Variable Dystrophin Content within Myofibers, between Myofibers, and between Regions of Muscles:

Determining the Level of Dystrophin that makes a Clinical Impact in BMD and Manifesting Carriers of DMD

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Dystrophin in drug development

- Parallel independent effort of Charley's Fund to address many similar questions
  - **Will 'riff off' their themes with review of literature supporting answers to three questions**
- *What does the literature say about the link between dystrophin expression and clinical outcomes?*
- *What questions in general need to be answered for dystrophin to be validated as a surrogate endpoint?*
- *Which might be real issues, but are not so critical that they can't be solved on more of a rolling basis?*

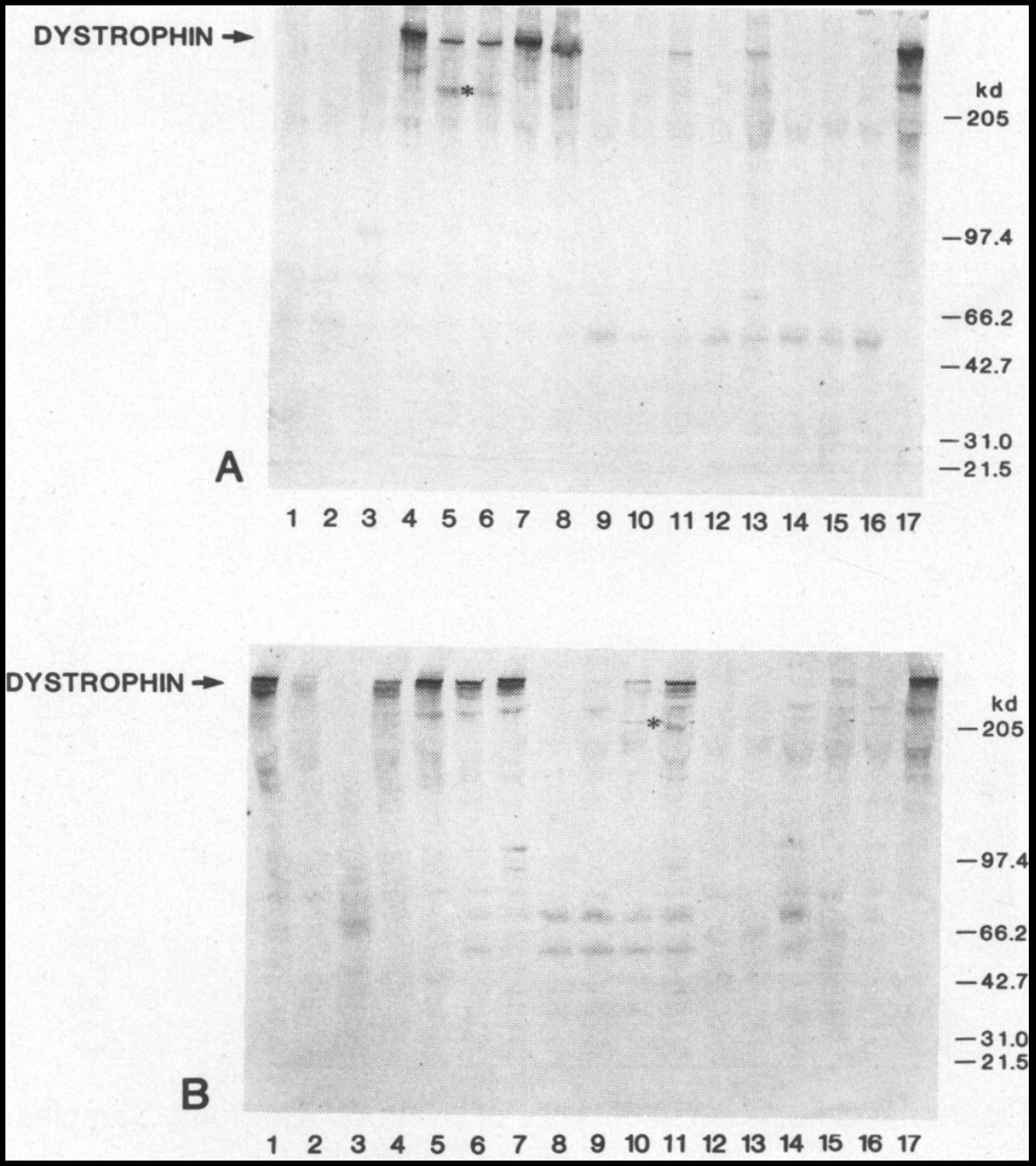
What does the literature say about the link between dystrophin expression and clinical outcomes?

- **Dystrophin loss of function is the cause of all dystrophinopathies – traditional names for groups**
  - **Duchenne**
    - Little or no dystrophin (<3%)
  - **Female manifesting carriers of DMD**
    - Varying amounts of normal dystrophin
      - **Key variable**: % of normal vs abnormal genes 'active'
  - **Becker**
    - Varying amounts of abnormal dystrophin
      - **Key variable**: Functionality of the protein (how abnormal?)

