Iowa Wellstone Center Muscle Tissue and Cell Culture Repository

Steven A. Moore, M.D., Ph.D.
The University of Iowa
Department of Pathology
and Iowa Wellstone Muscular Dystrophy Cooperative Research Center (MDCRC)

August 2012

history of muscular dystrophy testing at Iowa prior to MDCRC funding

- before 1996
  - muscle biopsies sent to Campbell Lab for research lab evaluation
- fall of 1996
  - set up immunofluorescence methods in Pathology (Campbell and Moore)
  - instituted monthly muscle pathology conferences (Campbell, Mathews, and Moore)
- 1999
  - started LGMD Study with Rochester, Penn, OSU, Vanderbilt, Wash U. (central evaluation of biopsies was done at Iowa)
  - set up FSHD clinical testing in Pathology (Mathews and Moore)
- 2002-2004
  - identified FKRP patients through Moore/Campbell (clinical lab/research lab) collaboration
  - set up ARMS assays for LGMD2D, 2E, and 2I in Pathology (Winder)
- 2005/2006
  - sequencing tests for *FKRP* and *LMNA* (Winder)
  - 4qA/4qB added to FSHD clinical testing (Winder and Moore)

integration and expansion of diagnostic services at Iowa

- Anatomic Pathology Histology Lab
  - muscle biopsy referrals for immunofluorescence staining of muscular dystrophy proteins
- Molecular Pathology Lab
  - CMD and LGMD gene sequencing
  - FSHD and DM1 Southern blots
  - LGMD ARMS-PCR for common point mutations in *FKRP, SGCA, SGCB*
- Cytogenetics Lab (Pediatrics)
  - assists with cell cultures for FSHD testing and for Core B
- Iowa MDCRC Core B, Campbell Lab, and other collaborators
  - western blots for dysferlin and calpain-3
  - cell culture studies to evaluate dystroglycan, collagen VI, and nuclear morphology (bleb assay)

Core B Resources

- muscle biopsy repository – residual frozen tissue from diagnostic biopsies
- cultured cell repository – skin fibroblasts established for diagnostic testing or research
- specialized diagnostic testing
  - new immunostains – R&D for clinical tests
  - western blots (dysferlin and calpain-3)
  - fibroblast assays

How are Repository cases accrued?

- muscle biopsies
  - consent waived for biopsies prior to submission of IRB protocol in 2005 (approx. 2800 biopsies)
  - monthly letters sent to individuals or referring physicians (approx. 2500 patients since 2005)
  - consents returned by patients (approx. 500 biopsies)
- cultured cells – nearly all skin fibroblasts
  - patients seen in clinic by Kathy Mathews
  - targeted referral patients following muscle biopsy evaluation or following email/telephone contacts
  - started at zero in 2005; now have >200 patients

muscular dystrophy referral biopsies 2005 through 2012

- 2005 – 115 dystrophies, 33% of 341 total
- 2006 – 102 dystrophies, 31% of 325 total
- 2007 – 132 dystrophies, 37% of 359 total
- 2008 – 186 dystrophies, 48% of 377 total
- 2009 thru 2012 – muscular dystrophies account for approximately 50% of the 450 to 500 biopsies seen per year

Repository resources - pediatric muscle biopsies

~15% of total

age at biopsy (yrs)	collagen VI	merosin	αDG[a]	DBMD	non-αDG LGMD	X-EDMD	congenital myopathy[b]	SMA or other neurogenic	metabolic myopathy[c]	non-specific diagnoses	total biopsies	% diagnostic
1 or under	2	18	18	6	3	0	7	6	12	57	129	55.8%
2	3	3	7	10	0	0	2	0	1	22	48	54.2%
3	8	1	5	2	3	0	2	0	0	24	45	46.7%
4	4	1	9	7	1	0	1	1	0	19	43	55.8%
5	1	0	9	15	2	0	1	1	0	25	54	53.7%
6	2	1	8	17	0	0	0	1	0	18	47	61.7%
7	3	0	3	17	0	0	0	1	2	17	43	60.5%
8	1	0	2	10	2	0	0	1	0	18	34	47.1%
9	3	1	5	9	5	0	0	2	0	15	40	62.5%
10	10	3	8	10	5	1	0	0	0	13	50	74.0%
total	37	28	74	103	21	1	13	13	15	228	533	57.2%

a - This column likely includes some patients, especially the older patients, with LGMD clinical phenotypes.

b - Congenital myopathies include nemaline myopathy, central core disease, multiminicore disease, myotubular myopathy, and congenital fiber type disproportion.

c - Metabolic myopathies include mitochondrial myopathies and glycogen storage diseases.

Core B cell culture resources

Iowa MDCRC Cell Culture Repository - >200 cases		
diagnosis	cases with genetic diagnosis	cases without genetic diagnosis
calpainopathy (LGMD 2A) - 2 cases		
collagen VI disorders – Ullrich CMD and Bethlem myopathy	10 (one patient has both *COL6A1* and *COL6A2* mutations)	6
	COL6A1 - 4 cases	
	COL6A2 - 4 cases	
	COL6A3 - 3 cases	
dysferlinopathy (LGMD 2B/Miyoshi myopathy) - 3 cases		
dystroglycanopathy	46	15
	FKRP - 21 cases (11 are homozygous c.826C>A, p.L276I)	
	FKTN - 6 cases (and 2 related carriers)	
	POMT1 - 3 cases (and 1 related carrier)	
	POMT2 - 9 cases (and 1 related carrier)	
	POMGnT1 - 7 cases	
dystrophinopathy		
BMD	3	1
DMD	6	1
FSHD - 14 cases		
LMNA-associated dystrophies - 7 cases (and 1 partial lipodystrophy case)		
merosin-deficient CMD (MDC1A) - 3 cases		
myotonic dystrophy - 1 case		
misc. myopathies and other neuromuscular disorders - 72 cases		
normal controls - 4 cases		
sarcoglycanopathy - 1 case of LGMD 2C		

