MUSCLE BIOPSY AND RNA SEQUENCING TO DIAGNOSE MUSCULAR DYSTROPHIES

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CARRELL KRUSEN NEUROMUSCULAR SYMPOSIUM
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KU MEDICAL CENTER
The University of Kansas

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HEALTH
UNIVERSITY OF UTAH

CLINICAL HISTORY

- A 4-year-old male presented to clinic with a 1-year history of abnormal gait, frequent falls, difficulty climbing stairs, and thigh pain.
- Serum CK was 11,000 IU/l
- PMHx: Patient was product of a normal spontaneous vaginal delivery of a full term birth. Neonatal jaundice. Started walking between 18-24 months. Mild delayed speech
- FH: Unremarkable

PHYSICAL EXAMINATION

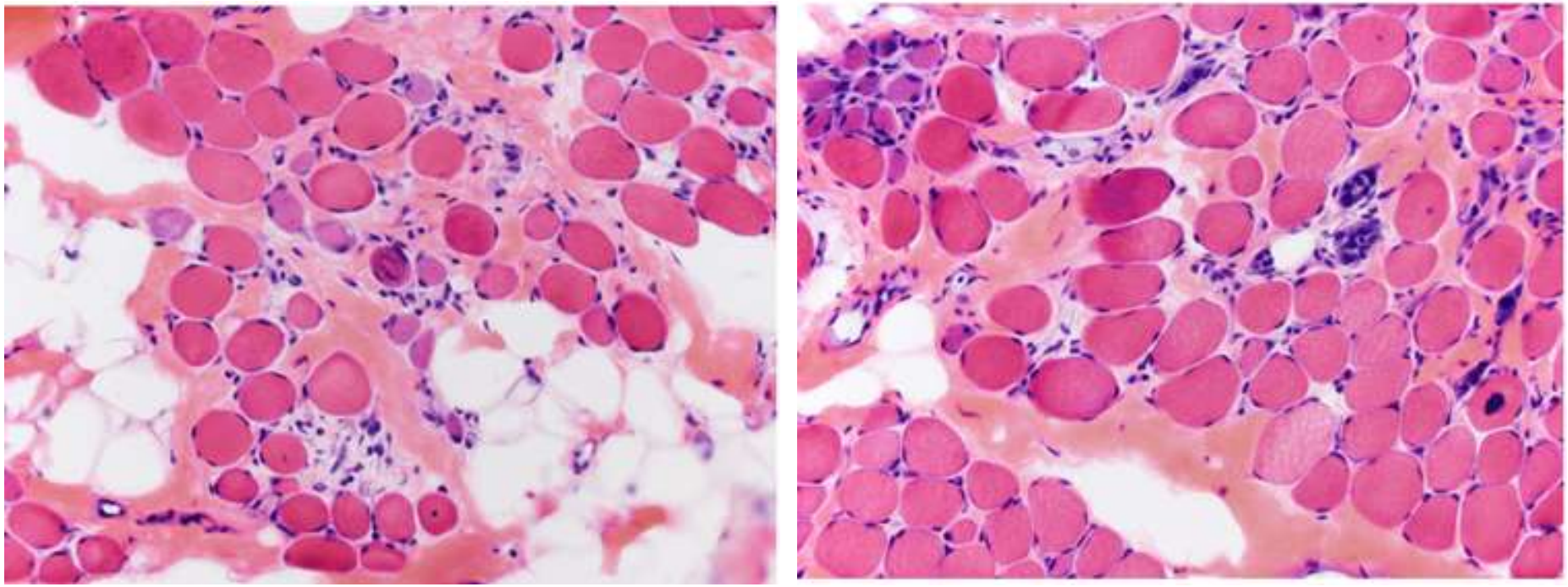
- Calf pseudohypertrophy
- Positive Gowers sign
- Toe walking
- Mildly lordotic gait

GENETIC TESTING

- Sequence analysis and deletion/duplication testing for muscular dystrophies was negative
- Two large Neuromuscular Next Generation panels at Invitae (Neuromuscular Disease Panel and Cardiomyopathy Panel) that included *DMD* and limb girdle muscular dystrophy genes were also negative

