NEUROLOGY
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Paul & Sheila Wellstone
Muscular Dystrophy Center
UNIVERSITY OF MINNESOTA
Driven to Discover℠

Translational Process

MDA Muscular Dystrophy Association
Fighting Muscle Disease

Peter B. Kang, MD

MDA Engage
Minneapolis, Minnesota
September 23, 2023

Disclosures (commercial entities, past year)

- Neurogene (consulting)
- Novartis/AveXis (consulting)
- NS Pharma (advisory board)
- Sarepta Therapeutics (advisory board)
- Teneofour (advisory board)

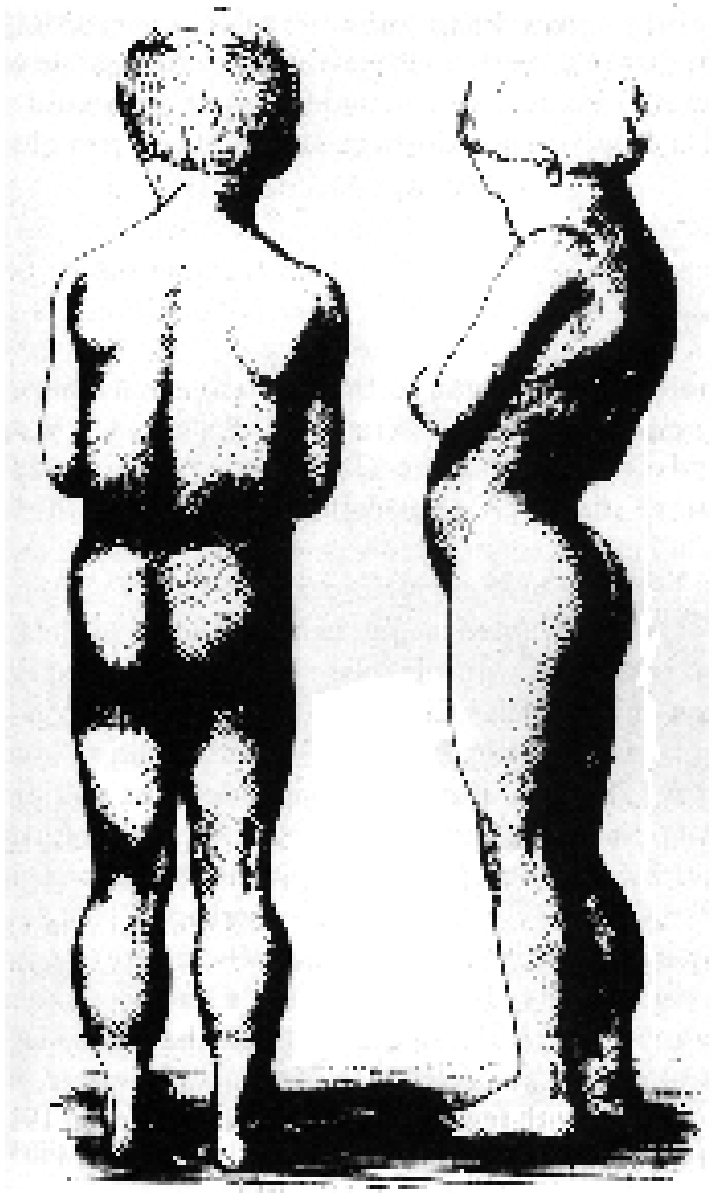
Outline

- Shorter diagnostic journeys
- Classification
- FDA-approved therapies
- Therapies in development

Shorter diagnostic journeys

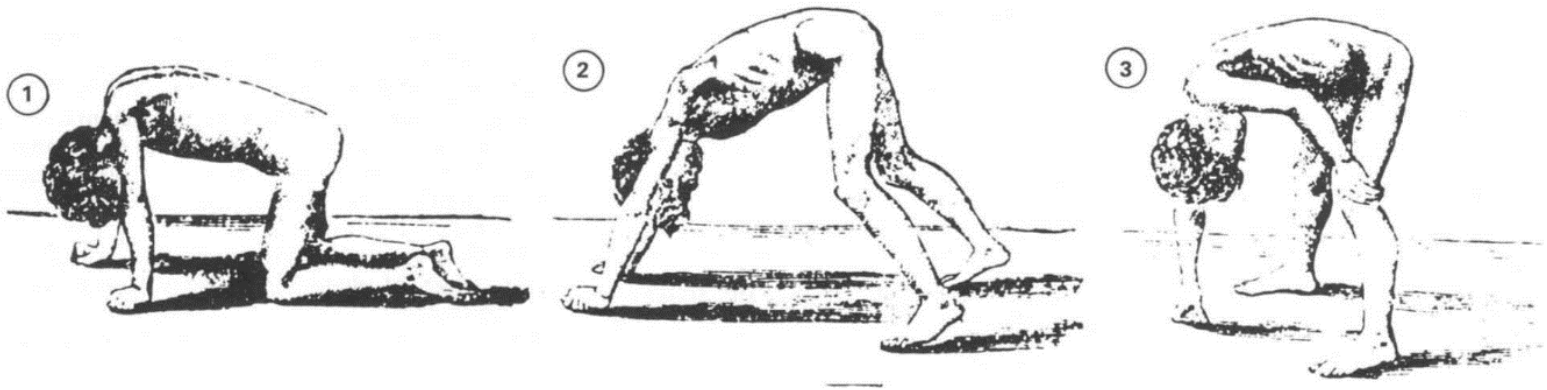
Looking ahead to the future

- Dr. Karachunski discussed newborn screening in depth
- Genetically based newborn screening is the wave of the future and will shorten many diagnostic journeys, though it will also raise new challenges
- For now, we still diagnose many neuromuscular disorders when children and adults become symptomatic



Duchenne GBA. De l'ectrisation localisee et de son application a la pathologie et a la therapeutique, 2nd ed. Paris: Bailliere;1861. p. 353-356.

Figure is a drawing based on a photograph of an early patient.



Gowers WR. Clinical lecture on pseudohypertrophic muscular paralysis. Lancet 1879;ii,73-5.

http://www.wikipedia.org

DMD clinical features

- Most common muscular dystrophy
- 1 in 5,000 live male births (not 1 in 3,500)
  - Moat et al, Eur J Hum Genet 2013;21:1049-1053
- Onset 3-5 years
- Initial presentation: gait difficulties, frequent falls, toe-walking
- Often no family history