Core B cell culture resources

Cultured Fibroblasts - FKRP Cases		
age (yrs)	muscle biopsy	genotype
compound heterozygous mutations		
10	yes	cmpd het FKRP c.430A>G (p.M144V) and c469G>C (p.A157P)
compound heterozygous mutations with one allele L276I – 10 patients		
4	yes	cmpd het FKRP c.826C>A (p.L276I) and c.946C>T (p.P316S)
9	no	cmpd het FKRP c.826C>A (p.L276I) and c.707T>C (p.L236P)
14	no	cmpd het FKRP c.826C>A (p.L276I) and c.1141del G (p.A381QfsX47)
16	no	cmpd het FKRP c.826C>A (p.L276I) and c.217C>T (p.Q73X); also c.341C>G (p.A114G)
18	no	cmpd het FKRP c.826C>A (p.L276I) and c.1000_1017dupGAGGCTGCGGGCGTGCGC (p.E334_R339dup that duplicates EAAGVR between codons 339 and 340)
19	yes	cmpd het FKRP c.826A>C (p.L276I) and c.469G>C (p.A157P)
22	no	cmpd het *FKRP* c.826C>A (p.L276I) and c.947delC (p.C317AfsX111)
29	yes	cmpd het *FKRP* c.826C>A (p.L276I) and c.947delC (p.C317AfsX111)
38	no	cmpd het *FKRP* c.826C>A (p.L276I) and c.661-662 insA(p.fsE257X)
homozygous L276I – 11 patients		
4	yes	homozygous *FKRP* c.826C>A (p.L276I)
10	yes	
12	no	
14	no	
23	yes	
31	yes	
39	no	
39	no	
43	no	
46	yes	
49	no	

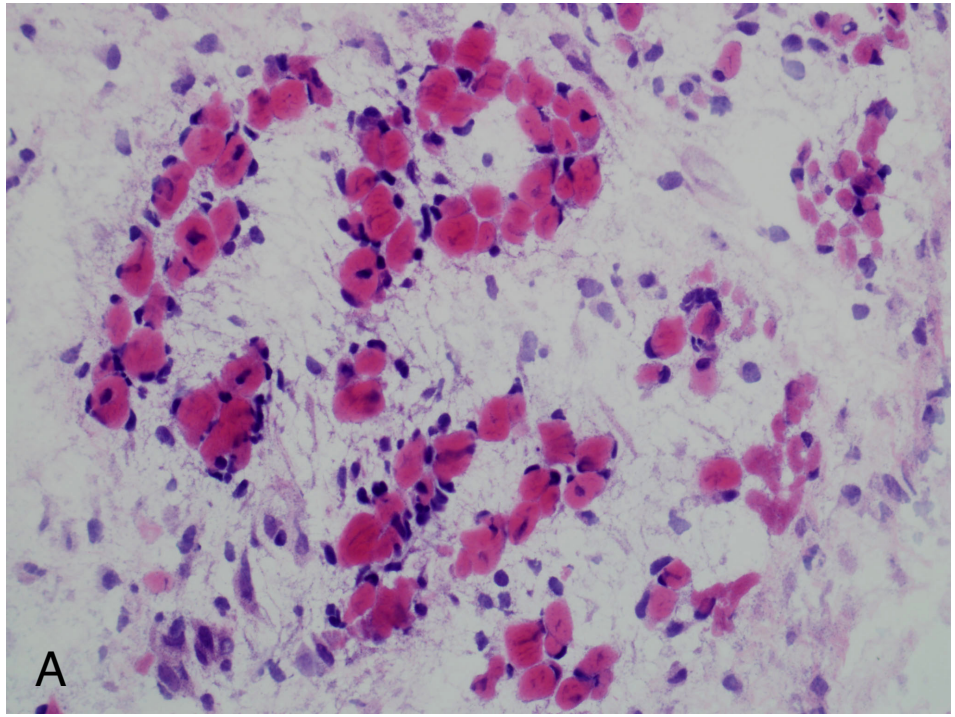
sharing Repository resources

- Non-collaborations
  - frozen muscle from DMD patients and age-matched controls
  - frozen muscle or muscle homogenates for western blot controls
  - cultured fibroblasts for a variety of research projects
  - cultured fibroblasts as a source of DNA for disease controls in diagnostic testing
  - institutions include Iowa, Tulane, BBRI, Hopkins, Cincinnati, Maryland, UCLA, and UTSW

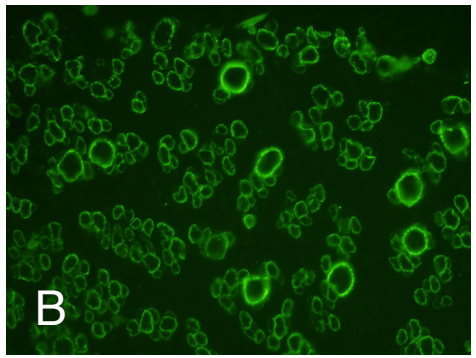
- Research collaborations with Wellstone and non-Wellstone labs at Iowa, Columbia, Northwestern, UCSF, Children's Hosp. of Calif., Wash. U., Harvard, Michigan, NIH, Melbourne, Sydney, Berlin, London and Bristol

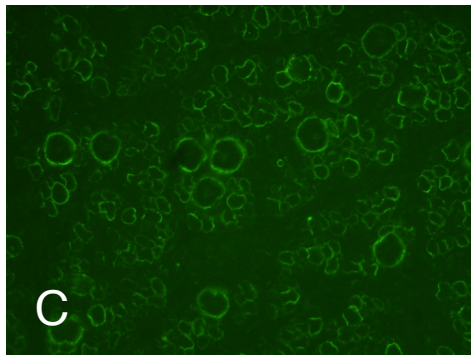
Ashkenazi Jewish founder mutation in *FKTN* causes WWS

exon 9 of *FKTN*
homozygous 1-base pair insertion (c.1167insA, p.F390IfsX14)



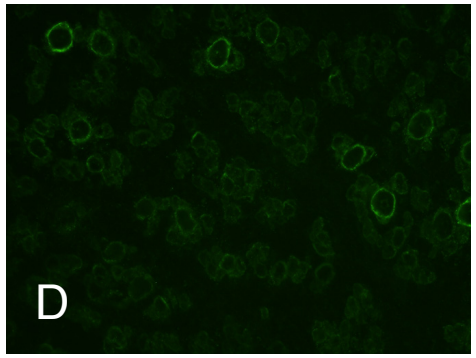
dystrophin

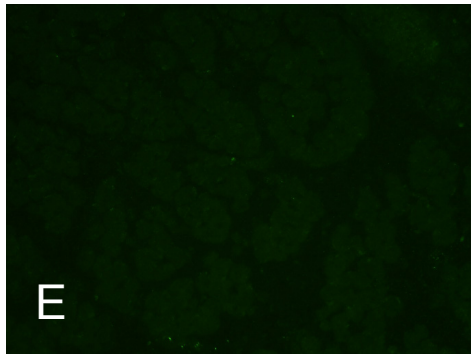




α-DG (GT20ADG)

