What we know about the biology and variability of Duchenne

Parent Project Muscular Dystrophy
JOIN THE FIGHT. END DUCHENNE.

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Nationwide Children's Hospital

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Disclosures

- Site principal investigator for PTC Therapeutics, Prosensa, Abeona Therapeutics, Akashi, and the NIH FOR-DMD study; site co-investigator for Sarepta

- Advisory boards for Sarepta, PTC, Audentes, Eli Lilly, 4D Therapeutics, and Dynacure

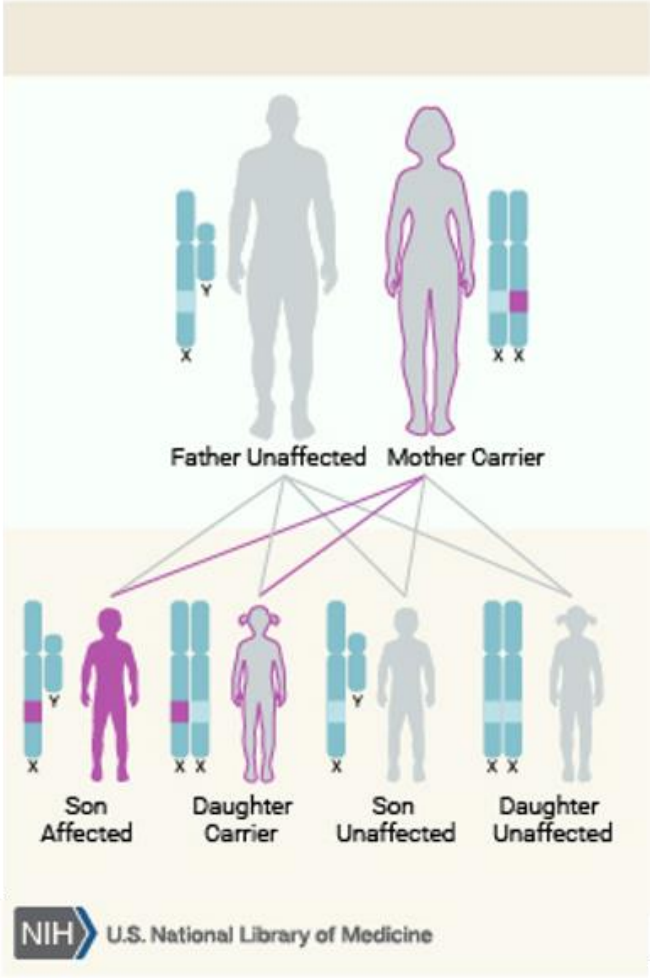
Dystrophinopathies: Duchenne and Becker muscular dystrophies