Dystrophin – clinical correlations

Duchenne

- 103 biopsies.
  - Hoffman et al. N Engl J Med. 1988 May 26;318(21):1363-8



Hoffman EP et al. N Engl J Med 1988;318:1363-1368.

- **Duchenne (n=40)**
  - **Loss of function**
  - **<3%**
- **Becker/Intermediate**
  - Partial loss of function
  - More variable

Table 1. Results of Dystrophin Analysis in 103 Muscle-Biopsy Specimens.

Dystrophin Data	Clinical Diagnosis			
	Duchenne's dystrophy	Intermediate dystrophy (outliers)	Becker's dystrophy	Other neuromuscular disorder
	number (percent) of specimens			
Normal size; 60–100% normal levels	1 (2.6)*	—	4 (22.2)*	38 (95.0)
Abnormal size; 40–100% normal levels	—	1 (14.3)	12 (66.6)*	2 (5.0)*
Normal size; 3–60% normal levels	1 (2.6)	4 (57.1)	1 (5.5)	—
Abnormal size; 3–40% normal levels	1 (2.6)	1 (14.3)	—	—
<3% Normal level, or undetectable	35 (92.1)	1 (14.3)	1 (5.5)*	—
Total	38	7	18	40

*The patients from whom these specimens were obtained were considered to represent unusual cases (see clinical histories in Methods).

Hoffman EP et al. N Engl J Med 1988;318:1363-1368.

Dystrophin-clinical correlations

Female carriers

- **Two girls with phenotype intermediate between those of Duchenne's and Becker's dystrophy.**
  - balanced X-chromosome translocation
    - 5 percent of normal
    - DMD-like
  - second girl had no detectable structural abnormality of her X chromosomes
    - Proximal weakness at the age of 5 years, ambulatory at age 12
    - 20 percent of normal

Genetic and biochemical normalization in female carriers of Duchenne muscular dystrophy:

Evidence for failure of dystrophin production in dystrophin-competent myonuclei

E. Pegoraro, MD; R.N. Schimke, MD; C. Garcia, MD; H. Stern, MD; M. Cadaldini, MD; C. Angelini, MD; E. Barbosa, MD; J. Carroll, MD; W.A. Marks, MD; H.E. Neville, MD; H. Marks, MD; S. Appleton, MD; H. Toriello, PhD; H.B. Wessel, MD; J. Donnelly, MD; S.M. Bernes, MD; J.W. Taber, MD; L. Weiss, MD; and E.P. Hoffman, PhD

April 1995 NEUROLOGY 45 677

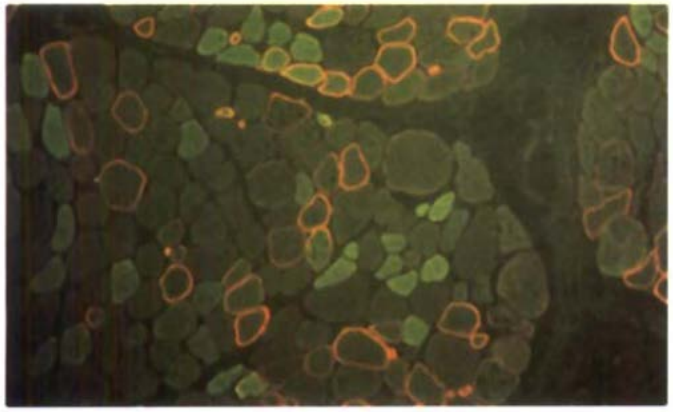
Dystrophin blots:

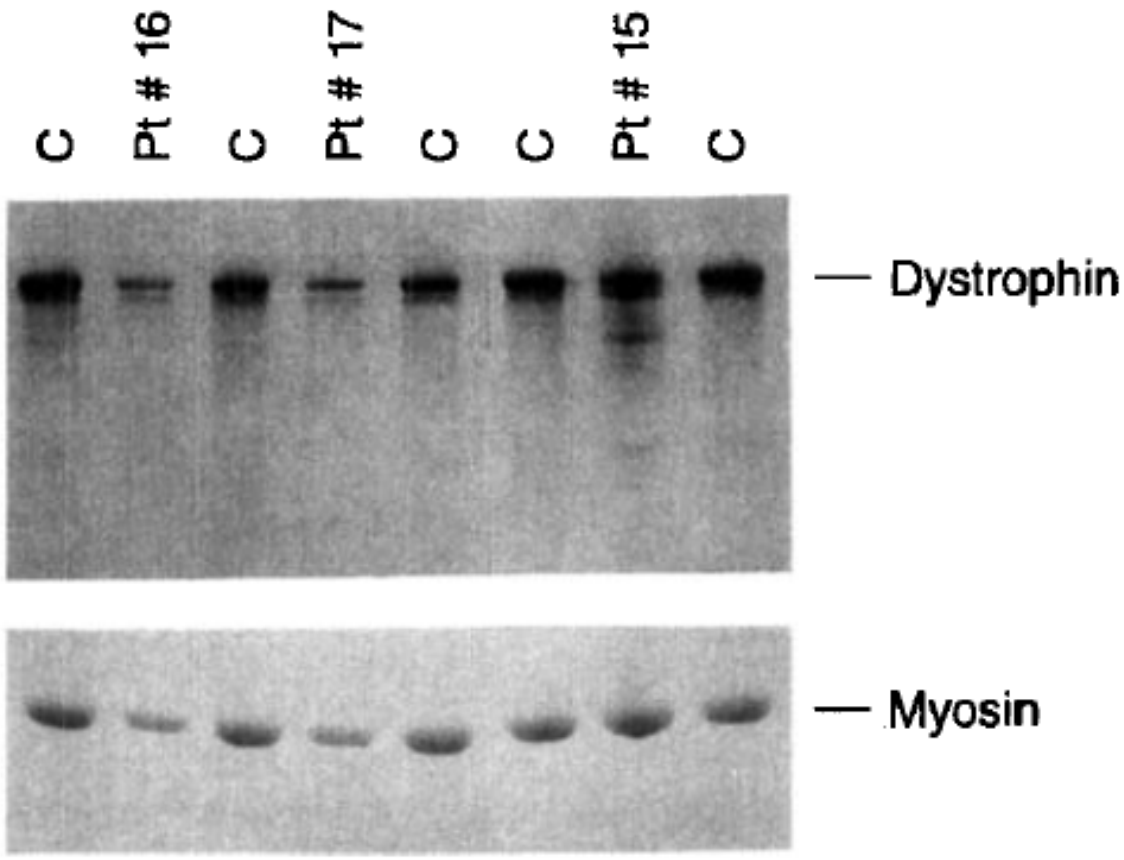
- 4 blots/patient
- vs. 2 Normal controls
- 8 measurements/patient
- Pre-flashed films; <5 s exp

N=19 patients

% normal vs DMD genes

- Quantitated muscle
- Quantitated blood





Pt	Inheritance of dystrophin gene mutations	Karyotype	Age at biopsy	Clinical severity	Dystrophin immunoblotting (% of normal)		% of active X in blood (height) Mutant dys gene	% of active X in blood (height) Normal dys gene	% of active X in muscle (height) Mutant dys gene	% of active X in muscle (height) Normal dys gene
1	P	46,X-X,+der(X) t(X;?)(p11;?)	2	Severe	18 ± 5	33%	80	20	55	45
2	M-F	46,X,Xq-(q25-qter)	4	Intermediate	3 ± 3	100%	85	15	75	25
3	P	46,X,t,(X;12) (p21.2;q24.33)	5	Mild	21 ± 5	24%	85	15	45	55
4	P	46,X,t(X;3) (p21;p21)	6	Intermediate	4 ± 3 (tr. 46 ± 13)	75%	95	5	90	10
5	M	46,XX	3	Mild	40 ± 15	37%	75	25	55	45
6	P	46,XX	4	Mild	32 ± 4	12%	100	0	70	30
7	M	—	8	Mild	24 ± 4	16%	75	25	75	25
8	M	46,XX	9	Severe	5 ± 3	60%	95	5	90	10
9	M	46,XX	10	Mild	3 ± 2	66%	75	25	80	20
10	P	46,XX	10	Intermediate	16 ± 9	56%	100	0	100	0
11	P	46,XX	12	DMD-like	48 ± 10	21%	75	25	65	35
12	—	46,XX	20	Severe	76 ± 23	30%	85*	15*	60	40
13	P	46,XX	29	Severe	70 ± 12	17%	95	5	15	85
14	M-F	46,XX	47	Intermediate	54 ± 15	28%	80	20	75	25