β-DG

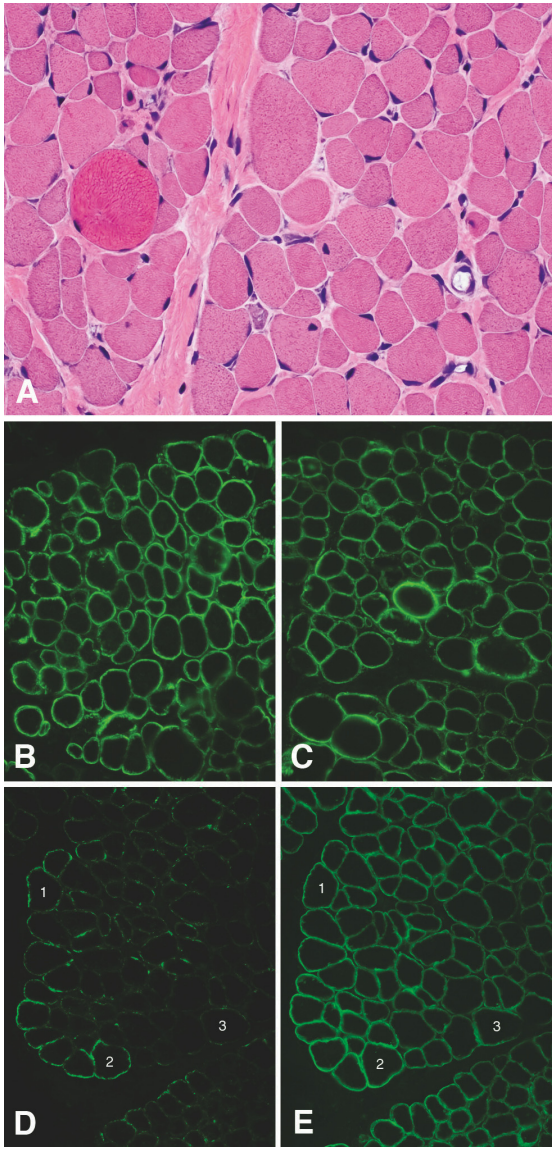


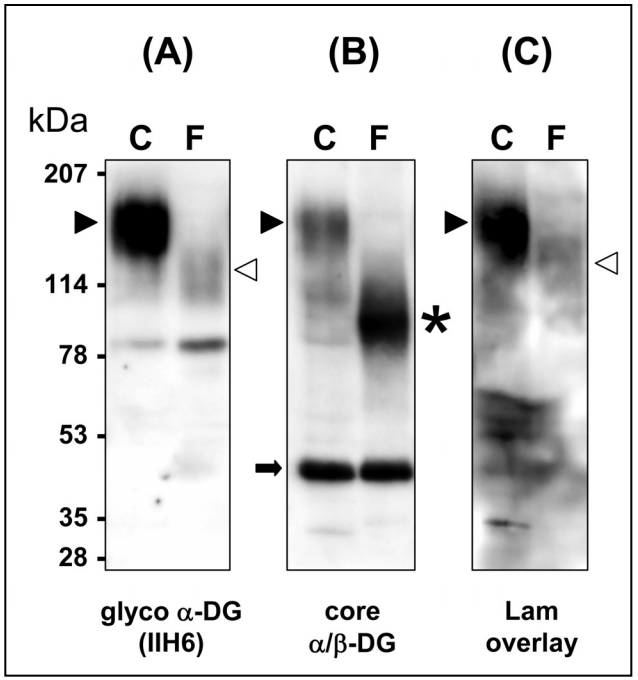


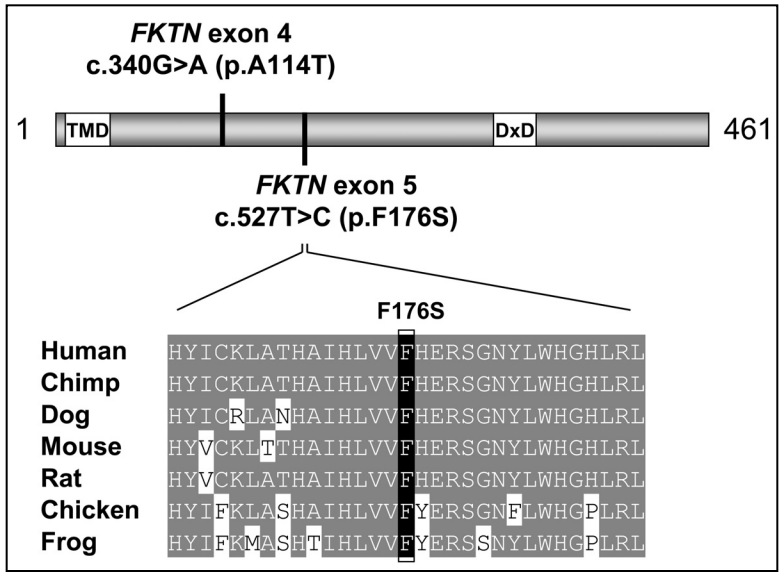
α-DG (IIH6)

Chang et al., Prenat Diagn 29: 560-569, 2009.