HIGH CREATINE PHOSPHOKINASE ACTIVITY OF SERA OF PROGRESSIVE MUSCULAR DYSTROPHY

Department of Pharmacology
Faculty of Medicine
University of Tokyo, Tokyo

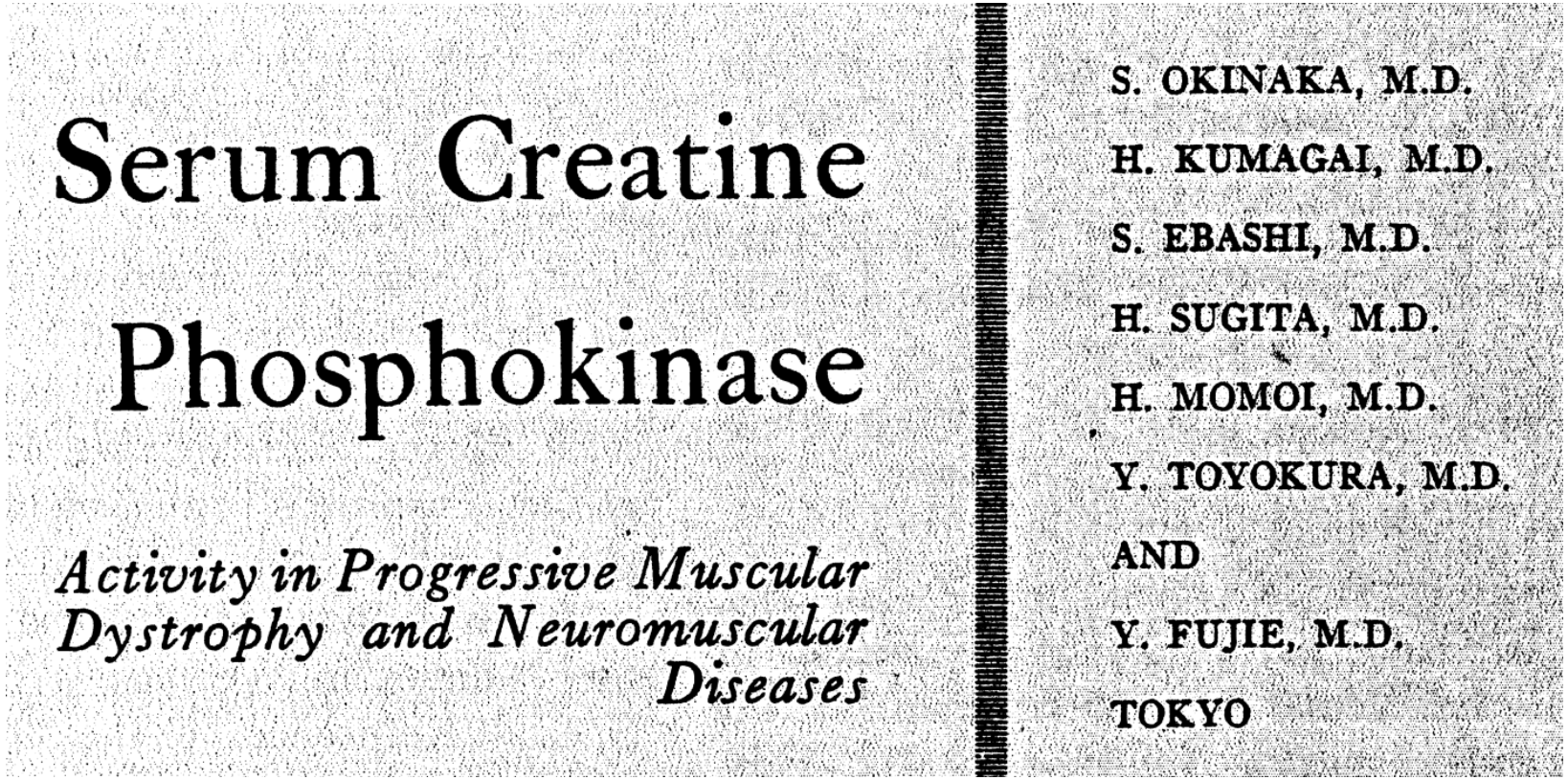
SETSURO EBASHI

Okinaka's Clinic, Faculty of Medicine
University of Tokyo, Tokyo

YASUO TOYOKURA
HIRONAO MOMOI
HIDEO SUGITA

J Biochem 1959;46:103-104

- CPK catalyzes conversion of creatine to phosphocreatine
- Serum CPK found to be elevated more consistently in muscular dystrophy compared to spinal muscular atrophy and amyotrophic lateral sclerosis



Serum Creatine Phosphokinase

Activity in Progressive Muscular Dystrophy and Neuromuscular Diseases

S. OKINAKA, M.D.
H. KUMAGAI, M.D.
S. EBASHI, M.D.
H. SUGITA, M.D.
H. MOMOI, M.D.
Y. TOYOKURA, M.D.
AND
Y. FUJIE, M.D.
TOKYO

Arch Neurol 1961;4:520-525

- Elevations of the serum CPK level were found to be more specific for muscular dystrophy than aldolase
- CPK levels highest in DMD, moderate in LGMD, lowest in FSHD

Serum CK levels in various LGMD subtypes

Limb-girdle muscular dystrophy type 1 (autosomal dominant)	
LGMD1A (*MYOT*)	1.6 to 9x ULN
LGMD1B (*LMNA*)	Normal to moderately elevated
LGMD1C (*CAV3*)	4 to 25x ULN
LGMD1D (*DNAJB6*)	Normal to 10x ULN
LGMD1E (*DES*)	Normal to 2x ULN
LGMD1F (*TNPO3*)	Normal to 20x ULN

Limb-girdle muscular dystrophy type 2 (autosomal recessive)	
LGMD2A (*CAPN3*)	6 to 84x ULN
LGMD2B (*DYSF*)	2 to 150x ULN
LGMD2C (*SGCG*)	8 to 150x ULN
LGMD2D (*SGCA*)	4 to 100x ULN
LGMD2E (*SGCB*)	3 to 209x ULN
LGMD2F (*SGCD*)	5 to 60x ULN
LGMD2G (*TCAP*)	1.2 to 17.5x ULN
LGMD2H (*TRIM32*)	1.4 to 24.5x ULN
LGMD2I (*FKRP*)	3 to 60x ULN
LGMD2J (*TTN*)	1.5 to 17x ULN
LGMD2K (*POMT1*)	20 to 40x ULN
LGMD2L (*ANO5*)	6 to 57x ULN
LGMD2M (*FKTN*)	6.7 to 343x ULN
LGMD2N (*POMT2*)	8.6 to 22x ULN
LGMD2O (*POMGNT1*)	28 to 68x ULN
LGMD2Q (*PLEC*)	19 to 29x ULN

Kang PB, Mercurio E. Laboratory assessment of the child with suspected neuromuscular disorders. In: *Swaiman's Pediatric Neurology: Principles and Practice*, Swaiman KF, Ashwal S, Ferriero DM, Schor NF, Finkel RS, Gropman AL, Pearl PL, Shevell M, editors. 6th editon. London: Elsevier 2017. Chapter 136, p.1038-1043.

Diagnosis of occult muscular dystrophy: Importance of the "chance" finding of elevated serum aminotransferase activities

Richard P. Morse, MD, and N. Paul Rosman, MD

From the Departments of Pediatrics and Neurology, Division of Pediatric Neurology, Floating Hospital for Children, New England Medical Center Hospitals, Boston, Massachusetts

We report our experience with four children, including one girl, in whom the eventual diagnosis of muscular dystrophy was made because of persistent, unexplained elevated serum aminotransferase values. Measurement of serum creatine kinase activity and careful physical examination are the most useful and cost-effective means of correctly identifying these patients. (J PEDIATR 1993; 122:254-6)

TABLE 2 Mathematical Modeling for Prediction of Log(ALT) and Log(AST) Values for Boys With DMD and BMD

Patients with DMD
$\text{Log(ALT)} = 5.70 - (0.05 \times \text{age}) + (0.000026 \times \text{CPK})$ (± 0.56; 95% CI)
$\text{Log(AST)} = 5.36 - (0.04 \times \text{age}) + (0.000036 \times \text{CPK})$ (± 0.52; 95% CI)
Patients with BMD
$\text{Log(ALT)} = 5.44 - (0.05 \times \text{age}) + (0.000026 \times \text{CPK})$ (± 0.56; 95% CI)
$\text{Log(AST)} = 5.25 - (0.04 \times \text{age}) + (0.000036 \times \text{CPK})$ (± 0.52; 95% CI)

Serum Transaminase Levels in Boys With Duchenne and Becker Muscular Dystrophy

Hugh J. McMillan, Matt Gregas, Basil T. Darras and Peter B. Kang

Pediatrics 2011;127;e132-e136; originally published online Dec 13, 2010; DOI: 10.1542/peds.2010-0929