CV

With 8 dystrophin immunoblot measures/sample

Average CVs – range **FDA guidance – CVs**

HLOQ (>20%): 30% range: 12%-37% **< 15% (at HLOQ)**

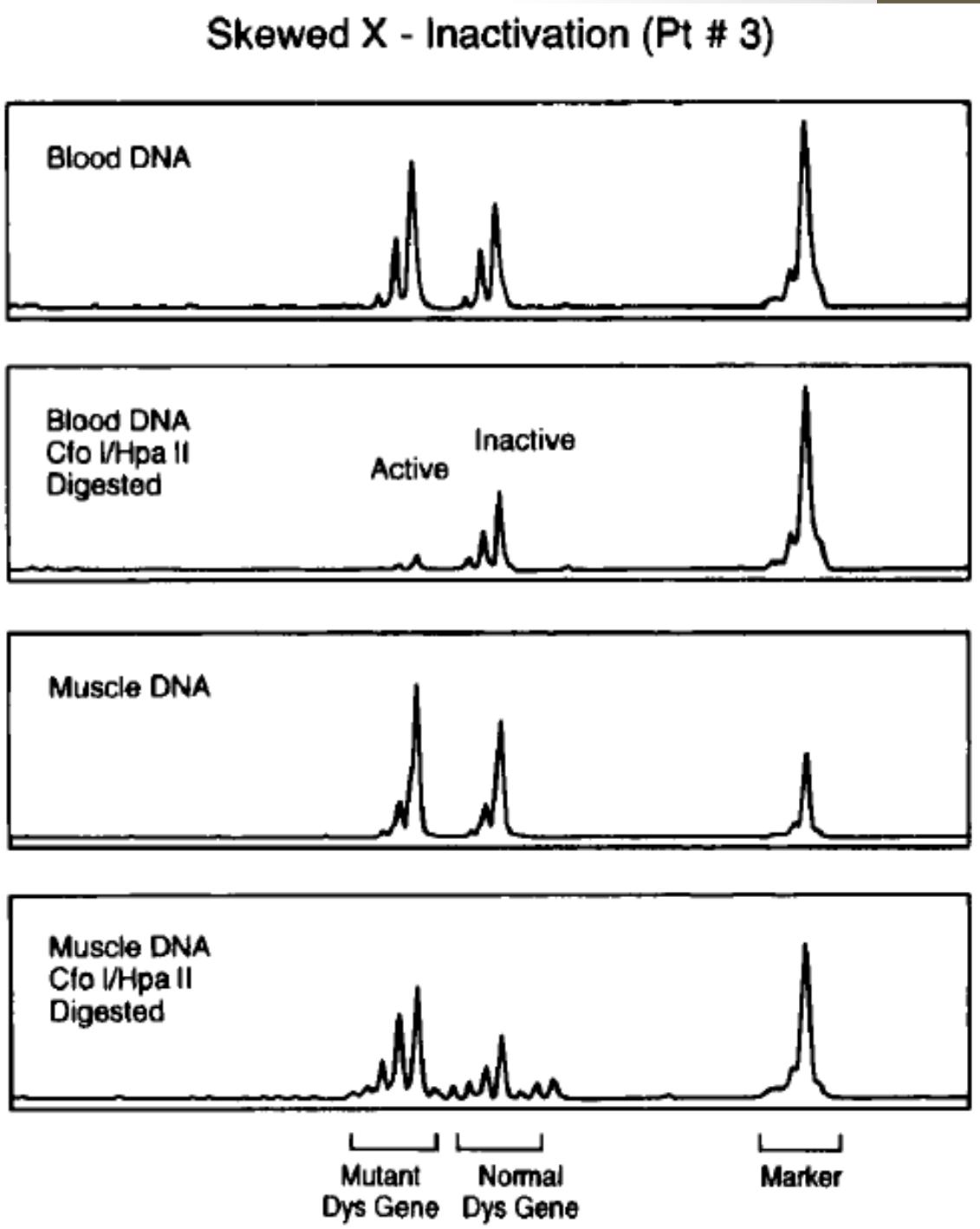
LLOQ (<20%): 65% range: 33% - 100% **< 20% (at LLOQ)**