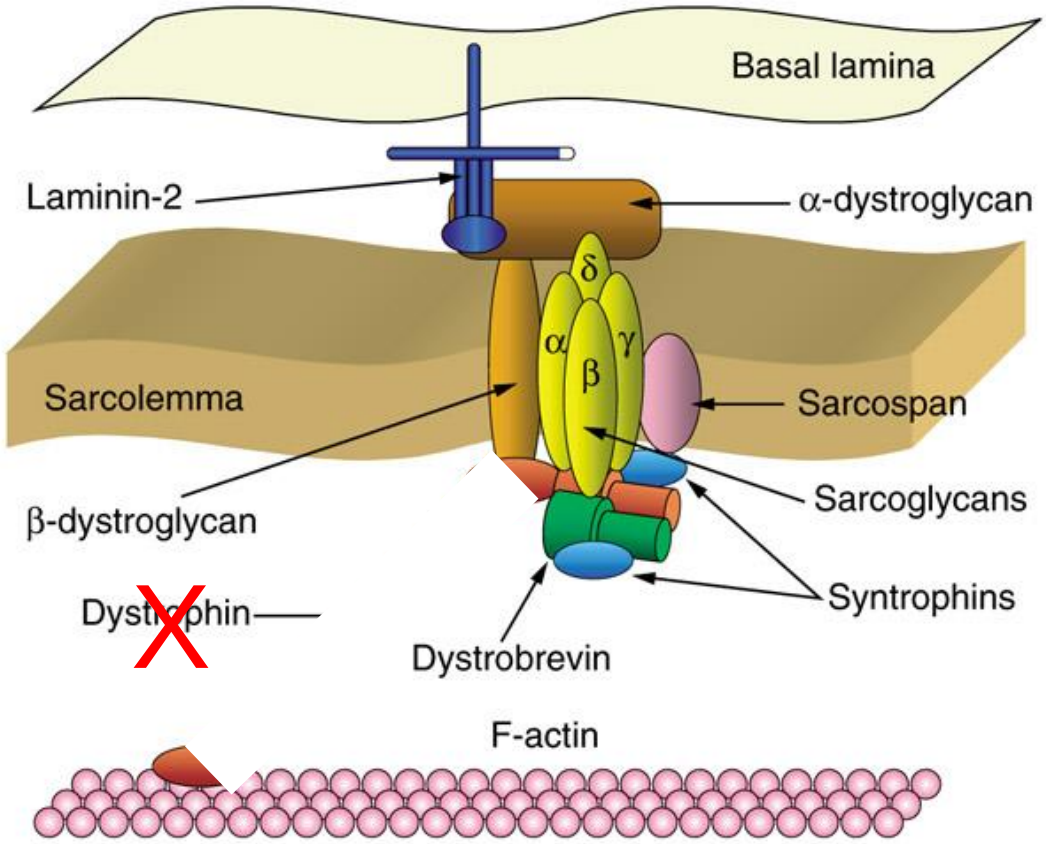
- Duchenne muscular dystrophy (DMD):
  - Onset age 3-5
  - Pelvic girdle weakness
  - Tight heel cords
  - CK 50-100X normal
  - Loss of ambulation by age 12 (range 7-12)
  - Death by age 20 (historically)

- Becker muscular dystrophy (BMD):
  - Classic definition: loss of ambulation > age 12
  - Alternatively:
    - “intermediate muscular dystrophy” for loss of ambulation ages 12 through15
    - BMD for loss of ambulation >age 15
  - Limb-girdle syndromes in adulthood
  - Muscle aches (myalgias)
  - Isolated cardiomyopathy