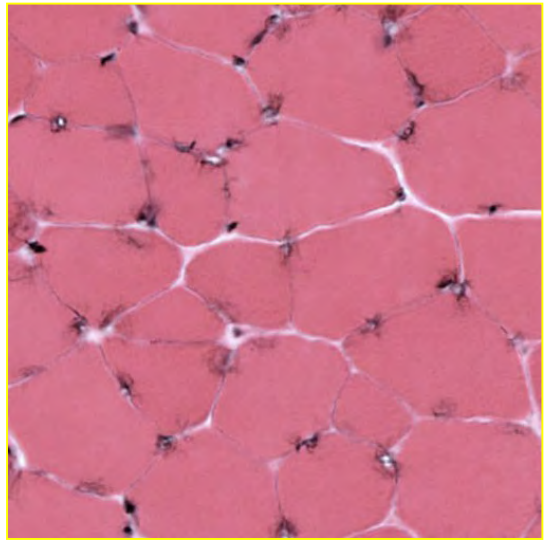
- Study of enzyme ratios
  - 82 enzyme data sets
  - 46 DMD patients
  - 9 BMD patients
- Could also use GGT to distinguish muscle versus liver disease

Muscle biopsy

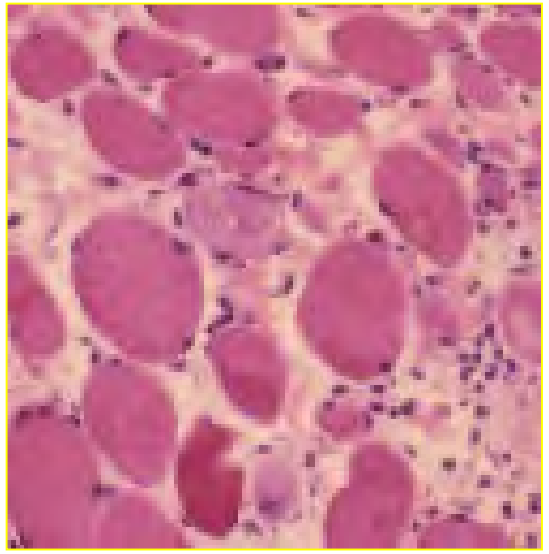
- Good for evaluation of myopathies
- Can also detect signs of neurogenic diseases

Hematoxylin and eosin

Control

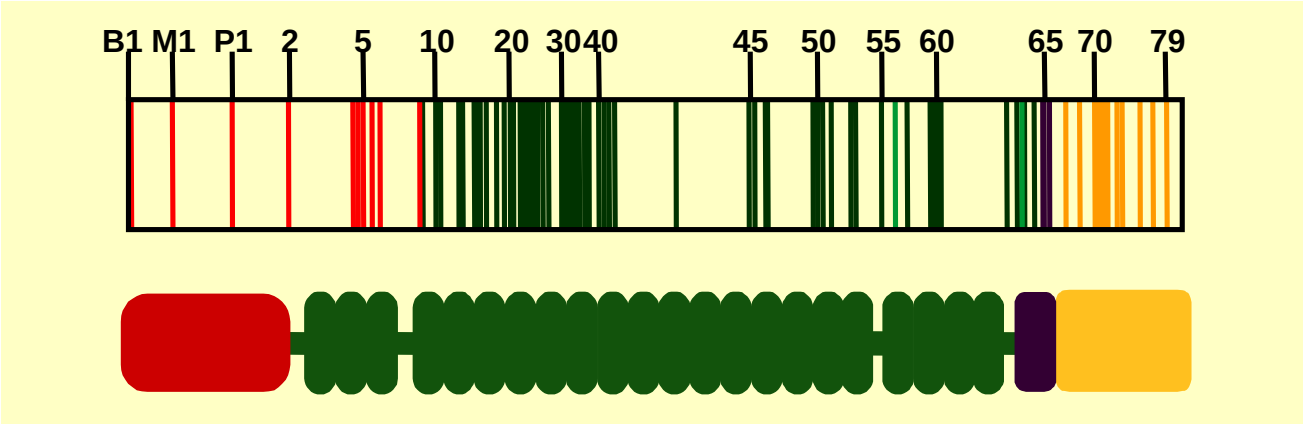


Affected



DMD laboratory studies

- CK ↑↑, typically > 10,000 U/L
- CK < 1,000 inconsistent with the diagnosis (normal range < 200)
- EMG: myopathic or normal
- Muscle histochemistry: necrosis, degenerating and regenerating fibers, inflammatory infiltrates
- Muscle immunohistochemistry: typically absent dystrophin staining (not available until 1980s)



Isolation of candidate cDNAs for portions of the Duchenne muscular dystrophy gene

Anthony P. Monaco*†, Rachael L. Neve*†, Chris Colletti-Feener*, Corlee J. Bertelson*, David M. Kurnit* & Louis M. Kunkel*†‡

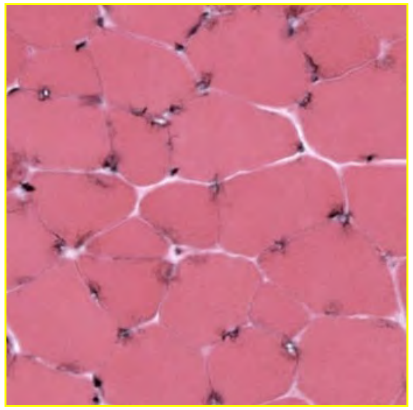
Nature 1986;323:646-650

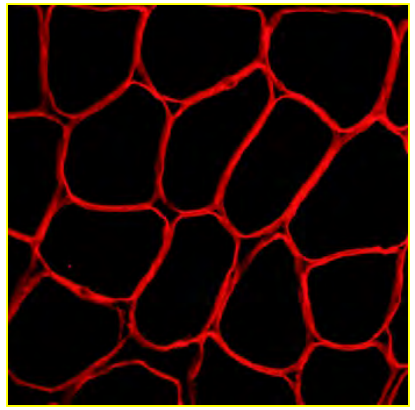
- Dystrophin (DMD) is the largest gene in the genome
- 79 exons, 2.3 million base pairs on Xp21
- 427 kDa protein product