novel *FKTN* mutation causing LGMD

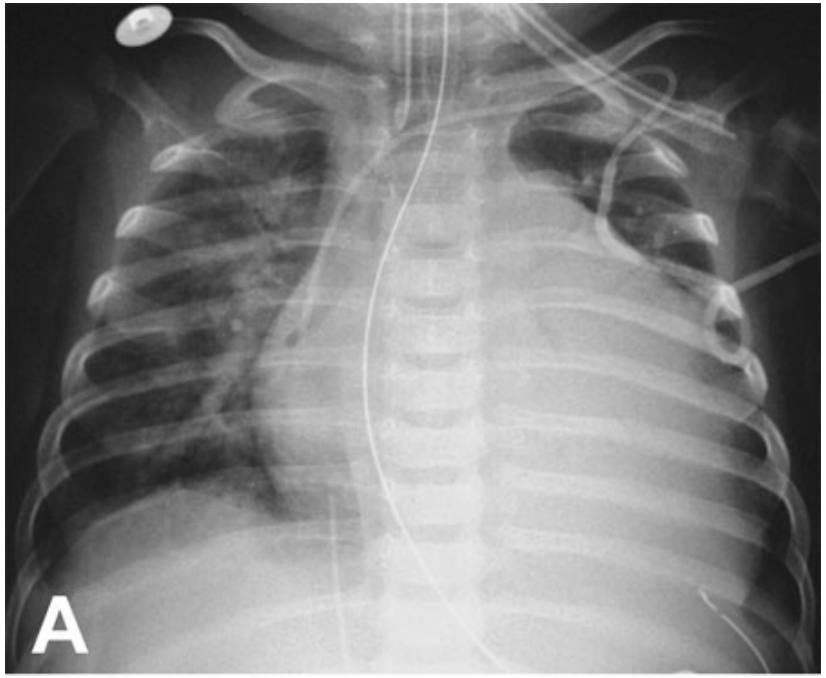




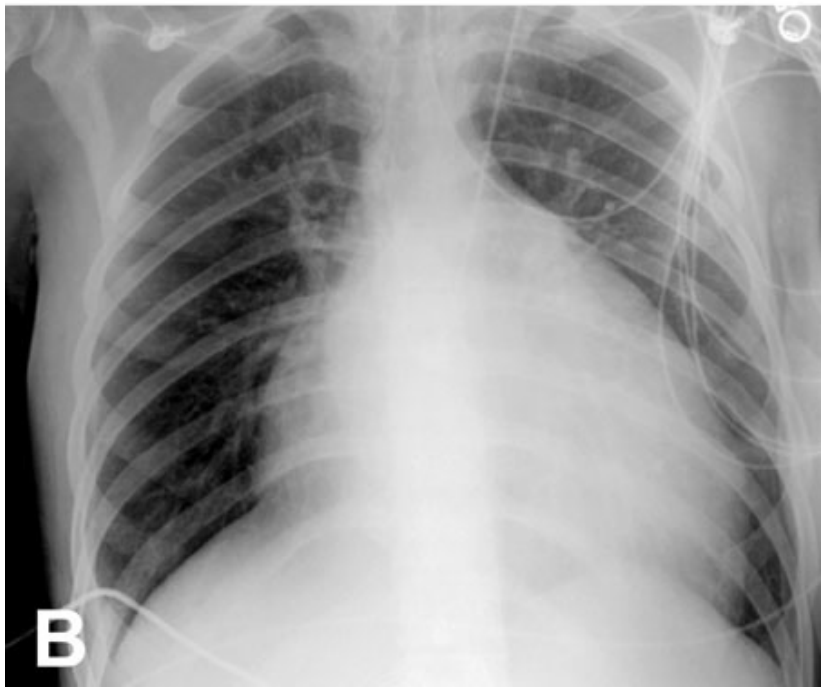


Puckett et al., Neuromusc Dis 19:352-356, 2009

severe cardiomyopathy can occur in LGMD patients homozygous for the *FKRP* common mutation