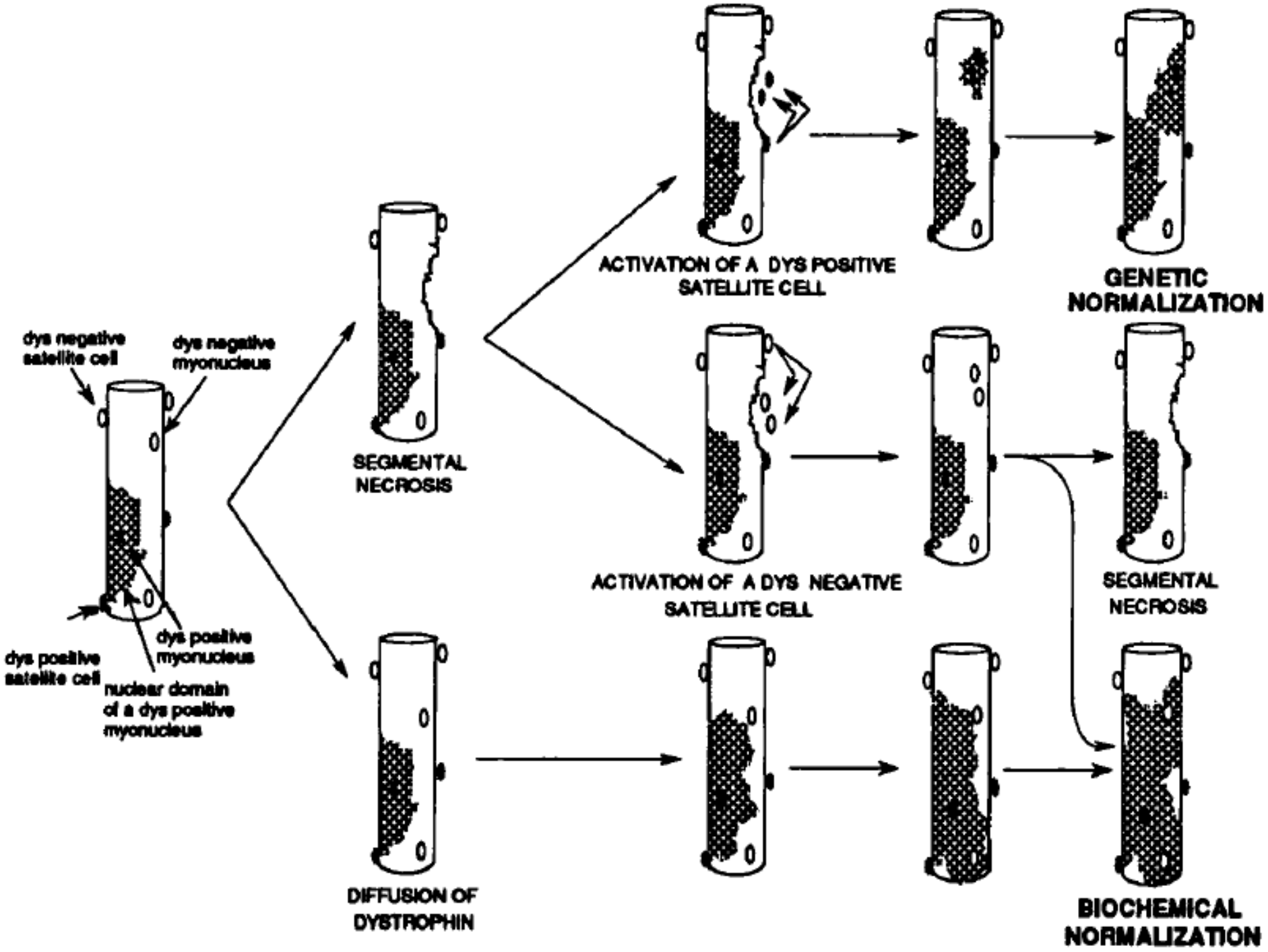
Determine % normal genes

Use blood as ‘initiation point’

Use muscle as ‘set point’

Correlate with Dystrophin production

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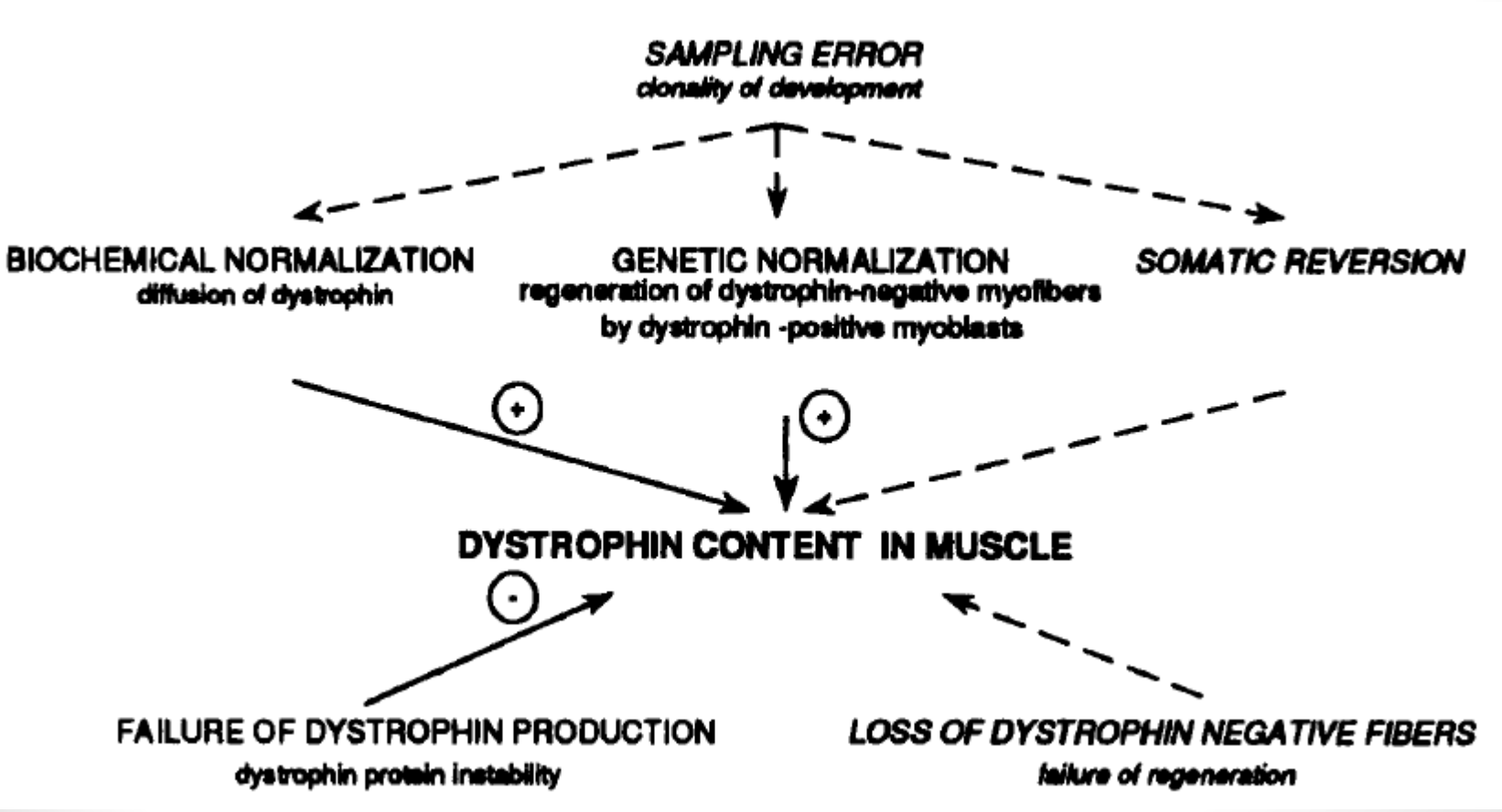
dys negative myonucleus
dys positive satellite cell
dys positive myonucleus
nuclear domain of a dys positive myonucleus
SEGMENTAL NECROSIS
DIFFUSION OF DYSTROPHIN
ACTIVATION OF A DYS POSITIVE SATELLITE CELL
ACTIVATION OF A DYS NEGATIVE SATELLITE CELL
GENETIC NORMALIZATION
SEGMENTAL NECROSIS
BIOCHEMICAL NORMALIZATION

