tissue in affected individuals. A characteristic phenotype observed during childhood is the use of arms and hands to “climb” up one’s legs when rising from a seated or prone position, a maneuver called the Gower’s Sign. Muscle weakness increases throughout childhood until the affected individual is wheelchair dependent by early teen years. Death typically occurs in the late teens and into the twenties,

though medical assistance can prolong life into the thirties. Cause of death is usually related to progressive weakness of respiratory muscles with subsequent pneumonia development.

Slide 4: Clinical Features, Continued

In addition to affecting patient skeletal muscles, smooth muscle of the gastrointestinal tract is also affected in Duchenne patients. As the disease progresses, patients can show scoliosis, cardiac arrhythmia or dilated cardiomyopathy. Cognitive impairment and decreased IQ are also observed in Duchenne populations, though variation is seen between individuals.

Individuals with Becker muscular dystrophy share clinical symptoms with Duchenne cases. However, symptoms are less severe and have later onset compared to Duchenne patients. Becker's patients typically live into their 40s or later.

While clinical diagnosis of Duchene is limited to males, carrier females may display mild symptoms including muscle weakness, seen in 17 to 20% of carriers; cardiac abnormalities or intellectual impairment, seen in a third of carriers; and scoliosis or lordosis, seen in up to 75% of carriers.

Slide 5: DMD Mutational Spectrum

Occupying 2.4 megabases, *DMD* is one of the largest genes - by length - in the human genome. The gene structure of the canonical transcript is comprised of 79 exons on the 21p region of chromosome X. The high mutation rate seen in the *DMD* gene is partially due to the large size of the gene.

The mutation type most commonly observed Duchenne cases are multi-exonic deletions. Large cohort investigations have identified deletions in approximately 60 to 70% of affected cases. Additionally, exonic duplications are observed to comprise around 10% of cases. Lastly, SNVs and small indels are observed in 25-30% of cases. Many of these are nonsense mutations leading to nonsense mediated decay and a loss of Dystrophin translation. Germline mosaicism is observed in as many as 2-10% of cases and should be considered during mutational analysis. Approximately 4-7% of individuals with a Dystrophinopathy do not have a pathogenic variant that is identifiable by the combined methods of sequencing and deletion/duplication testing.

Slide 6: Genotype/Phenotype Correlations: Reading Frame Rule

Determining which exonic duplication or deletion mutations lead to Becker vs Duchenne muscular dystrophy can be approximated using the "reading frame rule". The reading frame rule is based on evidence showing that the majority of patients that have a exonic duplication or deletion leading to an out of frame protein product, and thus a truncated protein, have a

Duchenne phenotype whereas patients with an in-frame exonic duplication or deletion are more likely to have a Beckers phenotype. Cohort studies have shown the reading frame rule correctly predicts phenotype nearly 90% of the time in Duchenne cases, but is less accurate for Becker cases. Importantly, some mutations have been observed in both Becker and Duchenne cases, indicating that caution should be used when predicting genotype/phenotype correlations.

Slide 7: Dystrophin Cellular Biology and Pathogenesis

At 3685 amino acids long, the Dystrophin protein is one of the largest proteins in the human proteome. The Dystrophin protein contains an n-terminal actin binding domain, an elongated rod domain comprised of 24 spectrin-like repeats, and a c-terminal domain that binds to Dystroglycan. The role of the Dystrophin protein includes linking intracellular actin to the Dystrophin-associated protein complex, which in turn interacts with the extracellular matrix. Under normal circumstances, Dystrophin is necessary for maintaining the structure and integrity of the muscle sarcolemma. When Dystrophin is greatly reduced or absent, as in Duchenne, muscle contraction leads to membrane destabilization and tissue damage. The disruption of the myofiber membrane is associated with calcium entry into the cell, inflammation, and depletion of myogenic stem cells. Over time, this leads to the formation of fibrotic and fatty tissue within the muscle, reducing contractile function, and causing the characteristic muscle weakness observed in Duchenne and Becker's patients.

Slide 8: Related Muscular Dystrophies

Genetic-based muscular dystrophies are not limited to Duchenne and Becker. There are several other muscular dystrophies associated with mutations in genes expressed in the muscle tissue and Dystrophin-associated protein complex.

Emery-Dreifuss muscular dystrophy chiefly affects skeletal and cardiac muscle. Early signs of this muscular dystrophy are restricted movement of joints: elbows, ankles and neck are commonly affected. Progressive muscle weakness and wasting are observed alongside cardiac disease and arrhythmias. Emery-Dreifuss muscular dystrophy is caused by mutations in the EMD, FHL1 and LMNA genes.

Limb Girdle Muscular Dystrophy (LGMD) can be childhood or adult onset and display a wide range of phenotypic variation due to the genetic heterogeneity seen in these individuals. Genes commonly affected in LGMD include the 4 sarcoglycan genes that code for proteins in the Dystrophin-associated protein complex. Dysferlin, Calpain-3 and other genes have also been associated with LGMD. The heterogeneity of genes associated with this phenotype highlights the necessity of multiple proteins for proper muscle function.

Other congenital muscular dystrophies are known, with varied genetic causes.

Screening and diagnosis of these other muscular dystrophies, described in the coming slides, have several similarities with Duchenne/Becker.