Hematoxylin & Eosin

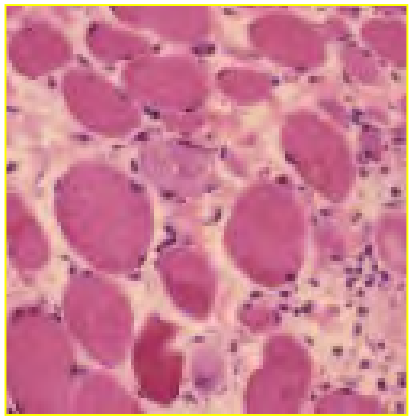
Dystrophin

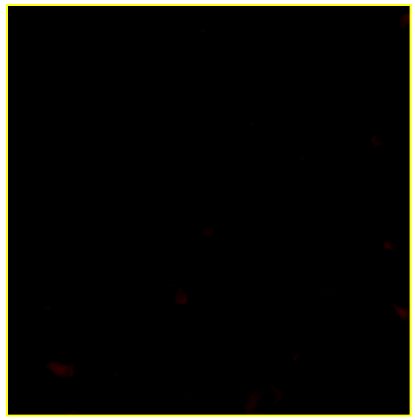
Control





DMD

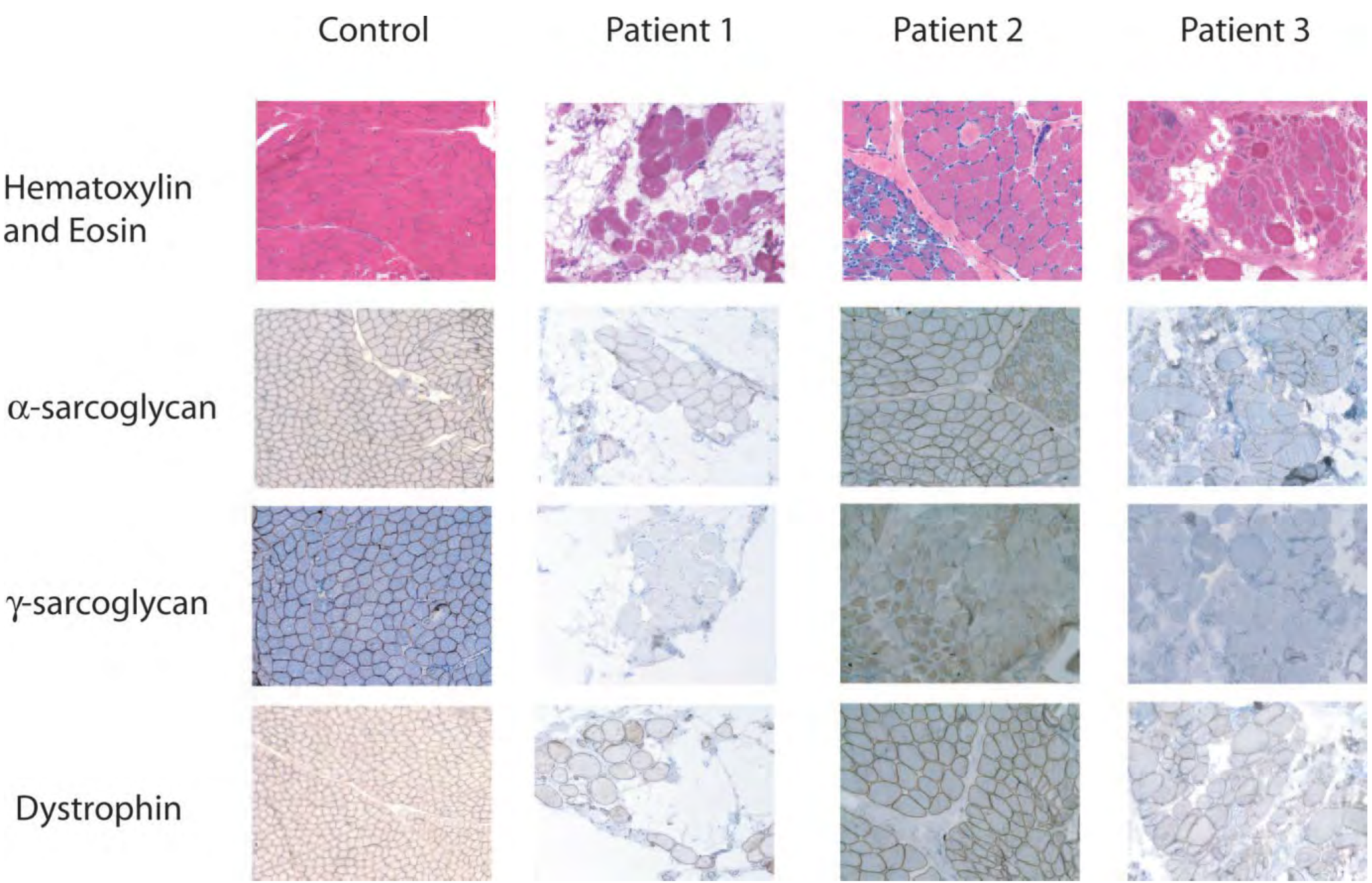




Cell, Vol. 51, 919–928, December 24, 1987, Copyright © 1987 by Cell Press

Dystrophin: The Protein Product of the Duchenne Muscular Dystrophy Locus

Eric P. Hoffman,* Robert H. Brown, Jr.,† and Louis M. Kunkel*‡§



Duncan et al, Neurology 2006;67:167-169

Initial sequencing and analysis of the human genome

Lander et al, Nature 2001;409:860-921.

The Sequence of the Human Genome

Venter et al, Science 2001;1304-1351.

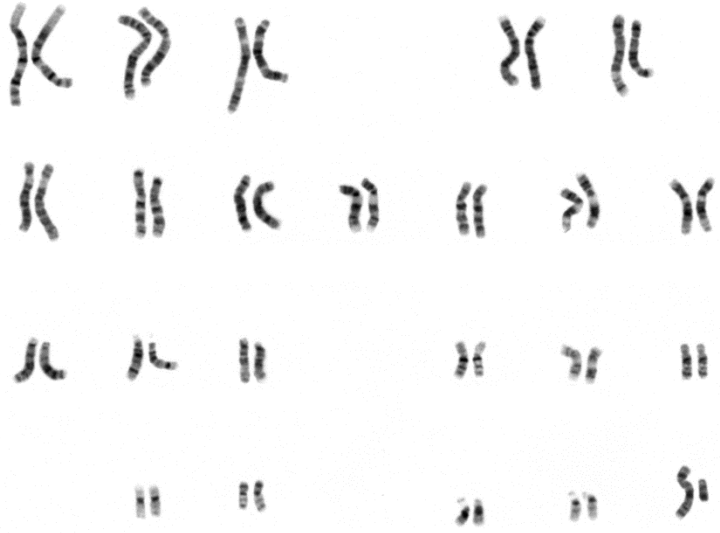
Genome-wide *in situ* exon capture for selective resequencing

Emily Hodges[1,4], Zhenyu Xuan[1,2,4], Vivekanand Balija[2], Melissa Kramer[2], Michael N Molla[3], Steven W Smith[3], Christina M Middle[3], Matthew J Rodesch[3], Thomas J Albert[3], Gregory J Hannon[1] & W Richard McCombie[2]

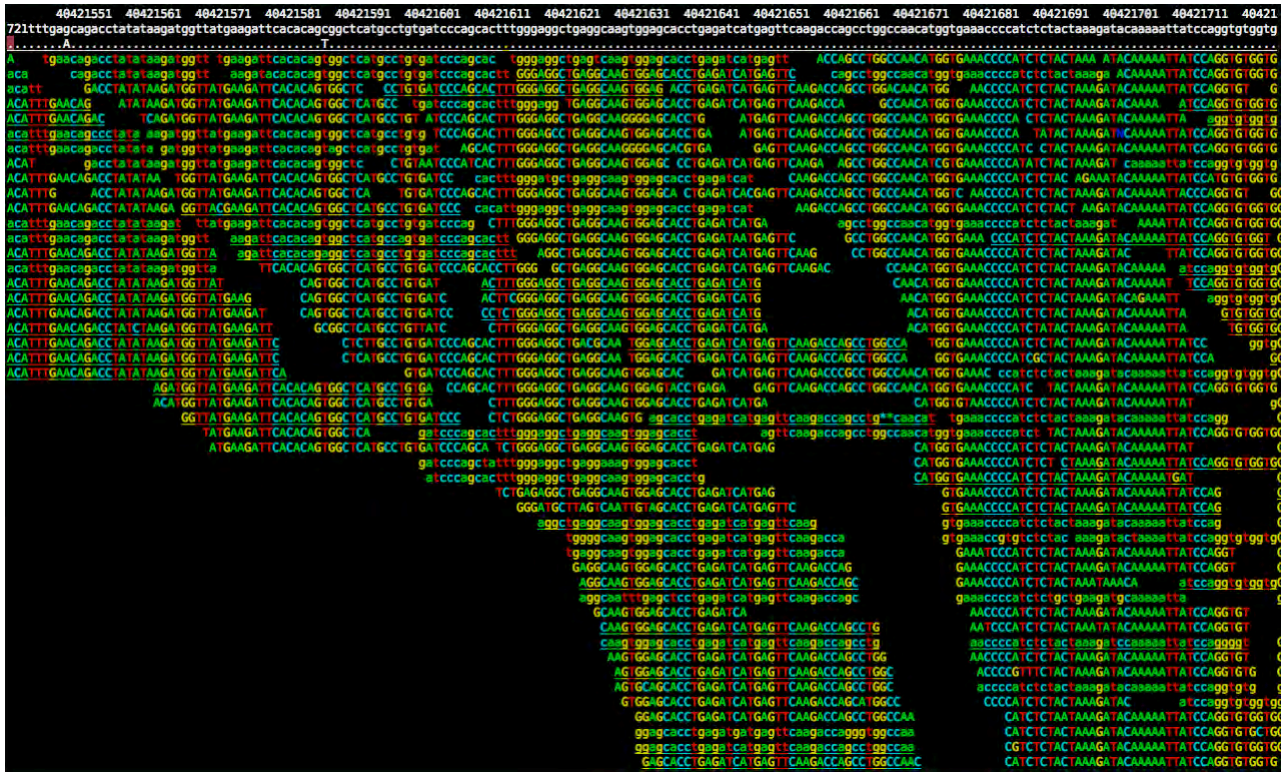
Nature Genetics 2007;39:1522-1527.

