

Causes of clinical variability in Duchenne and Becker muscular dystrophies and implications for exon skipping therapies

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Becker muscular dystrophy is caused by mutations in the *DMD* gene that permit significant residual dystrophin protein expression in patient muscle. This is in contrast to *DMD* gene mutations in Duchenne muscular dystrophy where little or no dystrophin is produced (typically < 3% normal levels). Clinically, Becker muscular dystrophy is extremely variable, from slightly milder than DMD, to asymptomatic hyperCKemia at old age. The factors driving clinical variability in Becker muscular dystrophy have now been studied in some depth, and the findings are likely highly relevant to anticipated clinical findings in exon skipping therapy in DMD. The specific mutations in Becker dystrophy play an important role, and clinical variability is less with high frequency mutations (deletions exons 45-47, 45-48). The percentage of dystrophin content in patient muscle is not well-correlated with clinical findings. Muscle MRI findings (degree of fibrofatty replacement) are very well-correlated with the degree of patient disability, regardless of mutation or muscle dystrophin content. Taken together, data to date suggest that the main determinant driving clinical disability in Becker dystrophy patients is the degree of fibrofatty replacement in muscle. Thus, as with DMD, *DMD* gene mutations and resulting dystrophin protein abnormalities initiate the disease process, but downstream tissue pathophysiology plays a dominant role in disease progression. Factors influencing the age-dependent rate of fibrofatty replacement of muscles are responsible for much of the clinical variability seen in Becker dystrophy, as well as Duchenne dystrophy. These fibrosis-related factors include genetic modifiers, degree of muscle inflammation, and induction of microRNAs in muscle that bind to dystrophin mRNA and down-regulate dystrophin protein content in patient muscle. Studies to date regarding clinical variability in Becker dystrophy suggest that exon skipping therapy in DMD may show variable efficacy from patient to patient.

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Conflict of interest

The author is a stock holder and holds management roles in ReveraGen BioPharma, AGADA BioSciences, and TRINDS LLC.

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Genotype/phenotype correlations in Becker dystrophy (or lack thereof)

Often genotype/phenotype correlations are discussed as ‘good’ or ‘bad’ – there is a strong correlation of specific genotypes with phenotypes, or not strong. The *DMD* gene is the largest in the human genome, and also complex with multiple transcriptional initiation points (alternative promoters), over 80 exons, all spread out over 2 megabases of the X chromosome (Fig. 1). With such complexity, and the highest spontaneous mutation rate of any gene (1 in 10,000 sperm and eggs), genotype/phenotype correlations might be expected to be more nuanced than “good or bad”.

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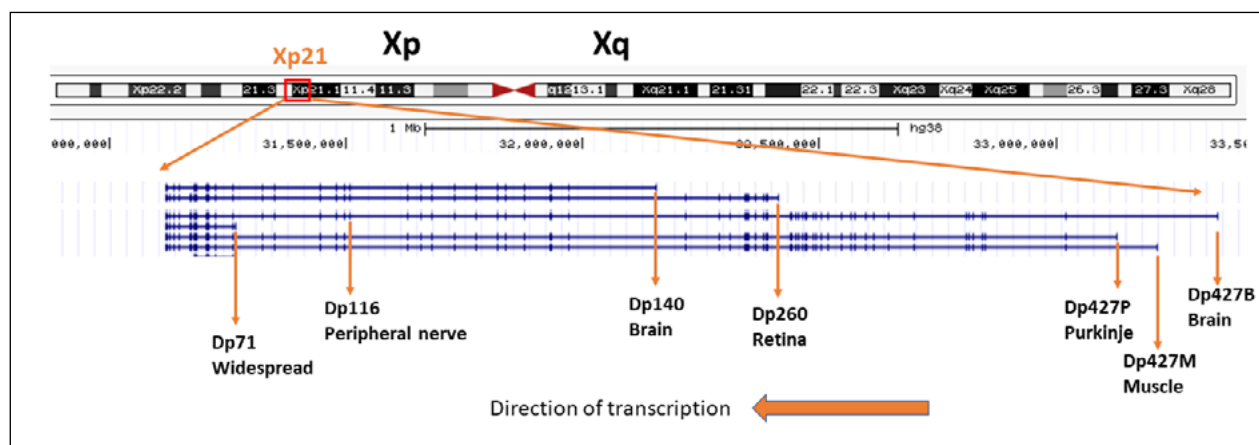