infant girl

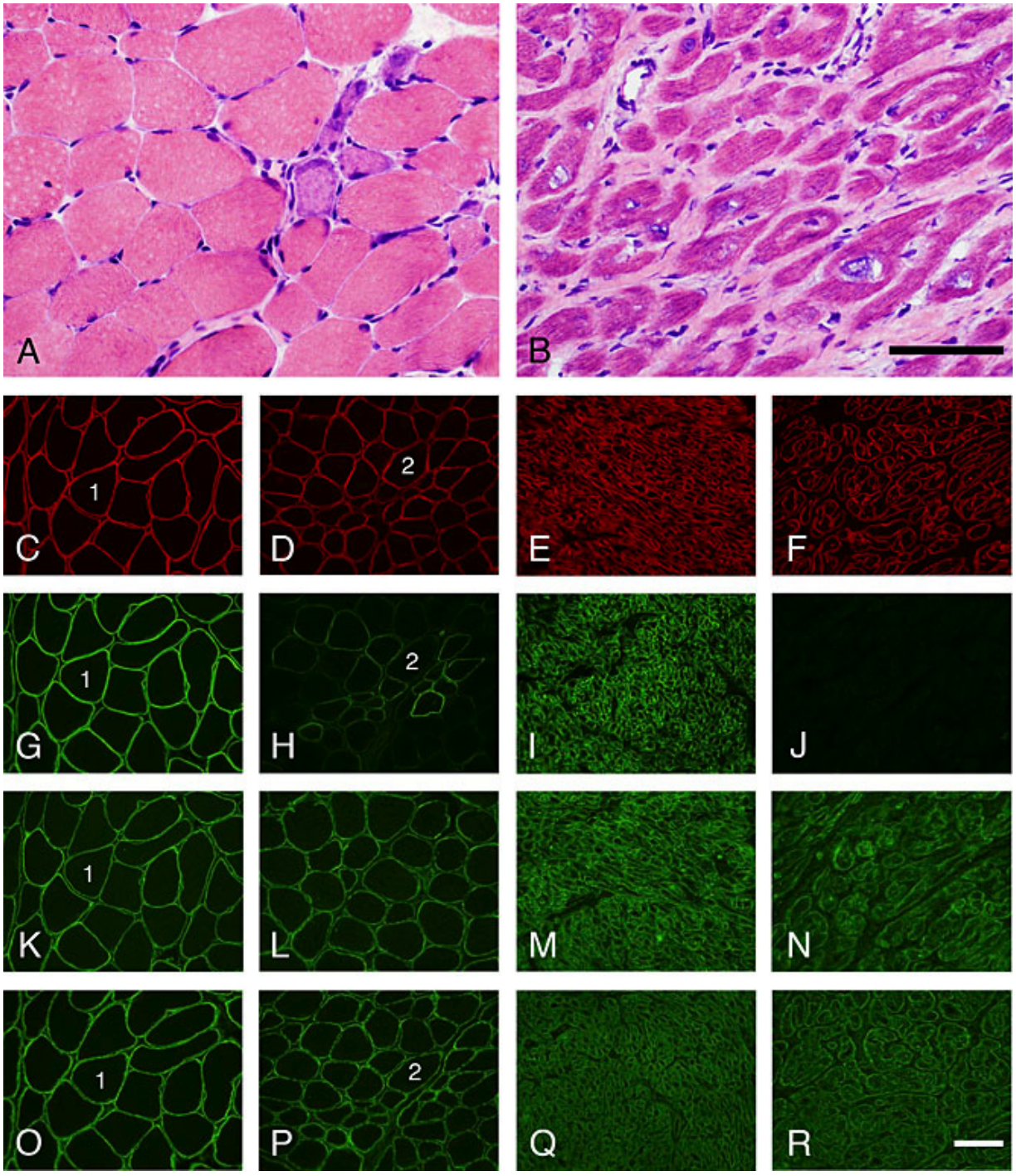


17 yo boy

Margeta et al.,
Muscle & Nerve,
40:883-889, 2009

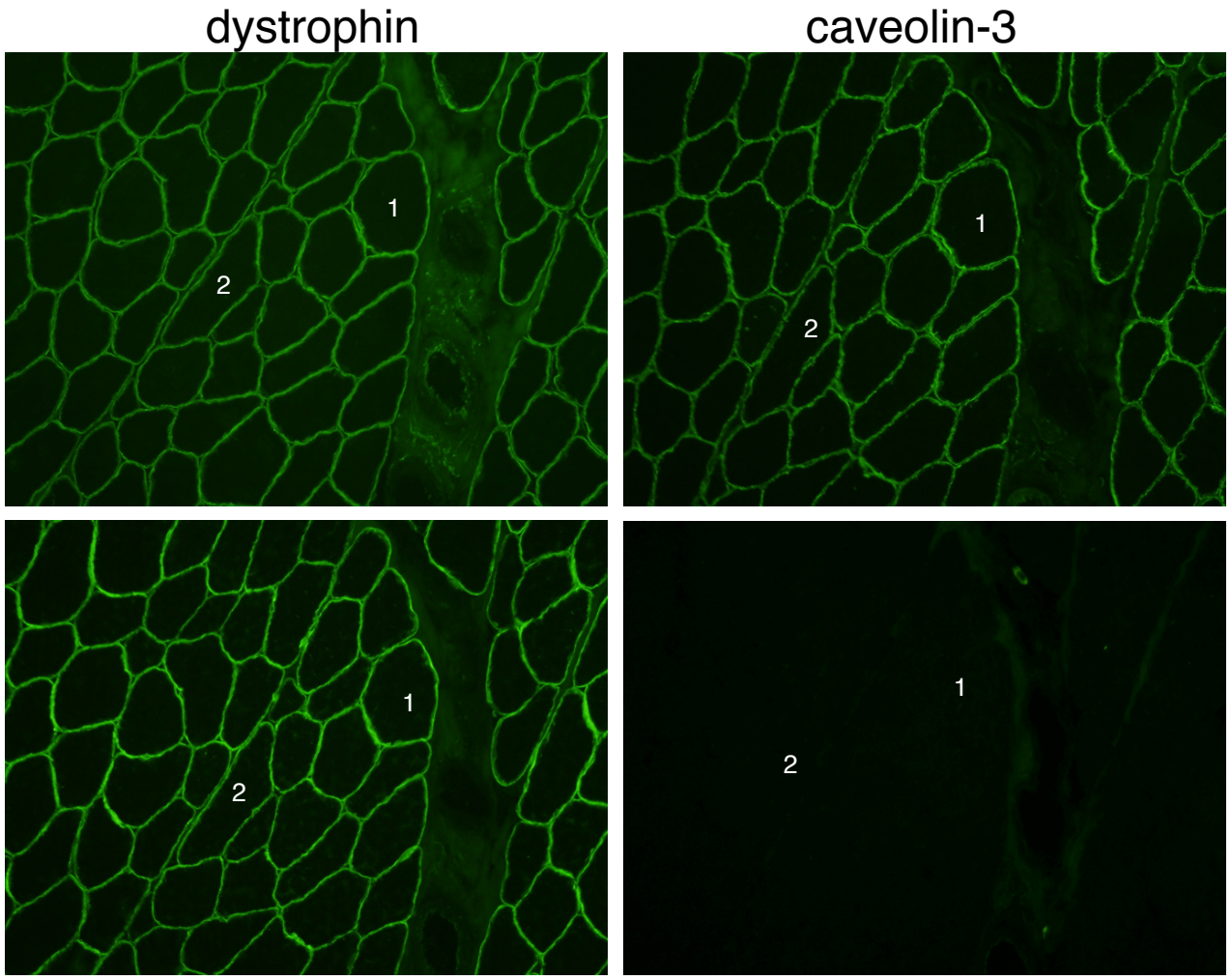