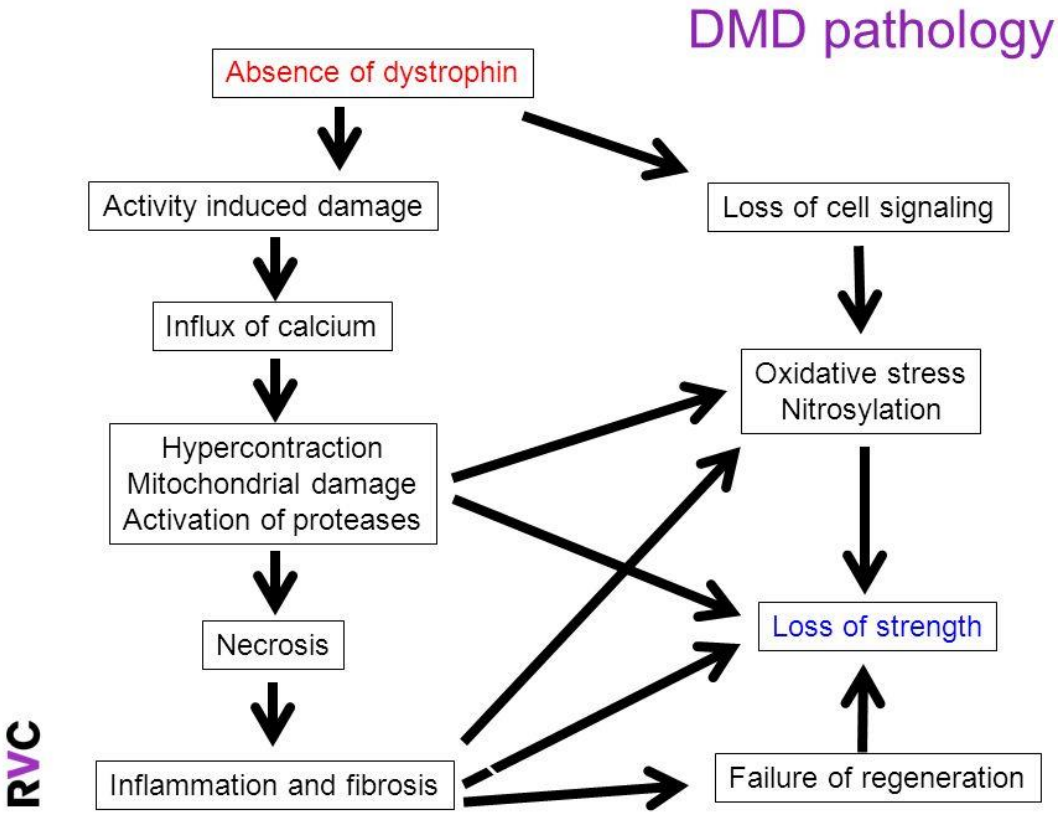
X-linked Recessive Inheritance

- 1/3 of cases occur *de novo* – that is, as a brand new mutation





Roberts, *Genome Biology*, 2001