Evidence for failure of dystrophin production in dystrophin-competent myonuclei

In older, more dystrophic muscle –

- 30% less dystrophin from normal dystrophin genes
- The gene is there, but dystrophin protein is not



Normal dystrophin: How much is enough?

Biochemical normalization

- Dystrophin diffusion in myofiber
- 2-fold increase
- Why CKs decrease

Genetic normalization

- Degen of (-) regen by (+)
- 3-fold increase
- Why manifesting carriers can improve

Failure of dystrophin production in end stage muscle

- About 30% less dystrophin than expect

How much is enough?

- Obvious sampling error
- In older more severe muscle, only dystrophin-positive myofibers still remain
- Given caveats, dystrophin seems like most any other protein
    - Threshold <5% severe, >20% normal

Becker muscular dystrophy

Hoffman EP[1], Kunkel LM, Angelini C, Clarke A, Johnson M, Harris JB. Neurology. 1989 Aug;39(8):1011-7.
Improved diagnosis of Becker muscular dystrophy by dystrophin testing.

97 patients (54 BMD): dystrophin-clinical correlations

Duchenne dystrophy

LoA ~11 years; dystrophin quantity less than 3% of normal

Severe Becker dystrophy

LoA 13 to 20 years; dystrophin 3% to 10%

Moderate/mild Becker dystrophy

LoA >20 years; dystrophin quantity greater than or equal to 20%

Am. J. Hum. Genet. 49:54–67, 1991

Exploring the Molecular Basis for Variability among Patients with Becker Muscular Dystrophy: Dystrophin Gene and Protein Studies

Alan H. Beggs,* Eric P. Hoffman,*,† Judith R. Snyder,* Kiichi Arahata,§ Linda Specht,†
Frederic Shapiro,‡ Corrado Angelini,‖ Hideo Sugita,§ and Louis M. Kunkel*