Genetic Testing

- Old-fashioned
  - Karyotype
  - Southern blot/FISH
  - PCR/MLPA
  - Sanger sequencing
- Newfangled
  - Chromosomal microarray
  - 2nd generation sequencing (NGS)
    - Targeted sequence capture
    - Exome sequencing
    - Genome sequencing
  - 3rd generation sequencing?
- <u>No genetic test method is perfect</u>



Courtesy: National Human Genome Research Institute - Extracted image from http://www.genome.gov/glossary/resources/karyotype.pdf., Public Domain, https://commons.wikimedia.org/w/index.php?curid=583512



Courtesy Tim W. Yu, MD, PhD

Different approaches to genetic analysis

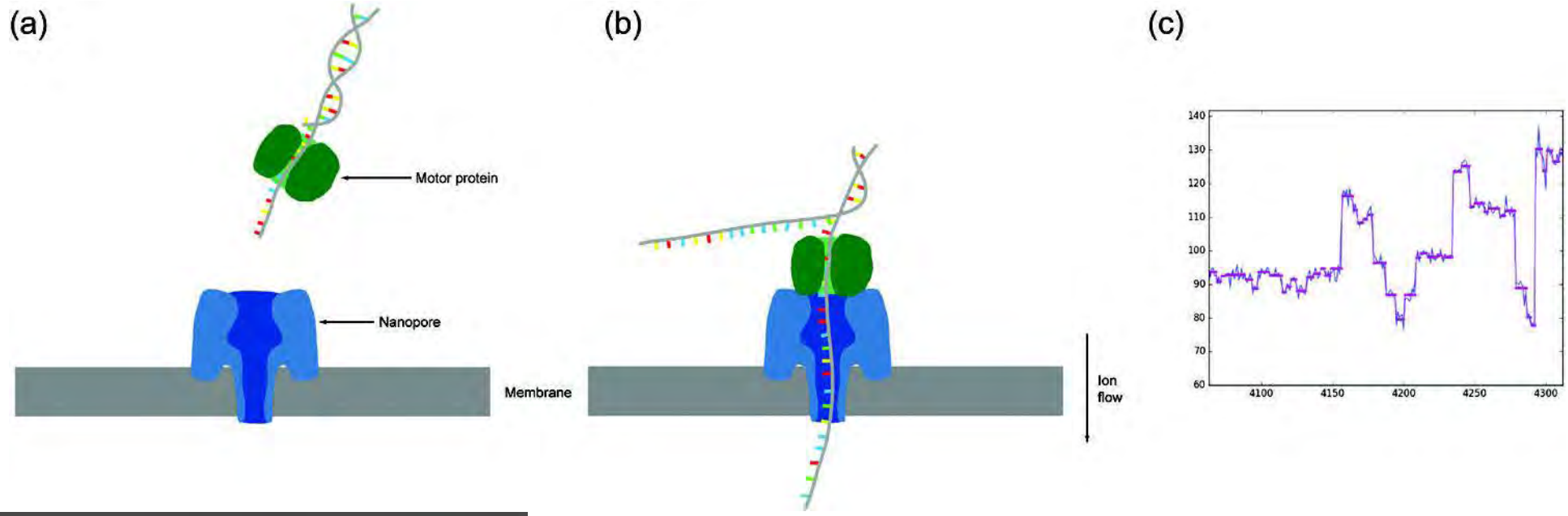
Clinical genetic testing

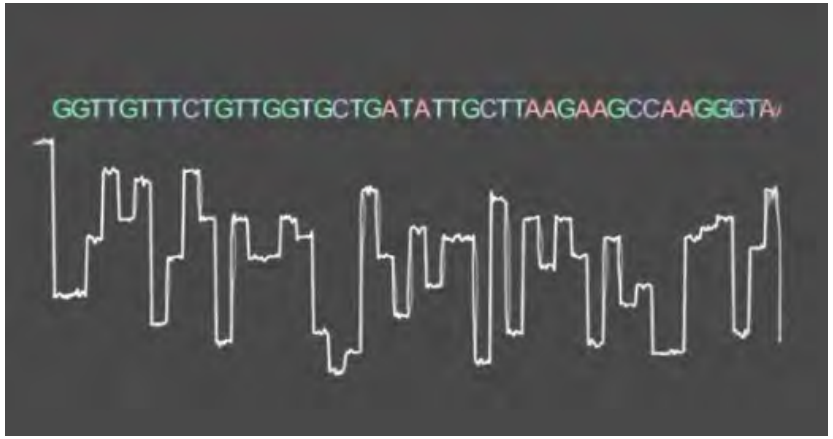
- Sanger sequencing: currently most useful for single genes
- Next generation sequencing
  - Targeted sequence capture
  - Exome sequencing
- CLIA-certified
- Official report issued
- Reliable timeframe for results

Research analysis

- Next generation sequencing
  - Exome sequencing
  - Genome sequencing
- Sanger sequencing: confirmation of candidate mutations
- Not CLIA-certified
- No official report
- No timeframe for results

Long read sequencing via the Nanopore system





Currents are measured in the pores

https://nanoporetech.com/learn-more

Long read sequencing (LRS) pilot project

- Enrolled 10 families affected by muscular dystrophy with incomplete genetic diagnoses
  - DMD patients with unclear clinical genetic findings
  - Autosomal recessive muscular dystrophy patients with single heterozygous pathogenic variants
- LRS whole genome sequencing using the Nanopore MinION and GridION systems
- Bruels CC et al, *Ann Clin Transl Neurol* 2022;9:1302-1309

LRS nanopore findings

- Routinely saw reads of 100-300 kpb
- Longest read length so far in our lab is 1.1 Mbp
- All clinically detected pathogenic variants (all single nucleotide variants) were also seen on LRS
- 4 individuals were found to have previously undetected pathogenic variants on nanopore sequencing
- 2 of these new pathogenic variants were structural variants (SVs)
- The other 2 new pathogenic variants were intronic splice variants

Table 1. Summary of nanopore LRS findings.