Dominick Wells,
Royal Veterinary College

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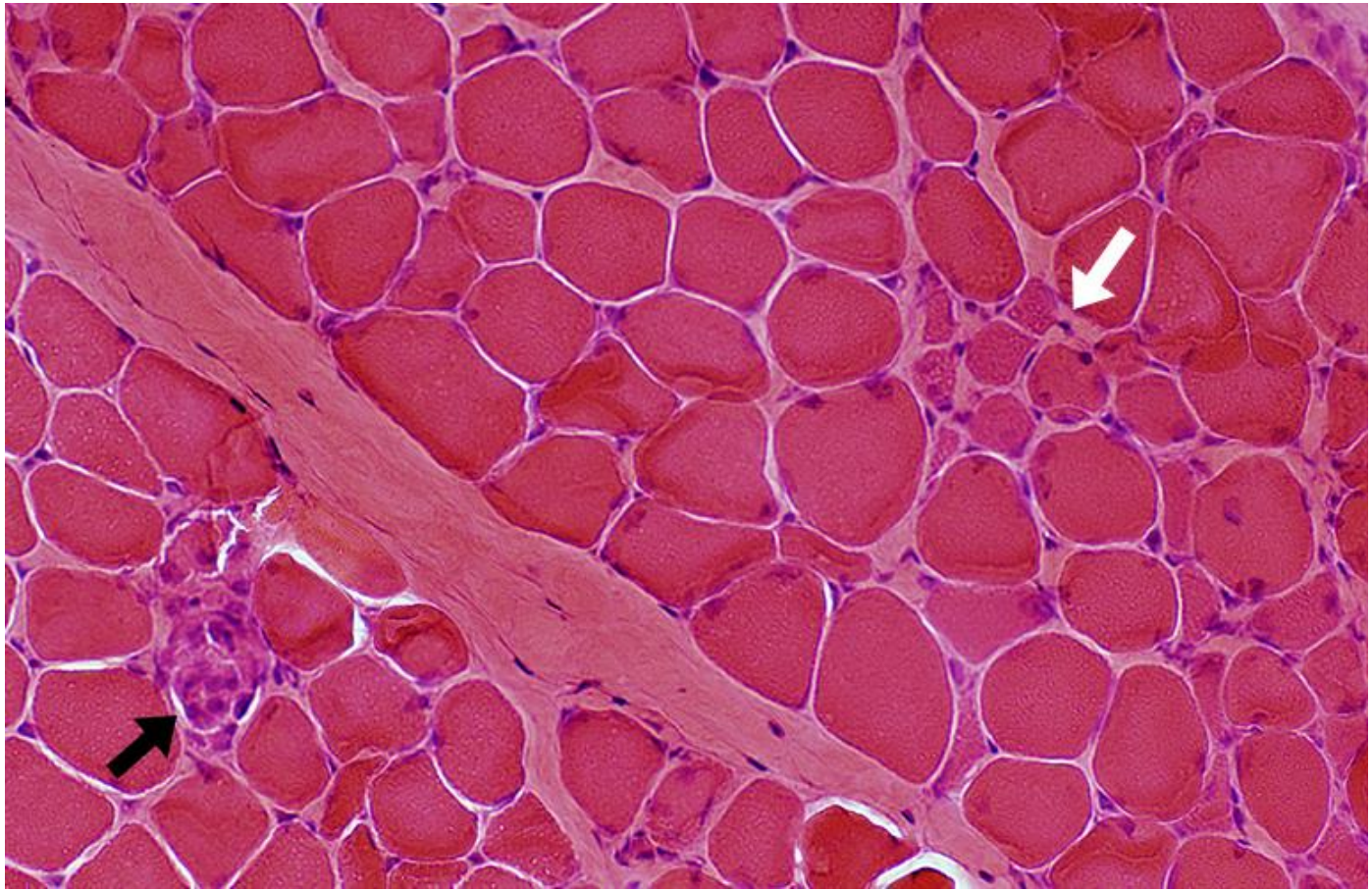
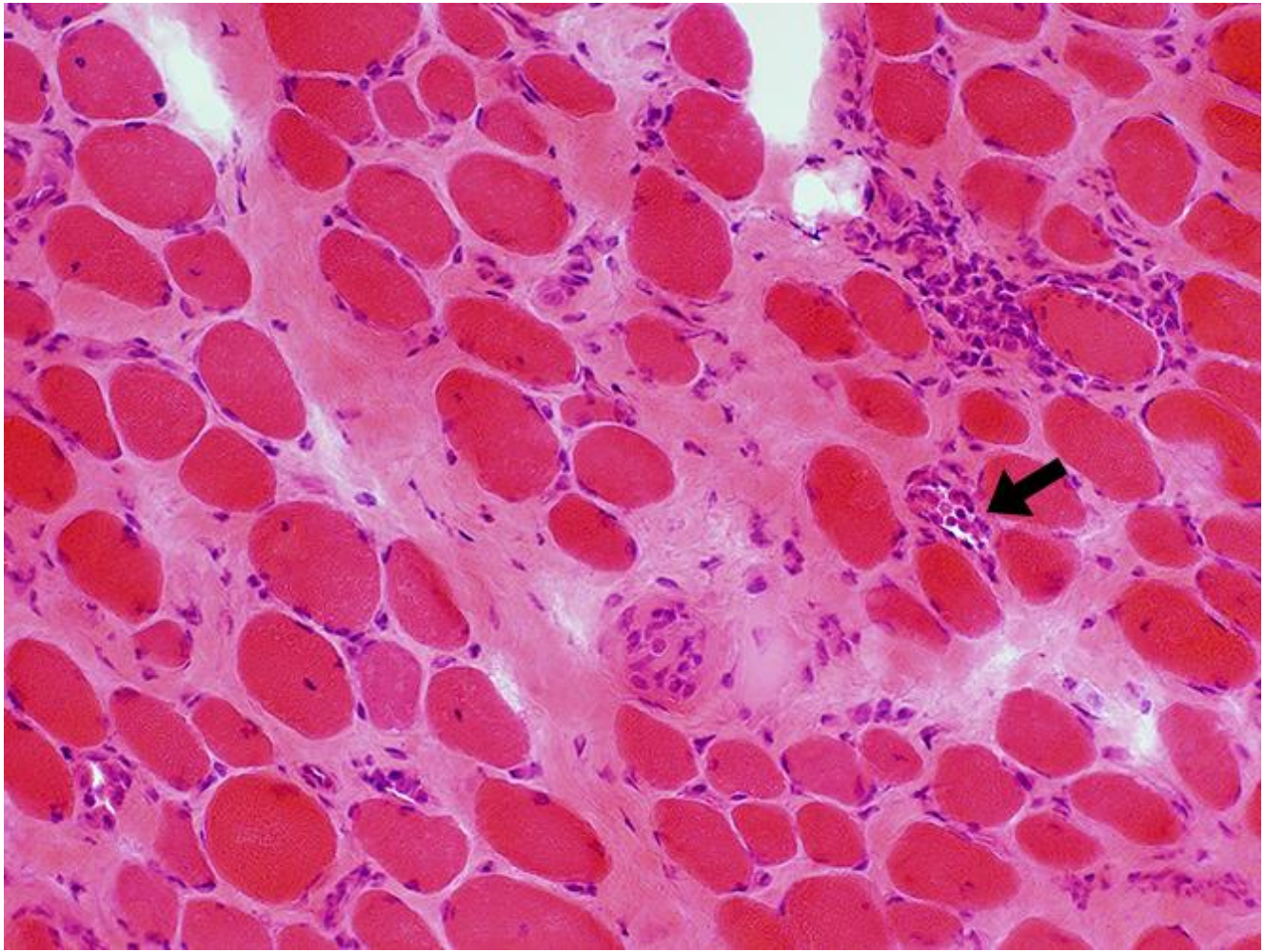