Figure 1. Schematic of the *DMD* gene. Shown is a genome browser visualization (www.genome.ucsc.edu), with labels added for the 6 different gene promoters, resulting mRNAs, and encoded proteins. Gene mutations in the first half of the gene may affect dystrophin expression driven from the first 3 promoters driving 'full length' dystrophin (Dp427B, Dp427M, Dp427P), but leave intact expression of downstream promoters (Dp71, Dp116, Dp140). Mutations located in the second half of the gene may disrupt expression of most or all dystrophin mRNAs and proteins driven from all promoters.

Excellent reviews of genotype/phenotype correlations in the dystrophinopathies have been published for both skeletal muscle and cardiac disease¹⁻⁵. Dystrophin isoforms and pathologies in non-muscle tissues have also been well-described, such as retina⁶, peripheral nerve⁷, vascular smooth muscle⁸⁻⁹, intestinal smooth muscle¹⁰⁻¹¹. Assessments of dystrophin mRNA transcript levels in human tissues show relatively high levels in most tissues with smooth muscle layers (visceral and vascular), skeletal muscle, cardiac muscle, and peripheral nerve (Fig. 2). Specific mutations should affect different dystrophin mRNAs, and cell-specific dystrophin function in variable ways. Indeed, the genotype/phenotype correlations of *DMD* gene mutations with the latter half of the gene showing greater cognitive involvement, and correlation with 3' mRNAs expression in brain, are quite compelling^{12,13}.

With DMD and BMD mutations distributed throughout the *DMD* gene, affecting changes to the dystrophin mRNA and protein in different ways, sometimes isoform-specific, in multiple organ systems (skeletal muscle, cardiac muscle, smooth muscle, peripheral nerves and neurons), things are 'quite complicated'. Thus, the response to the question of the reliability of genotype/phenotype correlations in the dystrophinopathies is not so much 'good or bad', but instead, "Well, it's complicated".

The definition of a syndromic disorder is that multiple organ systems are affected. To date, the dystrophinopathies have been defined as a disease restricted to skeletal muscle, and hence called a non-syndromic 'muscular dystrophy'. It is probably time to re-evaluate this and

acknowledge that both DMD and BMD are syndromic disorders, with involvement of multiple organ systems. This is likely an important distinction; the clinical phenotypes of syndromic disorders are acknowledged to be the interactive summation of perturbations of multiple organ systems. It is increasingly likely that the DMD and BMD phenotypes are interactive summation of perturbations of skeletal muscle, vascular smooth muscle, visceral smooth muscle, heart, peripheral nerve and central neurons. A corollary of this logic is that the *DMD* gene mutations initiate a process, but the downstream events leading to a phenotype are quite complex and variable; typical of syndromic disorders. Or stated in another way, if one views DMD and BMD as non-syndromic muscular dystrophies, then one could view the genotype/phenotype correlations as 'poor'. On the other hand, if one views DMD and BMD as syndromic disorders, the genotype/phenotype correlations could be considered 'quite good'.

What are known of drivers of clinical variability?