Individual	Clinically identified variant	Nanopore LRS results and (ACMG category)	Confirmation or supporting findings
1441-1	No variants identified in *DMD*	**Identified novel maternally inherited 5.9 Mbp inversion that disrupts DMD exons 3–79 (ACMG: P/LP)**	PCR and Sanger sequencing confirmed 1441-1 is hemizygous and 1441-2 (mother) is heterozygous for inversion
1441-2	Mother of 1441-1; asymptomatic	**Identified novel 5.9 Mbp inversion that disrupts DMD exons 3–79 (ACMG: P/LP)**	PCR and Sanger sequencing confirmed 1441-2 is heterozygous for inversion
1462-1	No variants identified in *DMD*	**Identified maternally inherited intronic splice variant *DMD* c.5548+67A>G (ACMG: P)**	Sanger sequencing confirmed 1462-1 is hemizygous and 1462-2 (mother) is heterozygous for the *DMD* splice variant; PCR and Sanger sequencing of RNA from muscle specimen confirmed aberrant splicing
1462-2	Mother of 1462–1; asymptomatic	**Identified intronic splice variant *DMD* c.5548+67A>G (ACMG: P)**	Sanger sequencing confirmed 1462-2 is heterozygous for the *DMD* splice variant
1480-1	No variants identified in *DMD*	**Identified intronic splice variant *DMD* c.5155-16T>A (ACMG: P)**	Sanger sequencing confirmed 1480-1 is hemizygous for *DMD* splice variant; aberrant splicing confirmed via minigene assay
1466-1	Duplication of *DMD* exons 10–26, suspected to be nontandem	Determined duplication including *DMD* exons 10–26 was in tandem and identified breakpoints (ACMG: VUS)	PCR and Sanger sequencing confirmed 1466-1 is hemizygous for tandem duplication
120-1	*LAMA2* c.2962C>T; p.Gln988Ter (ACMG: P/LP)	**An identified novel heterozygous *LAMA2* 3463 bp duplication (chr6:129,339,012–129,342,475, hg38) (ACMG: P); confirmed clinical SNV**	PCR and Sanger sequencing confirmed maternally inherited SV; LRS confirmed previously reported paternally inherited SNV
1126-1	*LAMA2* c.2538-1G>C; (splice variant) (ACMG: LP)	Confirmed clinical SNV	NA
1443-1	Decreased *D4Z4* methylation; no FSHD1 or FSHD2 variants identified	Confirmed *SMCHD1* c.182_183 delGT heterozygous variant (ACMG: LP)	NA
110-1	*ANO5* c.692G>T; (p.Gly231Val) (ACMG: P/LP)	Confirmed clinical SNV	NA
122-1	*CAPN3* c.1505T>C; p.Ile502Thr (ACMG: LP)	Confirmed clinical SNV	Sanger sequencing results suggest SNV is paternally inherited
125-1	*CAPN3* c.640G>A; p.Gly214Arg (ACMG: P/LP)	Confirmed clinical SNV	Sanger sequencing confirmed SNV is paternally inherited

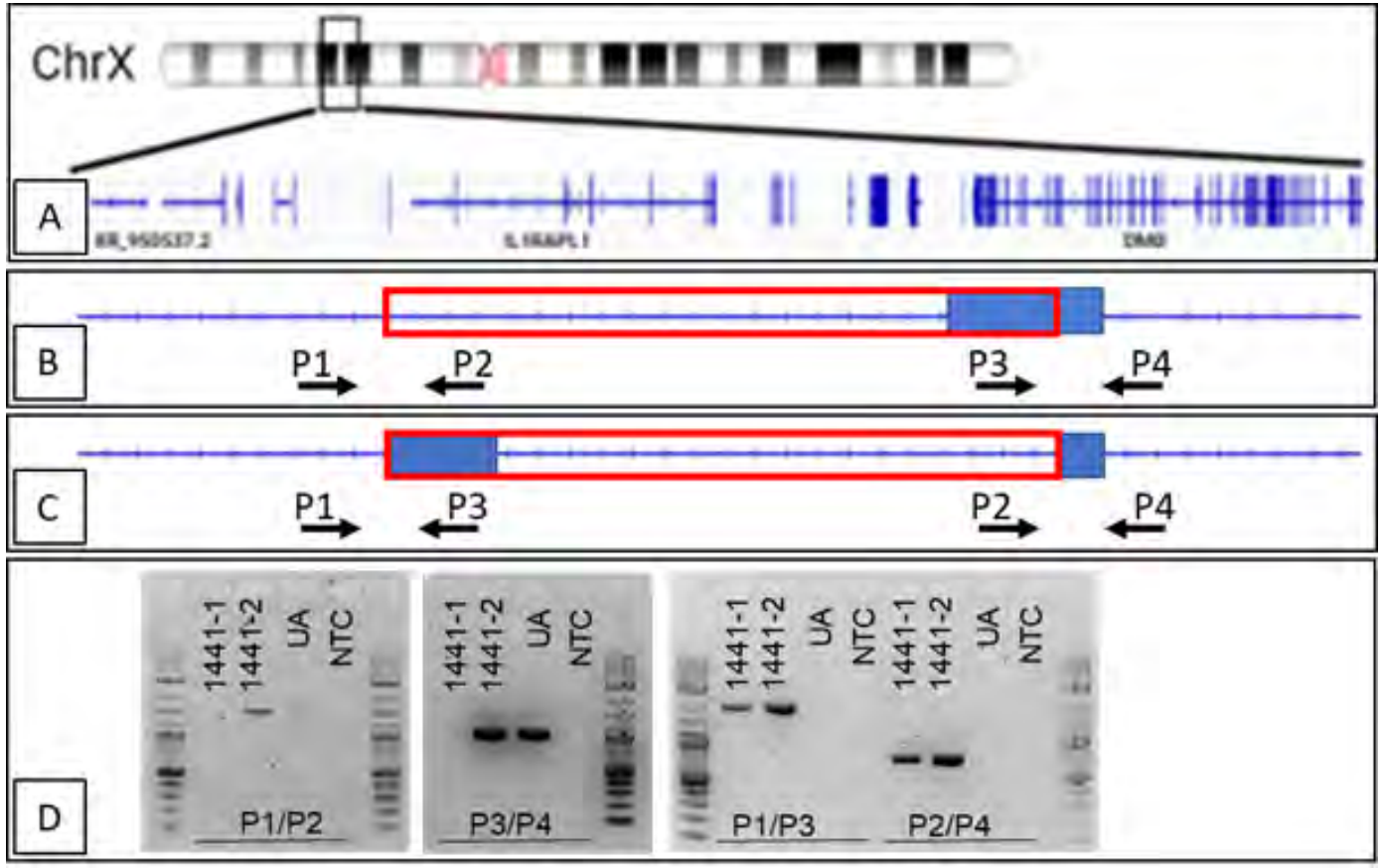
In 10 individuals, nanopore LRS identified four previously undetected pathogenic or likely pathogenic variants (shown in bold in families 1441, 1462, 1480, and 120), fully characterized a duplication noted on clinical testing, and confirmed all previously noted pathogenic SNVs. Variants identified in this study (in families 1441, 1462, 1480, 1466, 120, and 1443) were classified according to ACMG criteria; previously identified variants were classified by the reporting laboratory or according to their ClinVar designation. LRS, long-read sequencing; FSHD, facioscapulohumeral muscular dystrophy; P, pathogenic; LP, likely pathogenic; VUS, variant of unknown significance; SNV, single nucleotide variant.