Washington University Neuromuscular Website



Washington University Neuromuscular Website

Dystrophin Mutations

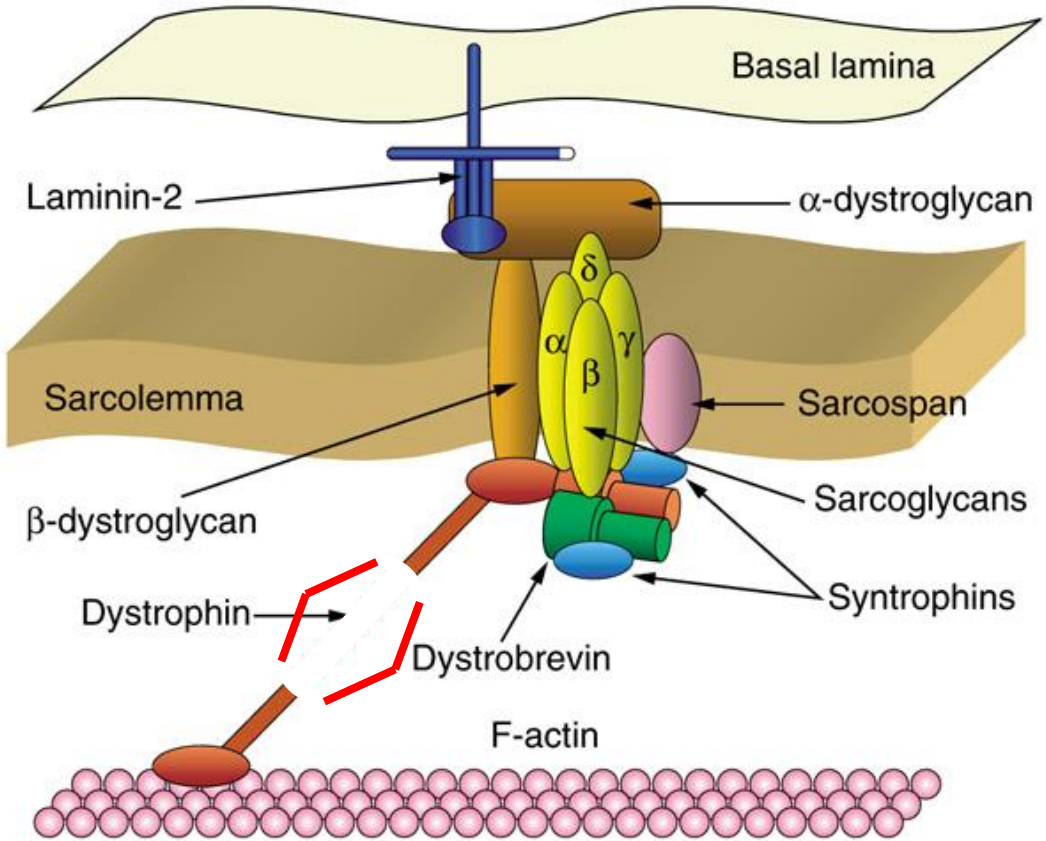
- Dystrophin gene (Xp21.1) is huge:
  - 2.4 million nucleotides
  - 79 exons and 8 promoters

- Large deletions (≥ 1 exon) account for ~65% of DMD/BMD patients

- ~5% have duplications
- ~15% of boys have nonsense mutations
- Remainder are frameshifting insertions/deletions, splice site mutations, missense mutations

Dystrophin mutations: Duchenne vs Becker

- **Size** of deletion does not correlate well with phenotype
- Best correlation is whether the deletion is “**in-frame**” or “**out-of-frame**”
- **In-frame deletions** are more likely to result in translation of a protein with partial function
  - (i.e., out-of-frame deletions are DMD ~90% of the time)