MUSCLE BIOPSY

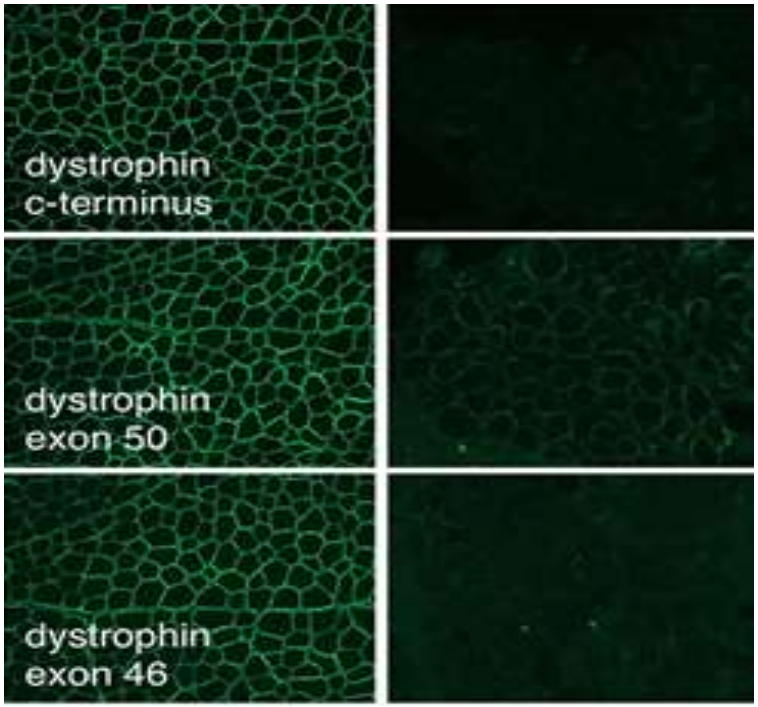
Dystrophic appearance



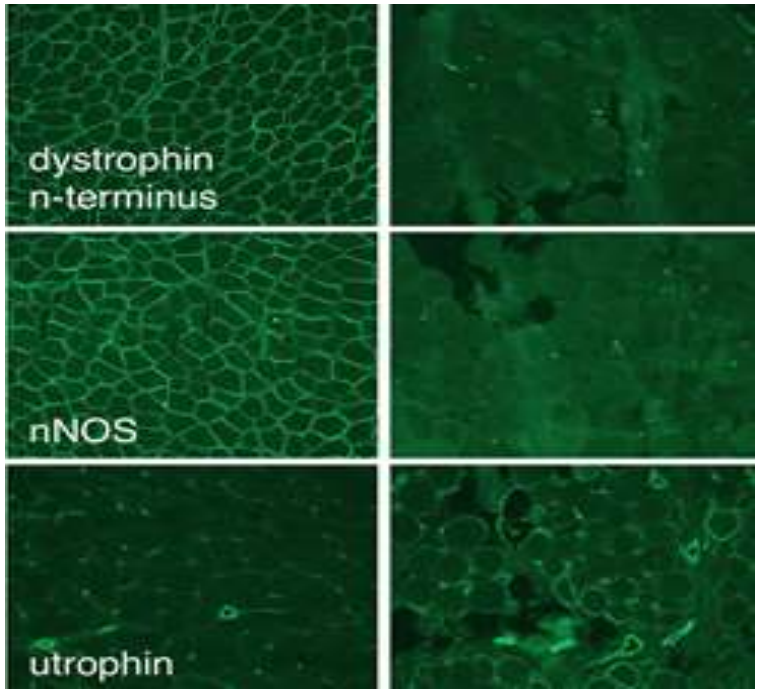
Left vastus lateralis muscle

IMMUNOFLUORESCENCE STUDY

CONTROL | OUR PATIENT



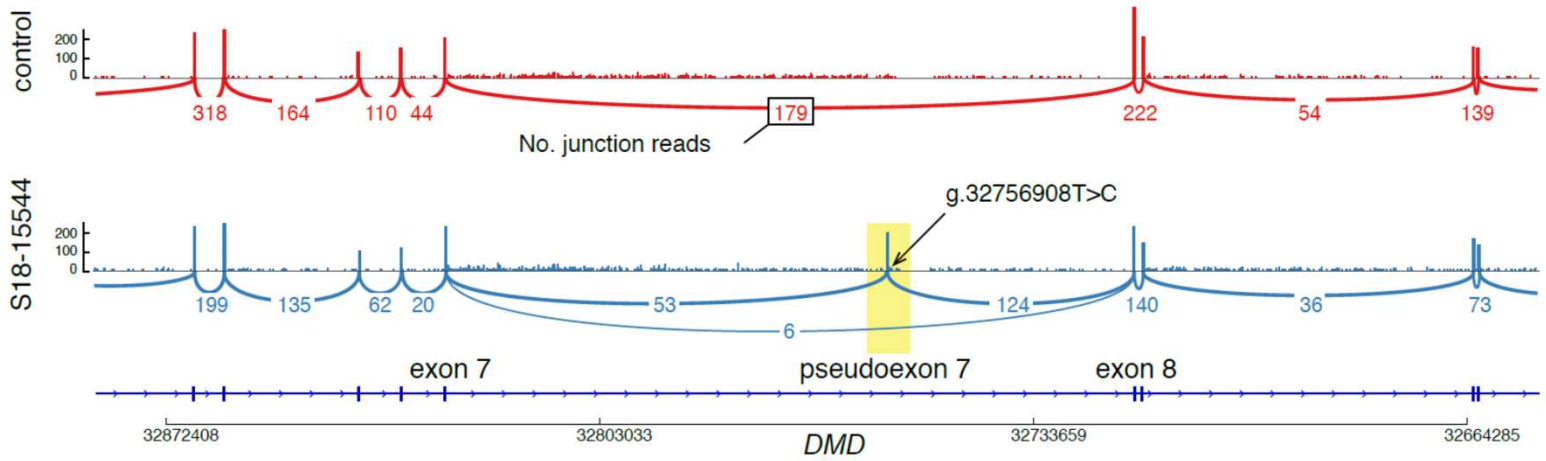
CONTROL | OUR PATIENT



Abnormal immunostaining pattern

Duchenne/Becker Muscular Dystrophy

RNAseq analysis



- RNA-seq results: Intron 7 cryptic splice donor mutation
- This mutation produces an inclusion of a 77 bp pseudoexon in intron 7 leading to a premature stop codon and, possibly, a truncated dystrophin protein
- The presence of a few normal reads suggest that a small amount of full length dystrophin may be made

CRYPTIC SPLICE SITE DONOR MUTATION (CSSD)

- Prevalence of DMD mutations:
  - 70% are deletions/duplications
  - 30% are “point mutations”
    - Non-sense, missense, frame-shifting small deletions or insertions, splice mutations
- 2-6% of point mutations correspond to CSSD mutations
  - Change in sequence in a non-coding region (intron), which adds a new splice site leading to the alteration of typical exons
  - One possibility is creating a new exon (pseudoexon)
    - Can lead to the inclusion of a premature stop codon, and thus, a production of a truncated protein
  - Depending on how strong a CSSD mutation is will determine the relative % with the pseudoexon (premature stop) and % normal dystrophin

Cryptic Splice Sites Donor Mutation Studies							
Studies	**Insertion between exons**	**Pseudoexon (bp)**	**Age at Onset**	**Symptoms**	**Cardiac Involvement**	**Intellectual Disability**	**Age at Ambulation lost**
Beroud, 2004[1]	60 - 61	89	18 mo.	-Gait abnormalities -Proximal weakness -Frequent falls -Macroglossia	X	X	15 yr.
	9 - 10	90	13 yr.	-Muscle cramps	X	--	40 yr.
	1M - 2	149	8 yr.	-Muscle cramps -Myalgia	X	--	--