68 BMD patients studied
DNA, biopsy, clinical correlations

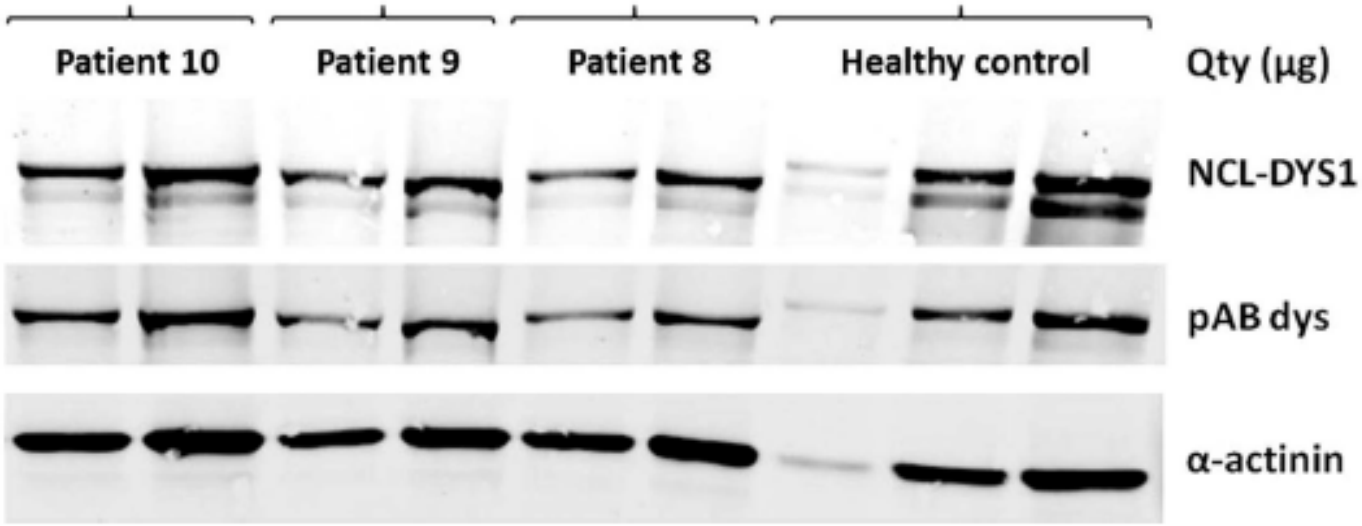
Table 2

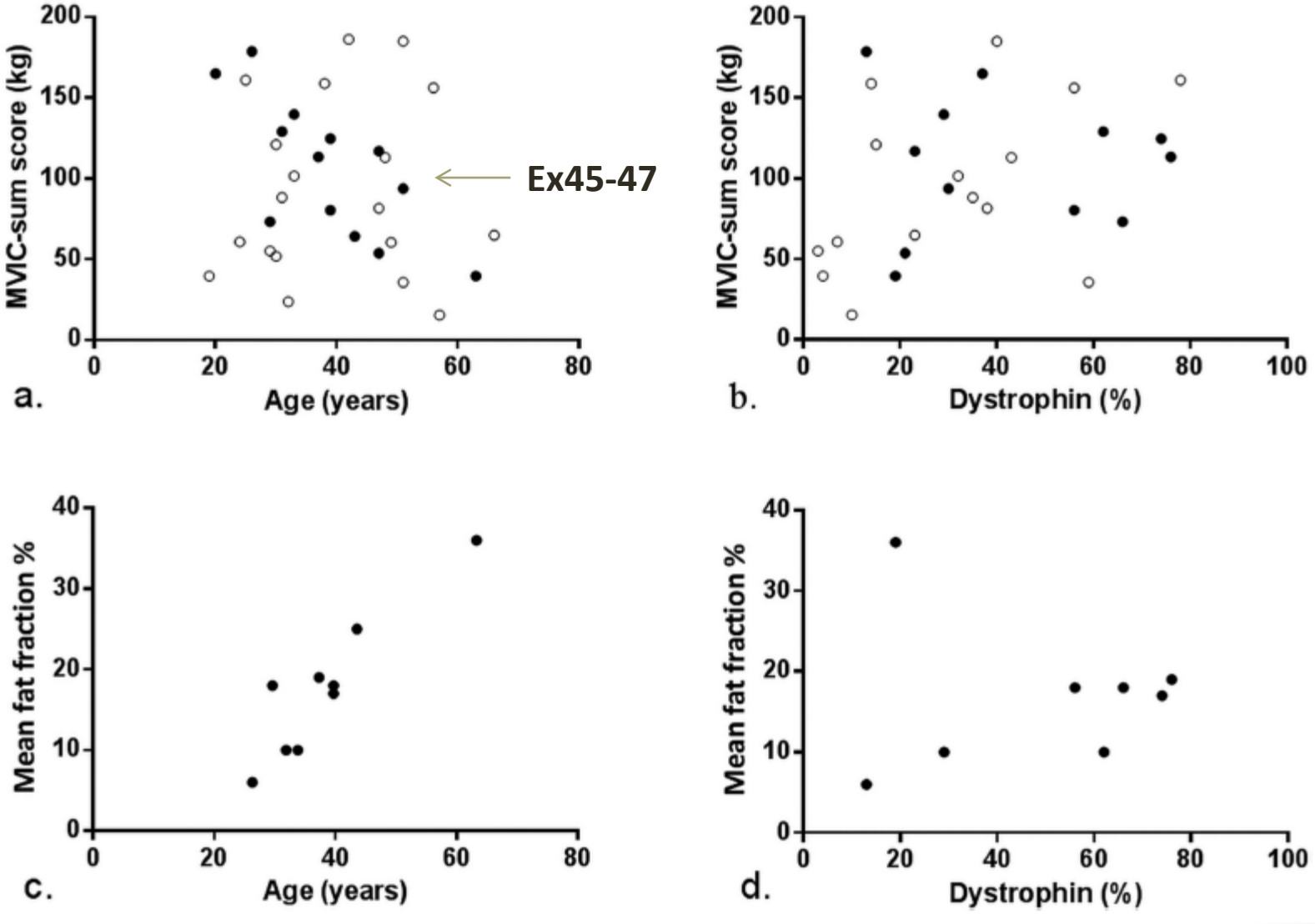
Comparison of Deletion/Duplication Sizes with Observed Dystrophin Sizes and Quantity