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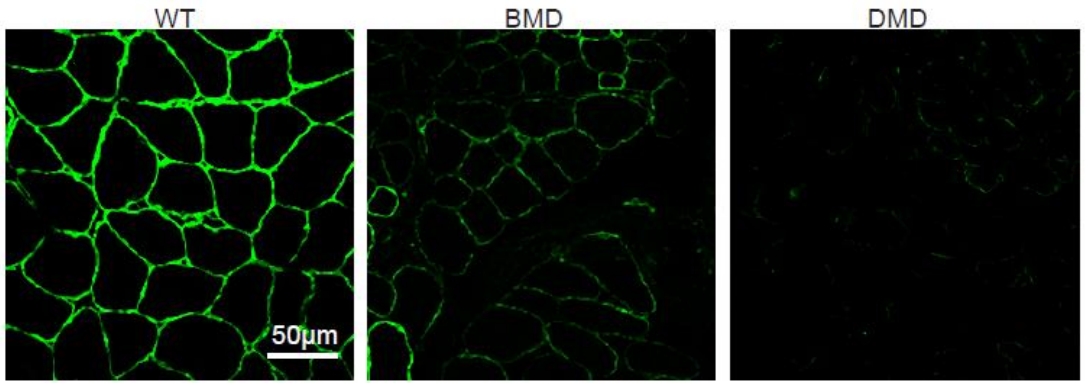
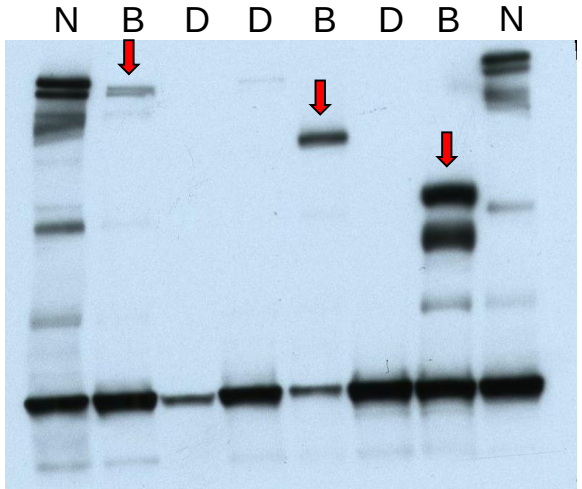




Wein et al, Pediatric Clin North Am 2015 Jun; 62(3):723-742

What accounts for patients who break the reading frame rule?

What accounts for variability among patients with DMD?

Reading Frame Rule in DMD/BMD

Table 2. The Value of Mutational Reading Frame in Predicting a Phenotype of Duchenne Muscular Dystrophy

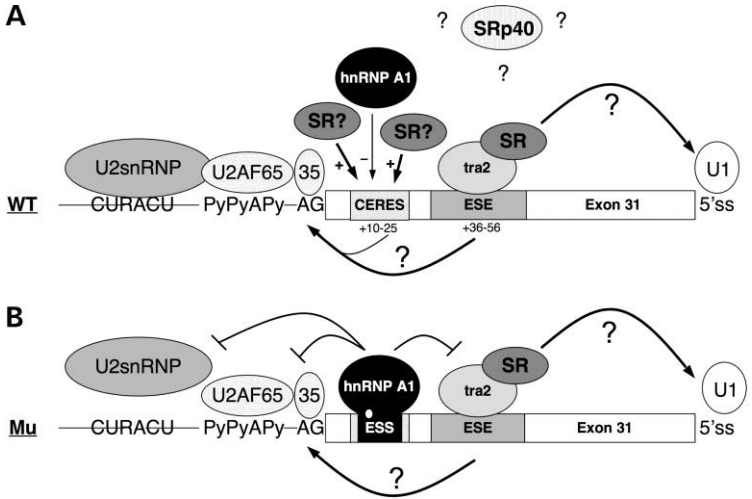
	DMD	I/BMD		
Exonic deletions only				
Truncating (out-of-frame) mutations	254	32	*88.8%*	*Positive predictive value*
Non-truncating (in-frame) mutations	30	38	*55.9%*	*Negative predictive value*
Sensitivity	89.4%	—		
Specificity	—	54.3%		
All mutations[a]				
Truncating mutations	519	79	*86.8%*	*Positive predictive value*
Non-truncating mutations	37	63	*63.0%*	*Negative predictive value*
Sensitivity	93.3%			
Specificity	—	44.4%		