Bruels CC et al, *Ann Clin Transl Neurol* 2022;9:1302-1309

1441: patient with DMD but no mutation

- 1441-1 was diagnosed clinically with DMD based on physical examination, serum CK levels, and absent dystrophin on muscle biopsy
- No detectable pathogenic variant on clinical genetic testing
- Nanopore sequencing revealed a 5.9 Mbp inversion on the X chromosome that included exons 2-79 of *DMD*

1441: inversion including *DMD*



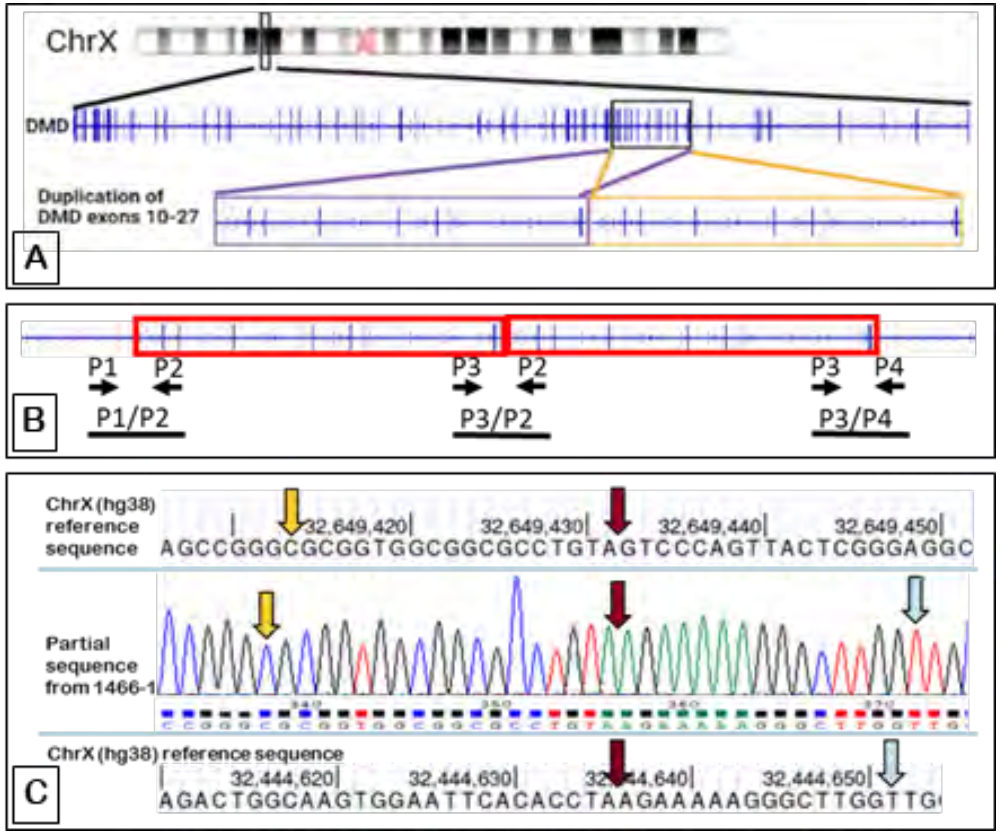
(A) Diagram indicates the location of the inversion.
(B) Positions of primer pairs P1/P2 and P3/P4 in gDNA without the inversion. Red box highlights the inverted region and blue box represents *DMD*.
(C) Positions of primer pairs P1/P3 and P2/P4 in gDNA with the inversion. Red box highlights the inverted region and blue boxes represent segments of *DMD*.
(D) PCR reactions using primer sets P1/P2, P3/P4, P1/P3, and P2/P4 on gDNA from 1441-1 (proband), 1441-2 (mother), and an unaffected individual (UA). A no template control (NTC) was included in all reactions. In 1441-1 and 1441-2, primers P1/P3 amplify an approximate 2,000 bp amplicon and primers P2/P4 amplify an approximate 800 bp amplicon, indicating they carry the inversion, while the unaffected individual does not. Primers P1/P2 and P3/P4 do not produce an amplicon in 1441-1, but do for 1441-2 and the unaffected individual, indicating that the proband is hemizygous for the inversion and 1441-2 (mother) is a heterozygous carrier.

Bruels CC et al, *Ann Clin Transl Neurol* 2022;9:1302-1309

1466: asymptomatic individual with “DMD”

- 1466-1 is an adult male who had prenatal genetic screening
- Normal muscle strength, normal serum CK level
- An in-frame duplication was detected in exons 10-26 in *DMD*
- Is the duplication in tandem?
- Nanopore sequencing confirmed that the duplication was in fact in tandem, indicating a likely benign variant

1466: *DMD* tandem duplication



(A) Diagram indicates the location of duplication in chromosome X, in *DMD*, and details of the duplication.

(B) Primer design strategy and expected results were similar to that for family 120. Red boxes highlight the duplicated region; black bars show expected amplicons. The relative positions of primers P1, P2, P3, and P4 in duplicated gDNA are indicated by arrows.

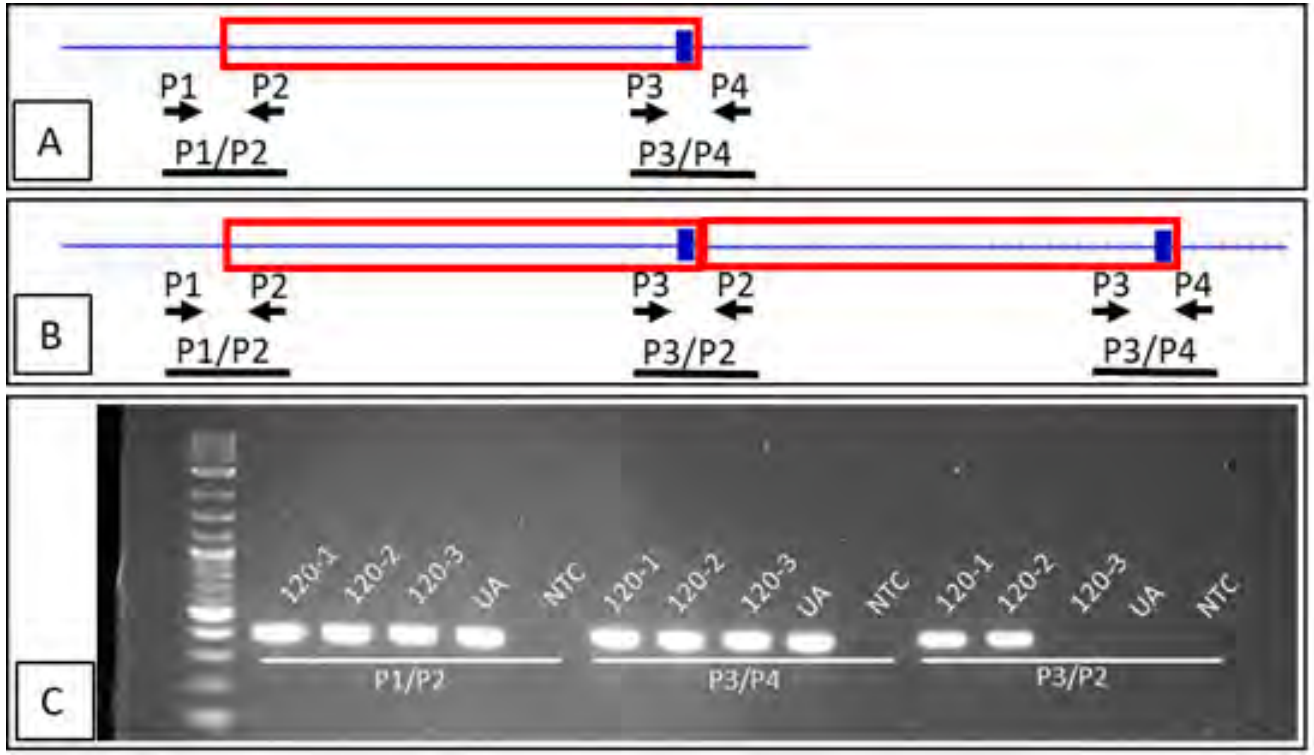
(C) The duplication breakpoints can be seen in the sequence obtained from the P3/P2 amplicon using primer P3, which includes the junction. Of the duplicated region. Burgundy arrows indicate the breakpoint location, gold or blue arrows highlight a single base in the reference sequence. Breakpoints are at chrX:32,444,637 and chrX:32,649,432 (hg38).

