Important facts about dysferlin and dysferlinopathy

Clinical

- Dysferlinopathy is an autosomal recessive form of muscular dystrophy caused by the presence of mutations in both copies of a person's dysferlin gene.
- There are multiple clinical presentations that are associated with dysferlinopathy, with the two must common being Limb Girdle Muscular Dystrophy type 2B (LGMD2B) and Miyoshi Myopathy type 1 (MM or MMD1).
- A patient is given a clinical diagnosis of LGMD2B if their initial weakness involves the proximal muscles of the leg; whereas they are given a clinical diagnosis of Miyoshi Myopathy if their initial weakness is more distal.
- The clinical presentation does not correlate with mutation type and studies have shown that both presentations can be seen within affected siblings of the same family.
- The natural history study of the dysferlinopathy (COS) has not identified any significant differences between patients having different initial clinical diagnoses and has therefore concluded they are the same disease despite the differences seen in initial muscle invovlement.
- In the US, dysferlinopathy is the second most common genetic type of Limb-Girdle Muscular Dystrophy, after LGMD2A (Calpain-3). In other parts of the world (Latin America, East Asia), it may be the most common form.
- The overall estimate of the prevalence of dysferlinopathy is between 5-10/million population.
- Dysferlinopathy is typically a slowly progressing, adult onset form of muscular dystrophy that doesn't affect life span.
- Onset of muscle weakness in dysferlinopathy typically begins in adolescence or young adulthood, with the median age of onset around 20. About 75% of patients have onset between 15 and 30.
- The first symptom patients often notice is the inability to stand on their tiptoes, due to weakness of the gastrocnemius muscles, which MRI data shows is one of the first muscles to be affected in all dysferlinopathy patients, including those with a diagnosis of LGMD2B.
- Prior to onset of muscle weakness, patients with dysferlinopathy typically have good muscle performance, and in many cases are athletic. The reason for the delayed onset is unknown.
- CK is typically markedly elevated in dysferlinopathy and can be 10-20X normal values. In a few cases were data is available, CK values have been shown to be normal at younger ages and then becomes elevated a few years prior to the onset of muscle weakness.
- Although dysferlin is highly expressed in heart, there isn't evidence of cardiomyopathy in most patients.
- Breathing is typically not impacted.
- Loss of ambulation typically occurs 10-20 years after onset of symptoms, although there is a wide range in rate of progression. This variation doesn't correlate with type of mutation or level of protein expression.

Gene

- The main muscle isoform of dysferlin contains 55 coding exons. There are 3 alternatively spliced exons (1A, 5A, and 40a) that are found in other isoforms of dysferlin.
- There aren't any mutational "hot spots" in dysferlin as there is in dystrophin. There are mutations identified is every one of the 55 dysferlin exons and no mutations account for a large proportion of cases.
- The most common types of mutations in dysferlin are point mutations that result in frameshift, nonsense, splicing, and missense mutations. Large duplications and deletions are seen but are not very common. In addition, deep intronic mutations that lead to aberrant splicing have recently been identified.
- There isn't a clear genotype-phenotype correlation in dysferlinopathy. The most likely stems from the fact that most mutations, including most missense mutations involving a single amino acid substitution, cause the same outcome at the protein level: greatly reduced or absence dysferlin protein expression.
- The dysferlin cDNA (with promoter) is a little more than 6kB, which is too big to fit in a single AAV. This accounts for the dual vector approach to gene therapy.
- The isoform currently being used in dysferlin AAV gene therapy is the predominant one in skeletal muscle, and the disease phenotype of a transgenic dysferlin-deficient mouse expressing this isoform was completely rescued in these mice.

Protein

- Dysferlin is a transmembrane protein of about 236 kD (depending on isoform). The dominant isoform in skeletal muscle contains 2080 amino acids.
- In addition to skeletal muscle, dysferlin is expressed in a number of other tissues. For instance, it is expressed in monocytes (white blood cells). This allows for RNA sequencing and protein expression studies from a blood sample without requiring a muscle biopsy.
- A few good diagnostic antibodies have been developed for the dysferlin protein which can be used in multiple techniques (western, IHC, pull-downs, FACS, etc).
- In muscle fibers, dysferlin is localized to the sarcolemma, and bracketing the Z-lines, where it is thought to be associated with the triad junction. Often, there is also diffuse localization inside the muscle fiber, possibly associated with internal vesicles.
- The dysferlin protein contains about ten functional domains spanning the length of the protein. In designing a mini-gene or exploring the possibility of exon skipping, it isn't as obvious as with the dystrophin rod domain which parts of dysferlin, if any, could be excised while retaining function. Structural studies of dysferlin are occurring now, but at present there isn't a complete, experimentally validated picture of dysferlin's folding structure.
- Studies in muscle tissue from dysferlinopathy patients or dysferlin-deficient mice point to a role for dysferlin in membrane repair. However, expression of a mini-version of dysferlin that restores sarcolemma repair function in a dysferlinopathy animal models fails to stop all aspects of the dystrophic phenotype such as centronucleation and muscle necrosis. This suggests that dysferlin plays other critical roles in muscle function. Other functional areas dysferlin has been linked to include Ca2+ homeostasis, vesicle trafficking, and membrane fusion. Which of these functions or combination of functions are the most relevant to the underlying pathophysiology of dysferlinopathy has yet to be determined.