Becker muscular dystrophy is extremely clinically variable. Certainly, the gene mutation and resulting perturbations of the dystrophin protein are a major component of this variability (e.g. the extent to which dystrophin function is retained). However, it is possible to hold the BMD mutation constant with the most common mutations (deletion exons 45-47, and exons 45-48). Excellent correlative studies of genotype/phenotype, dystrophin content of muscle, MRI findings, and clinical symptoms

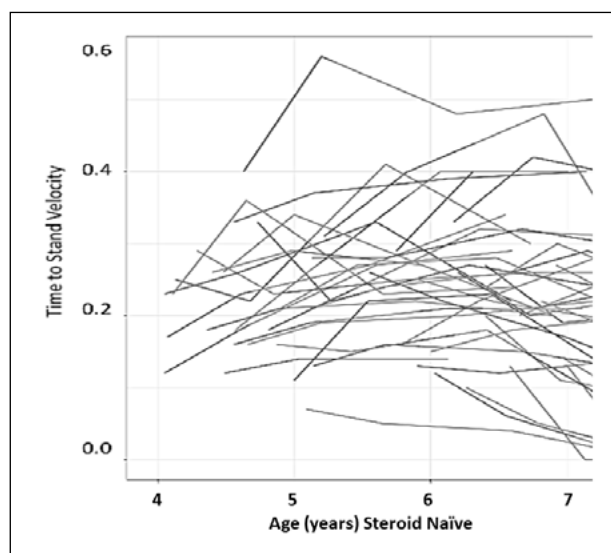


Figure 3. Clinical variability in 48 steroid-naïve Duchenne muscular dystrophy boys, age 4 to < 7 years. Shown is Time to Stand velocity (1/event) in DMD boys enrolled into vamorolone clinical trials. Extensive variability in disease severity is seen, with a 6-fold difference in velocity from the most severe to the mildest patient (from Dang et al., 2020 and Smith et al., 2020, mod.)^{38,39}.

and resulting dystrophin production is not irrelevant, as it clearly initiates the process. Critical here is relatively low levels of dystrophin seen with certain out-of-frame ‘leaky’ mutations can mitigate the severity to some degree (~3-4%), although generally not to the point where the patient would be clearly characterized as a mild/moderate Becker patient. For example, an intermediate DMD/BMD phenotype was seen in a patient with an out-of-frame deletion that expressed low levels of dystrophin (~4%), and also had the LTPB4 rare genotype associated with milder disease²³. Also, certain deletions seem generally ‘leaky’ permitting some low-level dystrophin, and many of these patients show later loss of ambulation. For example, exon 44 skippable mutations have about 3-5 year later loss of ambulation²⁴⁻²⁷, whereas exon 51 skippable about a 2-year earlier loss of ambulation²⁶⁻²⁷. Mutations involving the initial exons of the dystrophin gene (e.g. del exons 3-7) are known to show mRNA translation of dystrophin at alternative AUG initiation codons, and thus result in dystrophin production despite an out-of-frame mutation.

The multiple studies of genetic modifiers of loss of ambulation in DMD have been increasingly robust and relatively corroborative, considering that such genetic studies are challenging in rare diseases (lack of statistical power). Indeed, the ability to identify and replicate genetic modifiers in DMD suggests that the ‘effect size’ of the modifiers is surprisingly large (hence easy to detect). It

also speaks to the importance of the TGF β fibrosis pathways, as the majority of genetic modifiers modulate this pathway. It can be concluded that genetic modifiers lead to differences in the speed of the transition of skeletal muscle from successful regeneration to failure of regeneration (and fibrofatty replacement).

Effect size of genetic modifiers can be quantitated in DMD by the number of years change in mean age at loss of ambulation (Fig. 4). Genetic modifiers to date all show about 1-2 years change in mean age of loss of ambulation (where mean age is LOA at around 11-12 years). One must consider the genotype frequencies, the inheritance model (dominant [SPP1, CD40], or recessive [LTBP4]), and the calculated percentage of DMD boys that have the “at-risk” genotype (Fig. 4). These allele frequencies also vary in different ethnicities and world populations. For example, in China the SPP1 genotype associated with earlier loss of ambulation in Europeans is at a very low allele frequency, and thus does not show significant association with LOA in Chinese DMD boys. However, a different SPP1 promoter at high allele frequency in Chinese DMD boys shows highly significant association with LOA. In effect, one might consider this an independent validation of the importance of SPP1 (osteopontin) in the progression of DMD²¹.