skeletal muscle

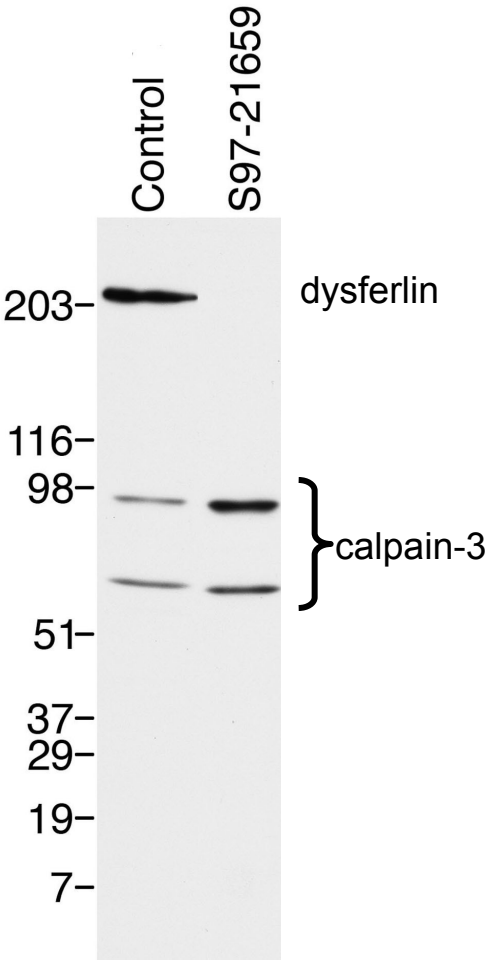
heart



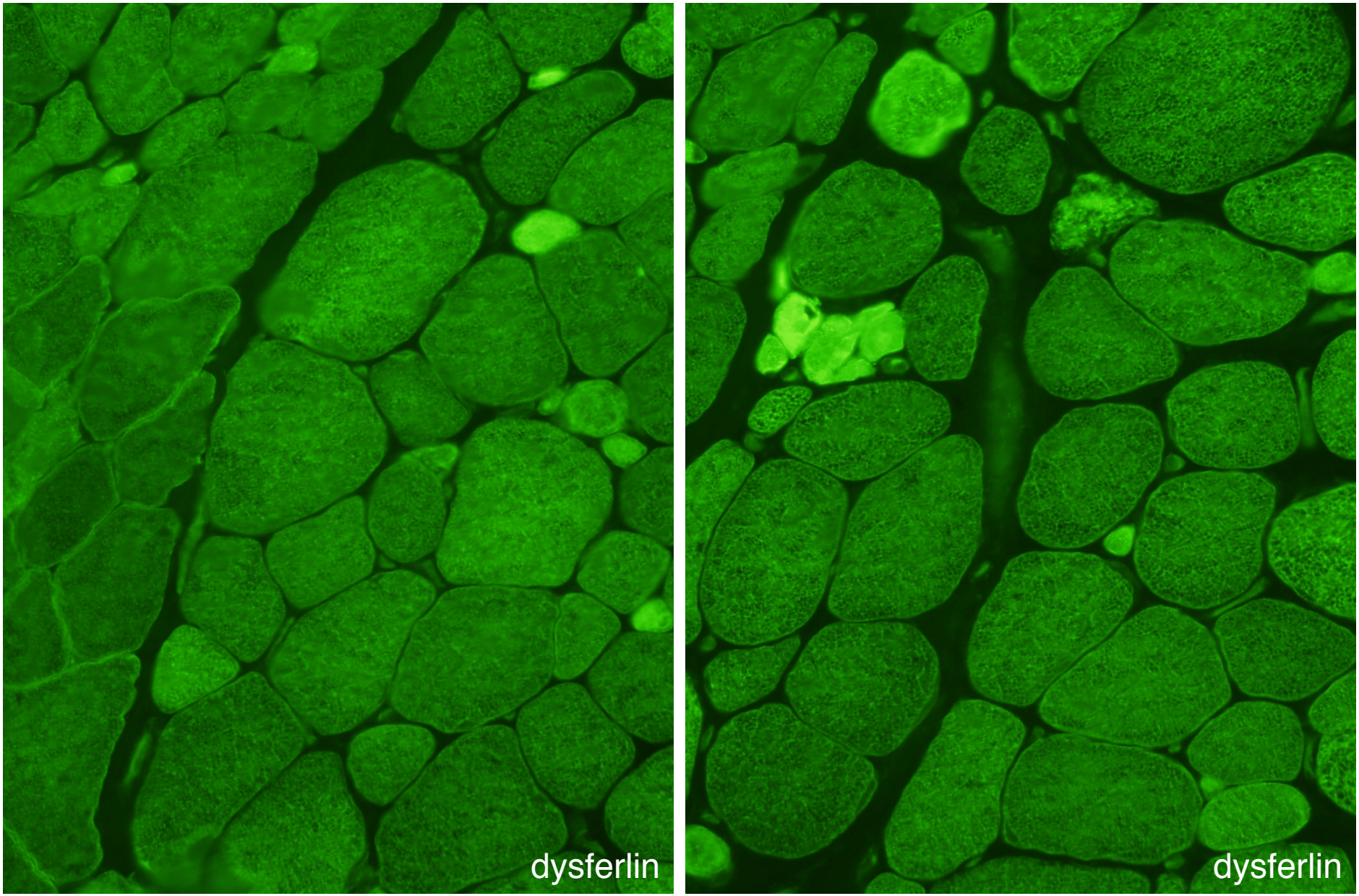
Margeta et al., Muscle & Nerve, 40:883-889, 2009