45%-55% of BMD patients have out-of-frame mutations

Flanigan KM, et al. *Hum Mutat*. 2009;30(12):1657-1666

Nonsense Mutations Do Not Always Predict DMD

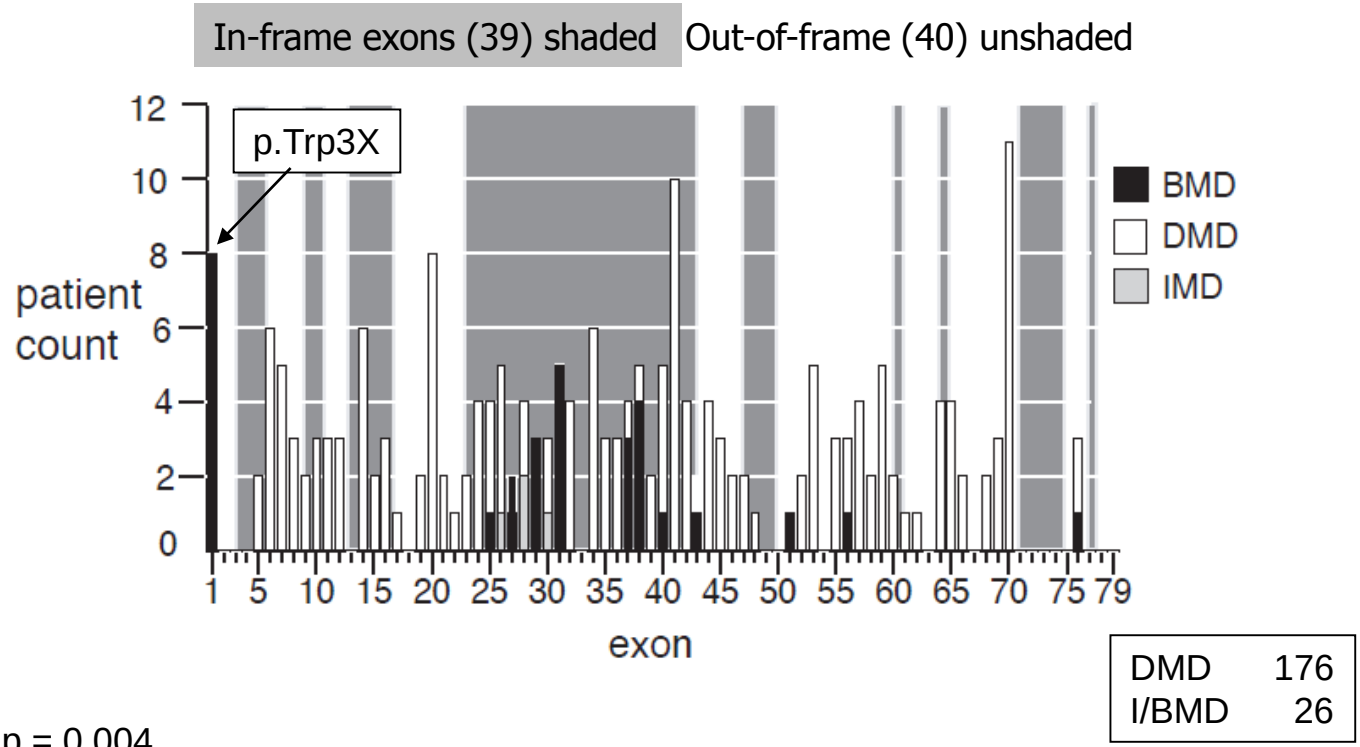
- Mutations predicted as nonsense mutations may instead affect exon splice regulatory signals[1,2]
  - This results in exclusion of exons
  - The remaining mRNA may be in-frame



Disset A, et al. *Hum Mol Genet.* 2006;15(6);999-1013.