Exons Deleted or Duplicated	No. of Patients	Predicted Size[a] (kD)	Observed Size[a] (kD)	Average Quantity[a] (%)
del 2–7	1	376	380	10
del 3	1	396	390	15
del 3–7	2	379	370 ± 0	10 ± 0
del 6–13	1	354	360	10
del 35–44	1	337	360	80
del 45–46	1	388	380	<5
del 45–47	11	382	380 ± 4	44 ± 17
del 45–48	9[b]	375	377 ± 5	53 ± 32
del 45–49	4	371	377 ± 5	30 ± 16
del 45–53	3	345	367 ± 6	70 ± 36
del 48	1	393	400	100
del 48–49	2	389	385 ± 7	45 ± 7
del 48–51	1	376	380	90
dup 2–7	2	421	420 ± 0	40 ± 14
dup 13–42	1	580	600	70
dup 14–18	2[c]	427	420 ± 0	15 ± 7

Dystrophin levels and clinical severity in Becker muscular dystrophy patients

J C van den Bergen,[1] B H Wokke,[1] A A Janson,[2] S G van Duinen,[3] M A Hulsker,[4] H B Ginjaar,[5] J C van Deutekom,[2] A Aartsma-Rus,[4] H E Kan,[6] J J Verschuuren[1]





Looking at <u>only</u> ex45-47 deletion patients (17%-80%)

- **Age – strength – fat in muscle well correlated**
- **Dystrophin % is not well correlated**

Threshold effect for abnormal dystrophin: <10% - Severe disease

- "Although we found no relation between (high) dystrophin levels and disease severity"

- "Our four patients with dystrophin levels **below 10%"**
  - low MVIC sum scores
  - early onset of symptoms

- "This finding implies a **threshold effect**, which is confirmed by several previous clinical studies suggesting that dystrophin levels below 10% are indicative of a more severe disease course. (*references 12, 14, 16)"*

Based on what we know from BMD, animal models, etc., what assertions should we feel comfortable making, and what major questions still need to be answered?

- Assertions:
  - Normal dystrophin – manifesting female carriers
    - 0-3% dystrophin = Duchenne muscular dystrophy (in a girl)
    - 3-5% dystrophin = severe disease
    - 10-20% = mild disease
    - >20% = asymptomatic
  - Abnormal dystrophin – Becker muscular dystrophy (all dystrophin replacement strategies)
    - 0-3% = Duchenne muscular dystrophy
    - 3-15% = Severe Becker muscular dystrophy (LoA 16-20 yrs)
    - >15% = mild/moderate/asymptomatic Becker muscular dystrophy
- What major questions still need to be answered?

- What questions in general need to be answered for dystrophin to be validated as a surrogate endpoint?
- Which are true "roadblocks" or hurdles that would be a mistake not to solve/answer before a drug were to be granted approval off a dystrophin surrogate?

  - Studies need replicates, determine CVs, report these
  - Options going forward: CVs of methods
    - Bend the rules for relatively poor CVs of immunoblot or immunostaining
    - Qualification of mass spec (CVs in range)

Blots: 8 dystrophin immunoblots Average CVs – range	**Mass spec**	**FDA guidance – CVs**
HLOQ (>20%): 30%	**6% HLOQ**	**< 15% HLOQ**
LLOQ (<20%): 65%	**22% LLOQ**	**< 20% LLOQ**

- **Which might be real issues, but are not so critical that they can't be solved on more of a rolling basis?**

- ***Acknowledgement of sampling error***
  - Muscle is largest organ system of the body
  - A 0.1 g biopsy is a tiny sampling
  - There is substantial variation within myofibers, between myofibers and between muscles

- Change discussion from
  - **Improved sensitivity for clinically insignificant levels of dystrophin**
- To
  - **Accurate detection of variable but clinically significant amounts in a subset of patients.**
  - **Pharmacodynamic marker**

- **Research:** Determine why dystrophin is not being made by some normal nuclei, and what this may teach about
  - Variability in dystrophin in Becker
  - Variability in dystrophin in exon skipping