Data emerging in genetic modifiers of heart involvement DMD and BMD are quite interesting. Dystrophin-deficient cardiac tissue shows distinct pathology and functional deficits compared to skeletal muscle. Heart does not show the repeated bouts of degeneration and regeneration, and fibrotic replacement is slowly progressive and limited initially to basolateral free wall of the left ventricle (likely due to inflammation and death of myocytes where functional load on the heart tissue is highest)²⁸. Also, dystrophin driven from the brain promoter can compensate for a deleted muscle gene promoter in skeletal muscle but not cardiac muscle²⁹. One might expect some genetic modifiers to be consistent between dystrophin-deficient heart and skeletal muscle, and some distinct. Consistent with this, genetic modifiers can be found that are specific to heart³⁰, and also shared with skeletal muscle³¹.

Pulling variables together into models of DMD and BMD disease progression

For DMD, the initiating event is loss of dystrophin in multiple tissues that likely interact to lead to gradual fibrotic replacement of skeletal muscle, and, later, heart. The initiating event is relatively homogenous, and generally leads to loss of ambulation about 11-12 years of age. Genetic modifiers, low level dystrophin ‘leakiness’ of the

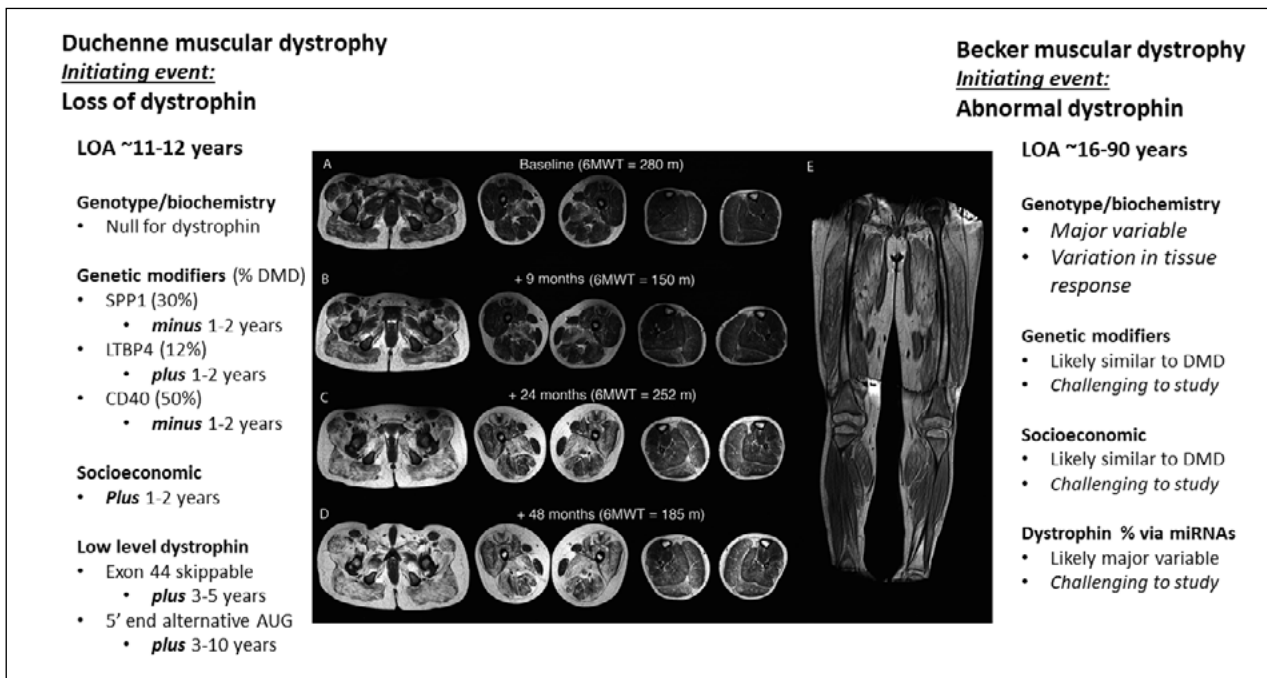


Figure 4. Factors contributing to clinical variability in Duchenne and Becker muscular dystrophies. Shown is a schematic of progressive MRI findings, with gradual fibrofatty replacement over time (from Godi et al., 2016, mod.)¹⁷. The progressive clinical phenotypes of both Duchenne and Becker muscular dystrophies is driven predominantly by the extent of fibrofatty replacement of muscle. The factors driving the fibrofatty replacement are listed for both Duchenne and Becker dystrophy, as discussed in the text. *Plus* and *minus* refer to later or earlier age at loss of ambulation.

mutation, and socioeconomic status³² all contribute to increased severity or decreased severity relative to this ‘average’ (Fig. 4). For each genetic modifier, the inheritance pattern, and the allele frequency can be calculated from the published papers, and define whether the ‘rare allele’ genotype causes earlier loss of ambulation (e.g. minus 1-2 years), or later loss of ambulation (e.g. plus 1-2 years) (Fig. 4). Indeed, all genetic modifiers have been found to change the age of loss of ambulation by 1-2 years, with SPP1 and CD40 rare allele causing a more severe progression (minus 1-2 years) (both dominant inheritance patterns), and LTBP4 rare allele a