western blots to assist with diagnosis - LGMD 2B patient classic test results

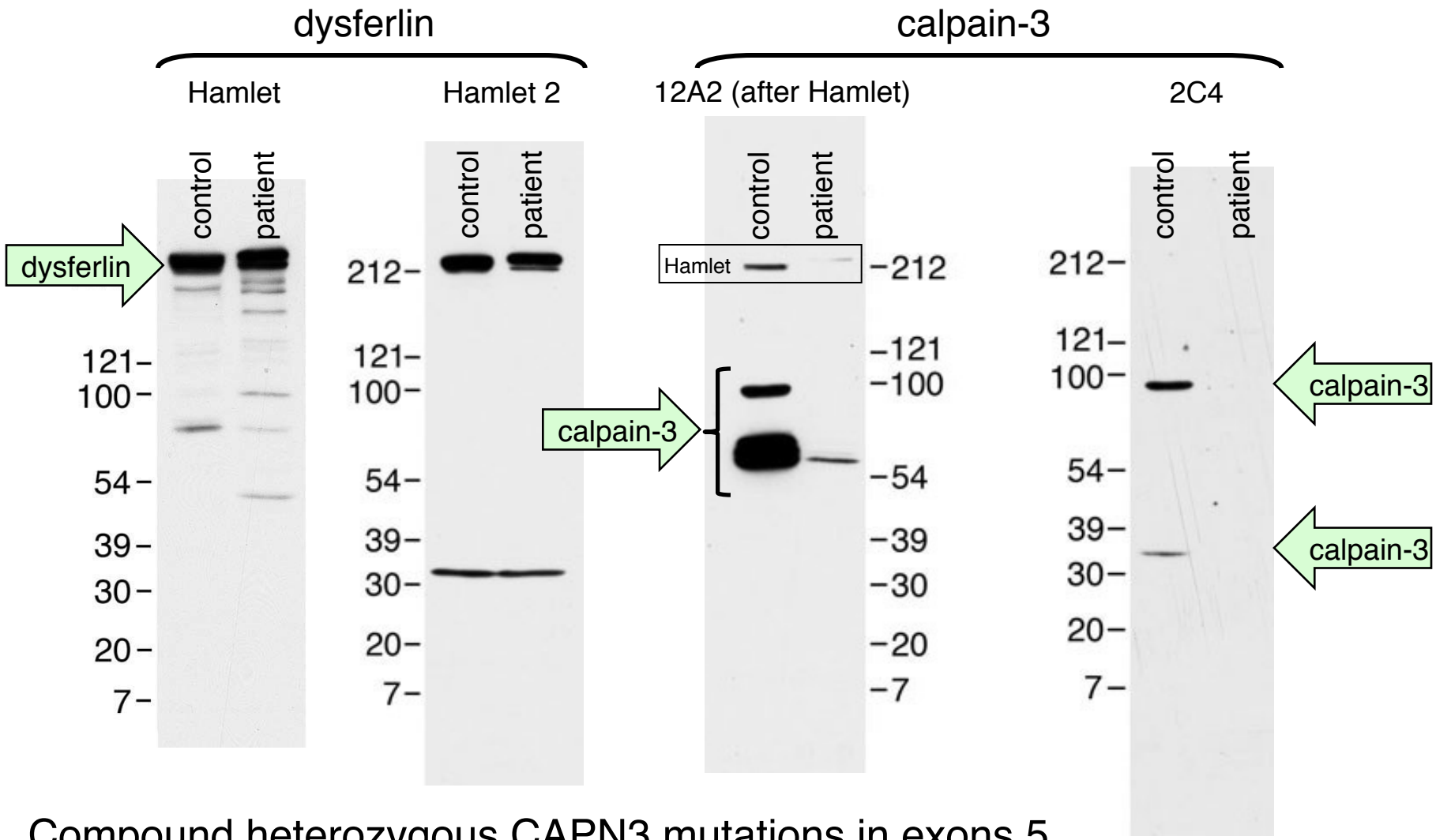




LGMD patient with reduced sarcolemmal dysferlin

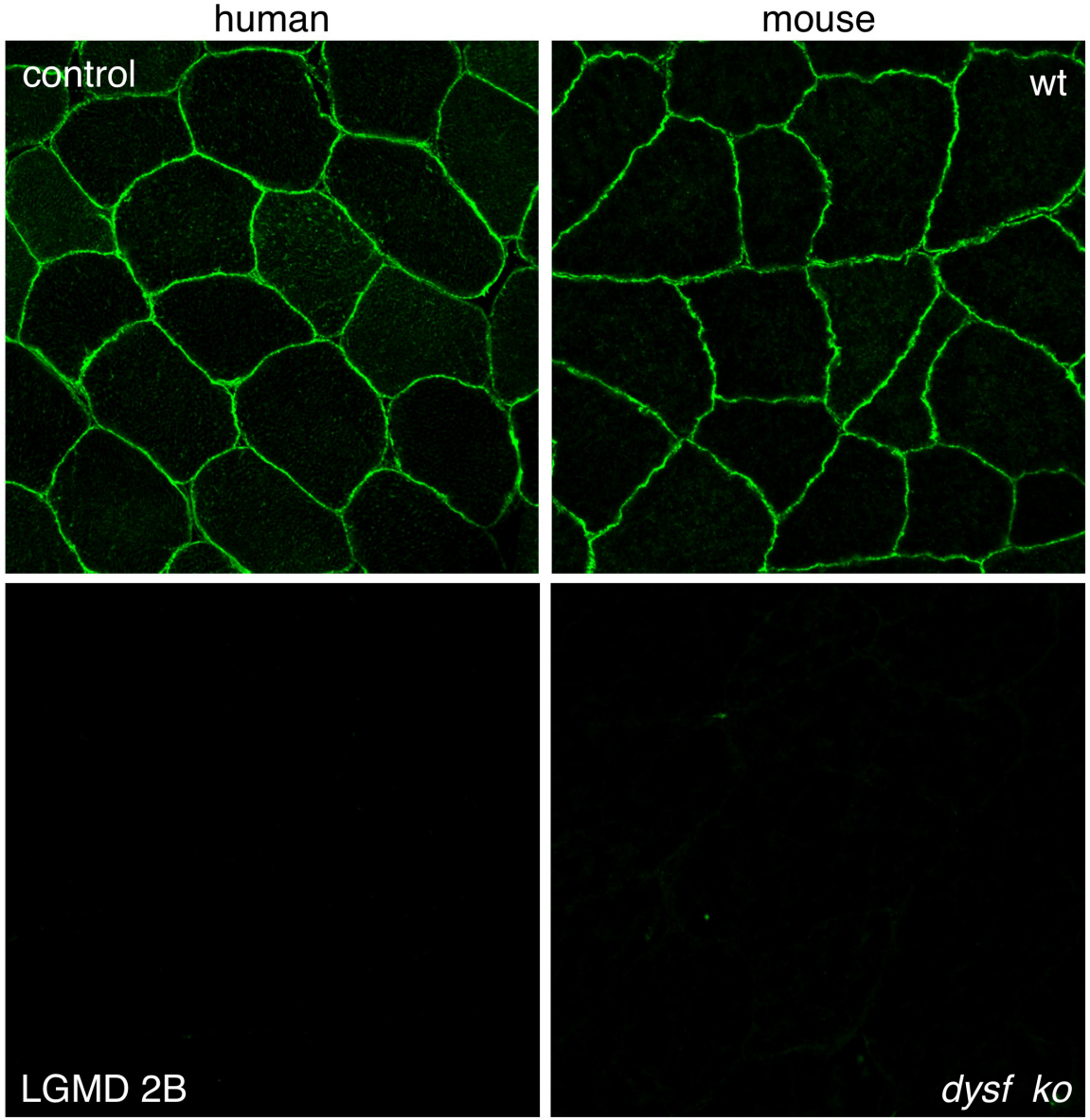


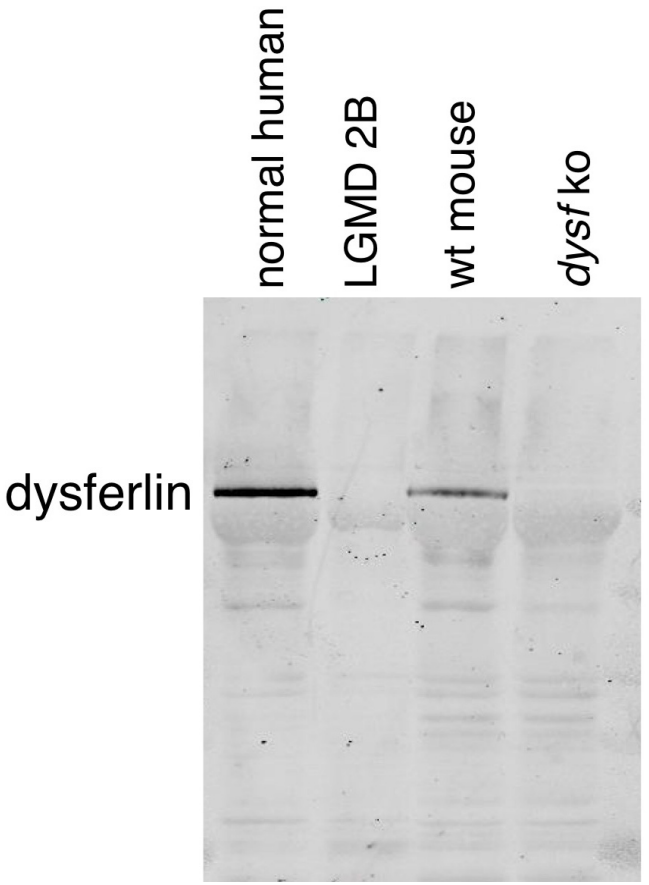
The patient with reduced sarcolemmal dysferlin has LGMD 2A, not 2B.