Slide 9: Diagnosis of Duchenne/Becker Muscular Dystrophy

DMD is the only gene that, when mutated, can lead to Duchenne or Becker muscular dystrophy. The DMD Care and Considerations Working Group has established that Duchenne muscular dystrophy should be suspected if: the patient has a negative family history and isn't walking by 17 months, the patient has a positive family history and displays abnormal muscle function or when the patient has increased transaminases. In many cases, prior to a genetic test, a physician evaluating a potential muscular dystrophy case, such as Duchenne or Becker, may test for elevated levels of serum creatine phosphokinase. Furthermore, the DMD Care and Considerations Working Group has established that diagnosis should be made by genetic testing, if available, or by muscle biopsy.

Slide 10: Screening/Creatine Phosphokinase Measurement

Creatine phosphokinase, or CK, is a dimeric protein present in muscle tissue. In affected individuals, damage to the sarcolemmal membrane allows CK to enter the vascular space. This abnormality leads to a significant increase in serum CK that can be detected by routine clinical laboratory testing.

Serum CK catalyzes the reversible reaction of $\text{ADP} + \text{creatine phosphate} \rightarrow \text{ATP} + \text{creatine}$. Testing of serum CK can use several methods to measure this reaction. One common means to test CK levels in a lab requires coupling the creation of ATP by CK to hexokinase and a glucose substrate to create glucose-6-phosphate. This, in turn, is coupled with NADP, which is reduced to NADPH in the presence of G6DPH. This last process can be measured by monitoring absorbance at 340nm using a spectrophotometer.

Due to difference in assay chemistry, age at sample collection and lack of standardization, an exact cutoff is not established. Individuals with Duchenne typically have CK levels 10-20 times higher than normal. The American Academy of Pediatrics has suggested affected individuals typically have a concentration above 1000U/L. Elevated CK levels is detectable early and can be measured within the first week of life.

Because elevated CK levels can be observed in other diseases, or due to certain environmental events, CK testing cannot be used for diagnosis of Duchenne or Becker Muscular dystrophy.

Slide 11: Molecular Genetic Testing – Copy Number Variation Testing

Diagnosis of Duchenne or Becker muscular dystrophy is usually made through genetic testing, if available. Since exonic copy number variation is the most common type of mutation observed in affected individuals, initial evaluation includes testing for large duplications or deletions within the *DMD* gene.

Several methods for determining copy number in a quantitative fashion exist. Multiplex ligation-dependent probe amplification, or MLPA, in which two probes specific for a region of interest are ligated and amplified in a sequential fashion and analyzed quantitatively, is a common method. Array CGH, SNP array, Real-time quantitative PCR and multiplex PCR are other measures of quantitative detection that can be used as well.

Abnormal results may arise or be missed due to mosaicism. Additionally, balanced translocations or inversions are mutation types that can be missed by the aforementioned technologies.

Slide 12: Molecular Genetic Testing – Sequence Level Testing

In instances where a copy number change is not detected, sequence analysis can be performed to identify coding or splice-site mutations via PCR on *DMD* exonic material. Multiplex PCR is often used for labor and cost savings. The continued development and advances offered by next-generation sequencing technology make it an active target in test development. For tests such as these, identification of variants predicted to be associated with the disease phenotype is followed by confirmation using Sanger DNA analysis.

Analysis of variants identified during sequencing for potential pathogenicity should consider the frequency of the observed variant in control populations, predicted effect on protein and previous publications supporting or refuting a deleterious effect in Duchenne or Becker individuals. The American College of Medical Genetics, or ACMG, has provided a series of sequence variant guidelines to assist evaluating laboratories.

During sequencing and analysis, relevant results may be missed due to mosaicism, intronic variation not detected during sequencing or other genetic complexities. Over 95% of affected individuals who receive genetic testing identify a pathogenic *DMD* variant.

Slide 13: Muscle Biopsy and Protein Evaluation

Through rare, in instances where: no pathogenic variant can be identified, a discordant or intermediate phenotype is observed, or genetic testing not available, Dystrophin abnormalities can be detected at the cellular or tissue level through a muscle biopsy. Dystrophin protein expression can be assayed using immunofluorescent or immunohistochemical analysis of muscle tissue. Clinical labs should use antibodies directed towards the n-terminal, central and c-terminal domain to account for altered protein structure that appears in some individuals. In some cases, biopsies from Duchenne patients show localized Dystrophin expression. These “revertant fibers” are observed in many patient biopsies, highlighting the need for thorough analysis.

Slide 14: Genetic Counseling and Considerations

The ACMG states that Dystrophin gene testing is considered for: a) males with clinical features of Duchenne or Beckers muscular dystrophy b) females at risk for being a carrier, and c) pregnancies of females known to carry a *DMD* mutation. Because of the X-linked nature of Duchenne, a carrier mother has a 50% risk of having an affected son and a 50% risk of passing the mutated allele on to a carrier daughter. Even with genetic counseling, as many as a third of Duchenne and Becker cases are caused by *de novo* mutations and will not be detected by screening or testing family members.

Slide 15: 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 sarcolemma, which leads to tissue damage and muscle weakness.

Duchenne and Becker muscular dystrophy are caused by mutations within the *DMD* gene. Diagnosis of the disease is accomplished through identification 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.

Slide 16: References

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[Slide 17: Disclosures

Current stockholder and employee at Natera.

Slide 18: Thank You from www.TraineeCouncil.org

Thank you for joining me on this Pearl of Laboratory Medicine on “Duchenne Muscular Dystrophy”]