milder disease progression (plus 1-2 years; recessive inheritance pattern). Likewise, the effect size of low level dystrophin expression due to exon 44 skippable mutations can be quantified (3-5 years later LOA), the effects of alternative AUG use and dystrophin expression in 5’ mutations (plus 3-10 years), and the effects of socioeconomic factors (minus 1-2 years for low socioeconomic status³² (Fig. 4). This begins to paint a more complete picture of factors influencing clinical severity in DMD, and also points to the multi-variate and complex nature of these factors.

For BMD, the initiating event of abnormal dystrophin is a much greater ‘driver’ of disease variability in onset and progression than it is in DMD where lack of

dystrophin is held constant. The great heterogeneity of gene mutations resulting in many different abnormal dystrophin proteins with variable residual function leads to variability in the initiation and progression of disease. Moreover, it could be expected that different dystrophin-expressing tissues and cells may respond differentially to specific abnormal dystrophins. For example, the abnormal dystrophin resulting from a deletion of exons 45-47 may lead to some cellular and physiological abnormalities in skeletal muscle, but different abnormalities in smooth muscle. Thus, the ‘syndromic’ nature of dystrophinopathies may be accentuated in Becker dystrophy due to differential perturbations of different tissues (Fig. 4).

It is highly likely that the same genetic modifiers and effects of socioeconomic status observed in DMD are relevant to BMD as well. That said, it is nearly impossible to study and prove effects of genetic modifiers and socioeconomic status in Becker dystrophy. This is because the major effects of genotype and biochemistry (abnormal dystrophin) on clinical symptoms in Becker dystrophy creates extensive population stratification, leading to precipitous loss of statistical power. To detect the effects of genetic modifiers and socioeconomic status on phenotype in Becker dystrophy would require the study of cohorts of a single

Becker mutation (e.g. only those with either of the common BMD mutations; e.g. del 45-47 or del 45-48). One would not want to mix the del 45-47 and del 45-48 patients as they have different dystrophin proteins (and this is too much of a variable). As each of these two genotype groups is only about 15% of all Becker patients, it will be challenging to assemble such cohorts. Until these studies can be done, it is probably safe to assume that genetic and socioeconomic modifiers of DMD are shared in BMD (Fig. 4).