	62 - 63	67	-Early symptoms: Neonatal hypotonia, feeding difficulties	-Difficulties running and climbing stairs -Frequent falls -Proximal muscle weakness -Hypertrophic calves	--	X	--
Trabelsi, 2014[2]	26	80	40 gog1	-Proximal muscular weakness -Calf hypertrophy -Elevated CK level	X	--	--
Greer, 2015[3]	45-47	82	Not mentioned	"Intermediate/ severe BMD clinical phenotype"	X	--	15 yr.

Slide 10

gog1 They do not mention the age at clinical onset.
gloria ortiz guerrero, 02/06/2019

INTRON 7 CRYPTIC SPLICE DONOR MUTATION

- Our *DMD* mutation has been reported in one patient from Germany[4]
- The mutation observed in our patient is identical to the reported German patient, although the first nucleotide of the pseudoexon sequence differed due to a common polymorphism
- The German patient was mildly affected in his teenage years compared to our patient. One of the hypothesis is a hypomorphic mutation in the published case

PUBLISHED CASE		OUR CASE
• Age at clinical onset: 13 yr • CK Level: 390 IU/l • Tiptoe walking • Behavioral problems	Vs	• Age at clinical onset: 3 yr • CK Level: 11,671 IU/l • Abnormal gait • Frequent falls • Difficulty climbing stairs • Thigh pain

CONCLUSIONS

This case illustrates:

1. The combination of muscle biopsy and RNA-seq can be diagnostic in DMD/BMD patients without mutations found by standard *DMD* genetic testing
2. The clinical spectrum of DMD/BMD associated with a specific pseudoexon mutation may be very broad

THANKS…

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REFERENCES

1. Beroud C, Carrie A, Beldjord C, et al. Dystrophinopathy caused by mid-intronic substitutions activating cryptic exons in the DMD gene. Neuromusucular Disorder 2004; (14):10-18 3.
2. Trabelsi M, Beugnet C, Deburgrave N. et al. When a mid-intronic variation of DMD gene creates an ESE site. Neuromuscular Disorders 2014; 24: 1111–1117
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