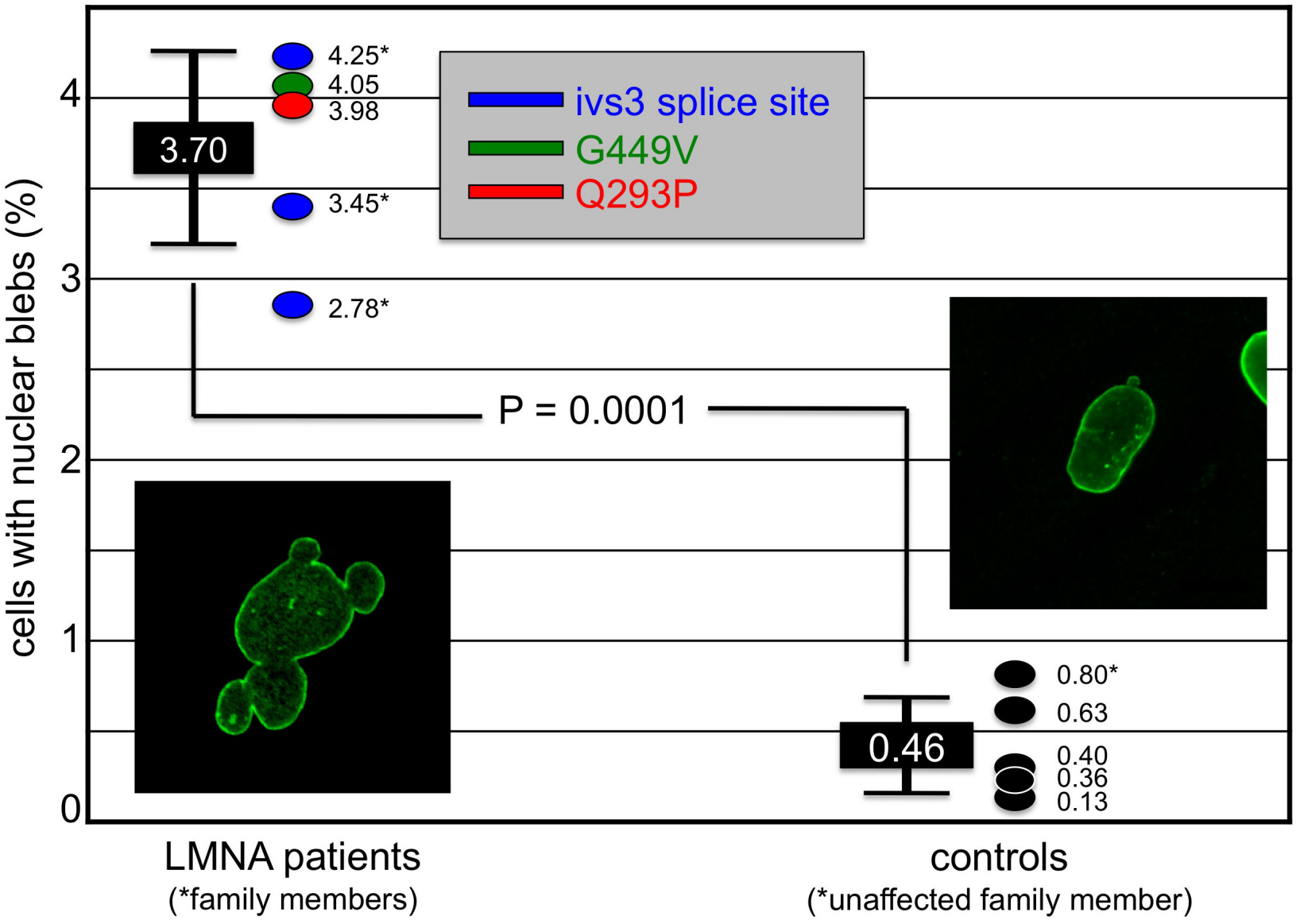
Compound heterozygous CAPN3 mutations in exons 5 and 10 were found by sequencing.

Core B assisted with the development and characterization of a new Epitomics, Inc. rabbit monoclonal anti-dysferlin antibody.





fibroblast nuclear bleb assay in *LMNA* patients



fibroblast α-dystroglycan assays

complementation
assay using
on-cell western blot

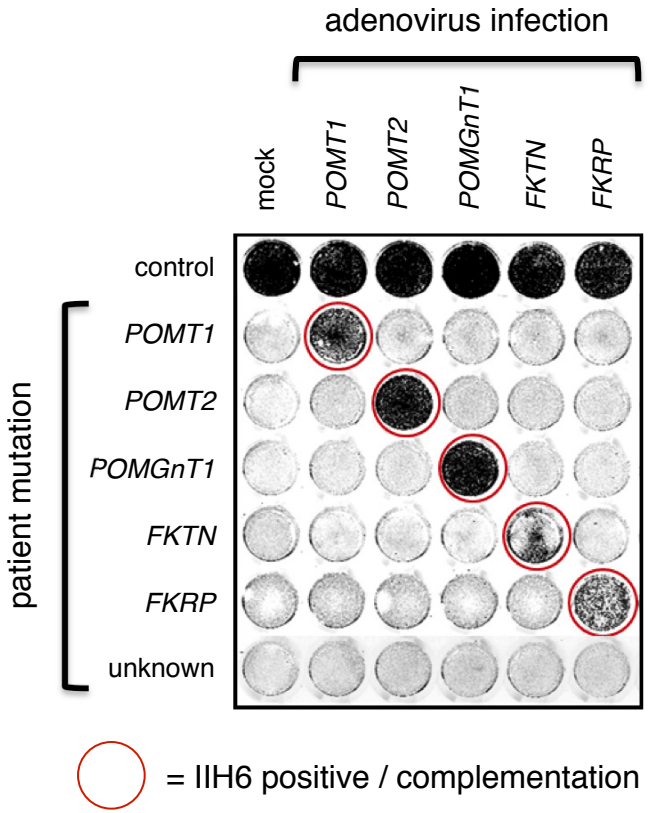


image similar to Figure 1 of
Willer et al., Nat Genet 44:575-580, 2012