1. Disset A, et al. *Hum Mol Genet.* 2006;15(6);999-1013.
2. Flanigan KM, et al. *Hum Mutat*. 2011;32(3):299-308.

Distribution of
BMD versus DMD nonsense mutations



p = 0.004

A little bit of dystrophin goes a long way

Available online at www.sciencedirect.com

ScienceDirect

Neuromuscular Disorders 28 (2018) 116–121

www.elsevier.com/locate/nmd

Case report

Low-level dystrophin expression attenuating the dystrophinopathy phenotype

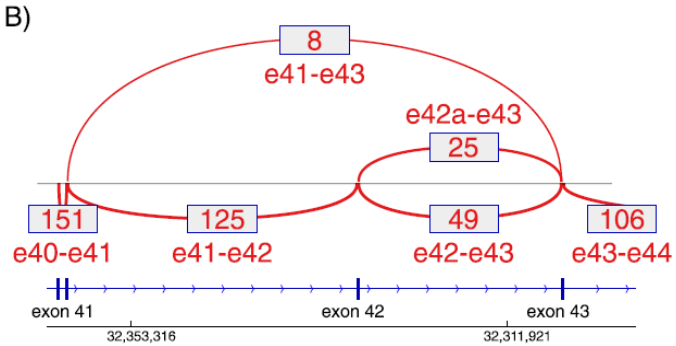
Megan A. Waldrop [a,b,c], Felecia Gumienny [a], Saleh El Husayni [d], Diane E. Frank [d], Robert B. Weiss [e], Kevin M. Flanigan [a,b,c,*]

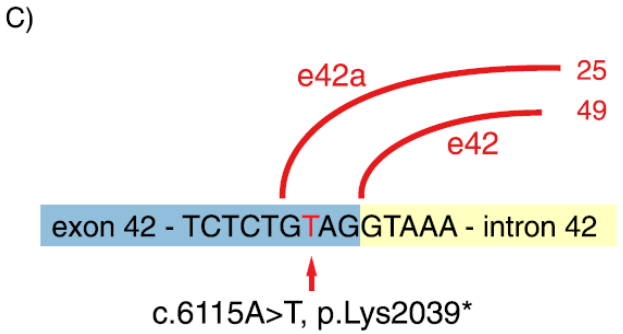
[a] *The Center for Gene Therapy, Nationwide Children's Hospital, Columbus, OH 43205, USA*

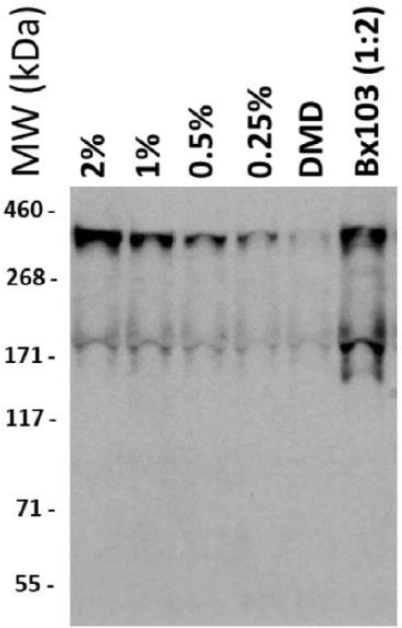
- 10 year old boy
- Toe-walking at age 7 years
- Diagnosed at age 9 years
- Predicted nonsense mutation in exon 42

- 6 minute walk distance = 575 meters
  - 157% predicted for age matched DMD patients
  - Normal controls = 630 meters
- North Star Ambulatory Assessement Score = 30/34

A little bit of dystrophin goes a long way







3.2% of normal dystrophin level is sufficient to greatly modify the disease

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Other genes modify other pathways and can influence disease severity

- Osteopontin (*SPP1)*
  - Cytokine involved in immune cell migration and survival
  - Implicated in fibrosis through the TGF-beta pathway
- Latent TGF-beta binding protein 4 (*LTBP4*)
  - Binds and sequesters TGFβ
  - Polymorphisms influence TGFβ activity
- *CD40*
  - co-stimulatory protein involved in T helper cell polarization
  - found on the surface of antigen-presenting cells
- *THBS1* (thrombospondin-1)
  - Upstream regulator of the TGFβ pathway

Takeaways

Takeaways

- DMD results from the absence of dystrophin
  - Mutations that allow a small amount of dystrophin can influence severity
- Not all nonsense mutations result in DMD
- We are learning about other genes that influence pathways important to disease progression
  - These may prove to be additional targets for interventional therapies


Questions?

Thank you!