A variable that may drive clinical severity in Becker dystrophy that is not relevant to DMD is effects of microRNAs. The dystrophin mRNA has a very large number (~80) putative microRNA binding sites that have the potential to individually and/or collectively decrease protein translation from the dystrophin mRNA, and thus in turn lead to decreased amounts of dystrophin (in muscle, heart, smooth muscle or nerve)³³⁻³⁵. It has been well-established that inflammation in muscle leads to induction of inflammation-associated microRNAs, and these in turn bind to the dystrophin mRNA and decrease dystrophin protein content in muscle.

This inflammation/microRNA/dystrophin pathway seems to explain the highly variable dystrophin levels seen in Becker dystrophy patients, even when controlling for the same causative exon 45-47 mutation³³. Further, inflammatory disease of muscle unrelated to dystrophinopathy appears to have the same pathway active, leading to reductions of dystrophin in skeletal muscle secondary to inflammation³⁵. It is intuitively attractive to consider that the microRNA pathway has effects on Becker patient disease severity, assuming that 'more dystrophin is better' (Fig. 4). However, there is no evidence for this. Indeed, in contrast, there is little evidence that dystrophin levels between 20-100% normal are in any way correlated with clinical severity in Becker dystrophy^{14,36}. Muscle biopsy studies include a high degree of 'sampling error', where only a small area of a single muscle, in a single stage of disease is studied for dystrophin protein amounts. Thus, a single biopsy is not representative of dystrophin levels in the patient as a whole. The lack of correlations of dystrophin content in Becker patient muscle biopsies with clinical phenotypes does not rule out the potential importance of the microRNA/dystrophin pathway. As with genetic modifiers, carrying out studies to prove the contribution of microRNA-mediated reductions in dystrophin in Becker patients would be important, but studies will be challenged to control for the mutation/protein stratification problem noted above.

Relevance of BMD models of clinical severity to exon skipping therapy in DMD

There are now 3 exon skipping drugs approved by the FDA in the USA, targeted towards exon 51 (Etiplersen, Sarepta), and exon 53 (Golodirsén, Sarepta; Viltepsó, NS

Pharma). The Sarepta drugs led to a mean ~1% normal dystrophin levels in treated DMD patient muscle (https://www.accessdata.fda.gov/drugsatfda_docs/nda/2019/211970Orig1s000SumR.pdf; https://www.accessdata.fda.gov/drugsatfda_docs/nda/2016/206488Orig1s000SumR.pdf).

The NS Pharma drug showed induction of a mean ~6% (https://www.accessdata.fda.gov/drugsatfda_docs/nda/2020/212154Orig1s000SumR.pdf). The NS Pharma drug has shown preliminary evidence of clinical benefit in 16 boys treated with viltolarsén, with drug-related improvements in multiple clinical motor outcomes³⁷. All 3 drugs were approved under an "accelerated" pathway dependent on surrogate outcome measures (dystrophin protein by immunoblot).

In the context of the above discussion regarding the variables contributing to clinical variability in both Duchenne and Becker dystrophies, it may be instructive to interpret the status and promise of exon skipping in DMD. First, one can interpret existing exon skipping data in the context of clinically meaningful dystrophin levels. Data to date suggests that dystrophin levels ~3 – 10% of normal levels typically lead to an intermediate phenotype between Duchenne and Becker muscular dystrophies (sometimes called "severe Becker dystrophy")^{14,36}. This is consistent with the ~3% dystrophin content of a leaky exon 44 mutation²³. Given this data, the two Sarepta drugs with ~1% dystrophin would not be expected to show significant clinical benefit. The report of a mean ~6% dystrophin induced from the NS Pharma drug is consistent with preliminary evidence of clinical benefit³⁷.

Assuming that exon skipping drugs were able to produce 3% or more of Becker-like dystrophin, one expects the extensive variability in patient clinical response given all the variables influencing clinical variability in Becker muscular dystrophy (Fig. 4). Functionalities of specific abnormal dystrophins, levels of dystrophin, microRNA/dystrophin pathways, genetic modifiers, and socioeconomic status are all likely to factor relatively heavily into the clinical response to exon skipping.

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