Bruels CC et al, *Ann Clin Transl Neurol* 2022;9:1302-1309

120: *LAMA2* partial diagnosis

- 120-1 was diagnosed with merosin deficiency based on clinical presentation, serum CK level, brain MRI
- Clinical genetic testing showed only a single heterozygous paternally inherited *LAMA2* c.2962C>T
- Nanopore sequencing detected a maternally inherited 3,463 bp duplication in *LAMA2* that included all of exon 30
- Exon 30 starts with a codon for asparagine and ends at the 2nd base for another codon for asparagine

120: *LAMA2* duplication



(A) Positions of primer pairs P1/P2 and P3/P4 in unduplicated gDNA. Red boxes highlight the duplicated region and black bars show expected amplicons.

(B) Relative positions of primers P1, P2, P3, and P4 in duplicated gDNA. Primer pair P3/P2 should only produce an amplicon if the duplication is present.

(C) PCR reactions were performed using primer sets P1/P2, P3/P4, and P3/P2 to amplify gDNA extracted from 120-1 (proband), 120-2 (mother), 120-3 (father), and an unaffected individual (UA). Primers P3/P2 amplify an ~400 bp amplicon in 120-1 and 120-2 indicating they carry the duplication, while 120-3 and the unaffected individual do not. NTC, no template control.

Bruels CC et al, *Ann Clin Transl Neurol* 2022;9:1302-1309

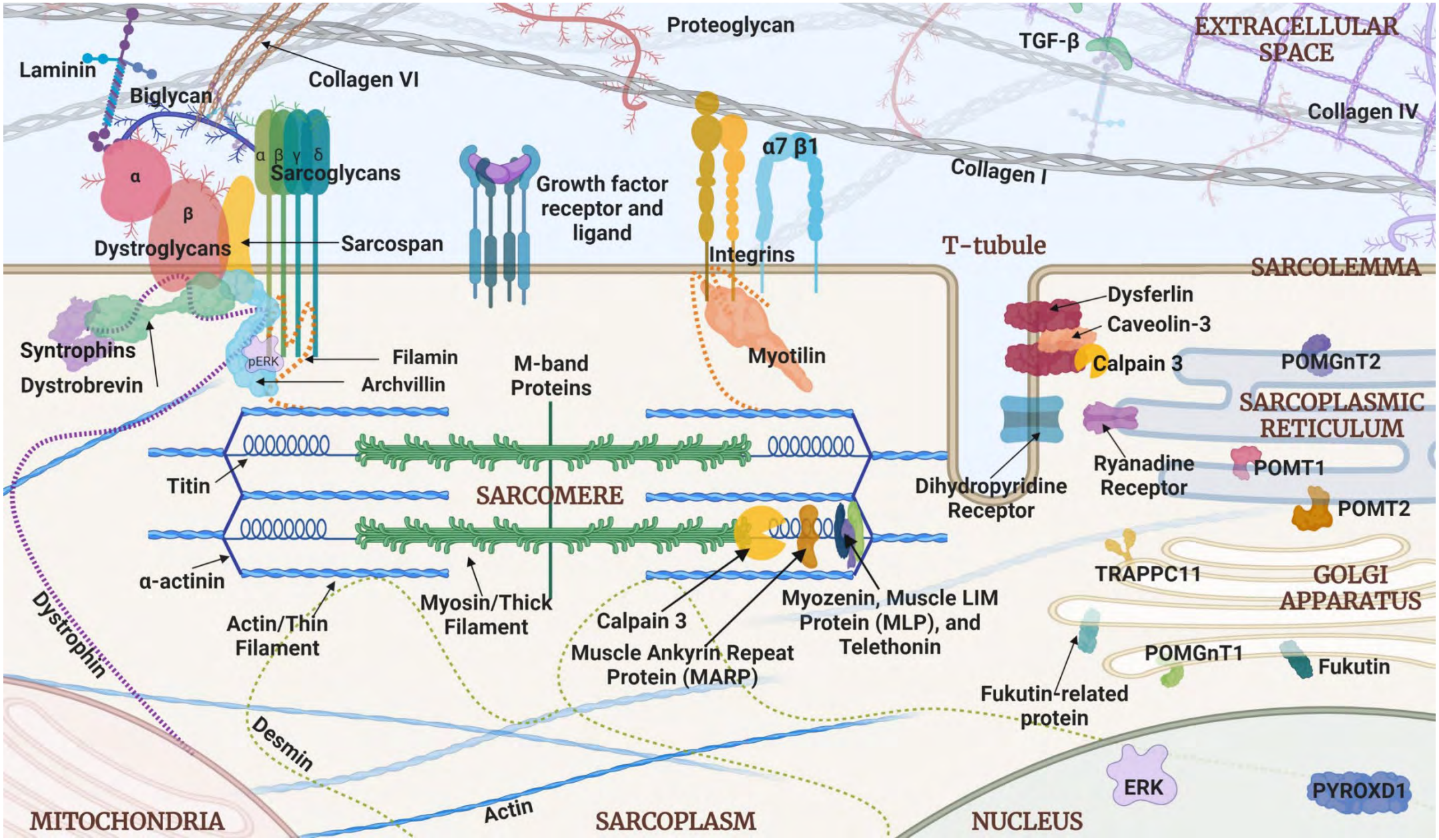
LRS study conclusions

- Nanopore LRS can accurately detect single nucleotide variants (SNVs) at 20-30X mean read depth
- Nanopore LRS can detect some SVs that are not apparent on short read sequencing (SRS) at 10-20X mean read depth
- There is potential for LRS to detect a broader range of pathogenic variants than SRS
- Cost issues should attenuate over time

Classification

Classification of neuromuscular disorders

- Motor neuron disease
  - Spinal muscular atrophy
- Neuropathy
  - Charcot-Marie-Tooth disease
- Neuromuscular junction disorder
  - Myasthenia gravis
  - Congenital myasthenic syndrome
- Muscle disease
  - Muscular dystrophy
  - Congenital myopathy
  - Metabolic myopathy



Barton ER et al, *Skeletal Muscle* 2020;10:22

Classification of muscular dystrophies

- Dystrophinopathy (DMD and BMD): X-linked
- Limb-girdle muscular dystrophy (LGMD)
- Congenital muscular dystrophy (CMD)
- Facioscapulohumeral muscular dystrophy (FSHD)
- Emery-Dreifuss muscular dystrophy (EDMD)
- Myotonic dystrophy (DM)
- Distal myopathy / distal muscular dystrophy
- Oculopharyngeal muscular dystrophy (OPMD)

LGMD: old classification

- There are currently over 30 associated genes
- Most LGMD mutations to date are single nucleotide variants or other small changes
- The traditional classification system divided LGMDs into types 1 (dominant) and 2 (recessive) + letters
- Recessive subtypes (LGMD2) were much more numerous than dominant ones (LGMD1)

The classification dilemma

- LGMD2Z was described a few years ago
- What now?
- LGMD2AA?
- It became apparent that the traditional classification system was no longer viable

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Neuromuscular Disorders 28 (2018) 702–710

www.elsevier.com/locate/nmd

Workshop report

229th ENMC international workshop: Limb girdle muscular dystrophies – Nomenclature and reformed classification Naarden, the Netherlands, 17–19 March 2017

Volker Straub[a,*], Alexander Murphy[a], Bjarne Udd[b,c,d], on behalf of the LGMD workshop study group

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Received 25 March 2018

Classification change explained

Old system

- LGMD1 = dominant
  - LGMD1A = *MYOT*
  - LGMD1B = *LMNA*
  - etc
- LGMD2 = recessive
  - LGMD2A = *CAPN3*
  - LGMD2B = *DYSF*
  - etc

New system

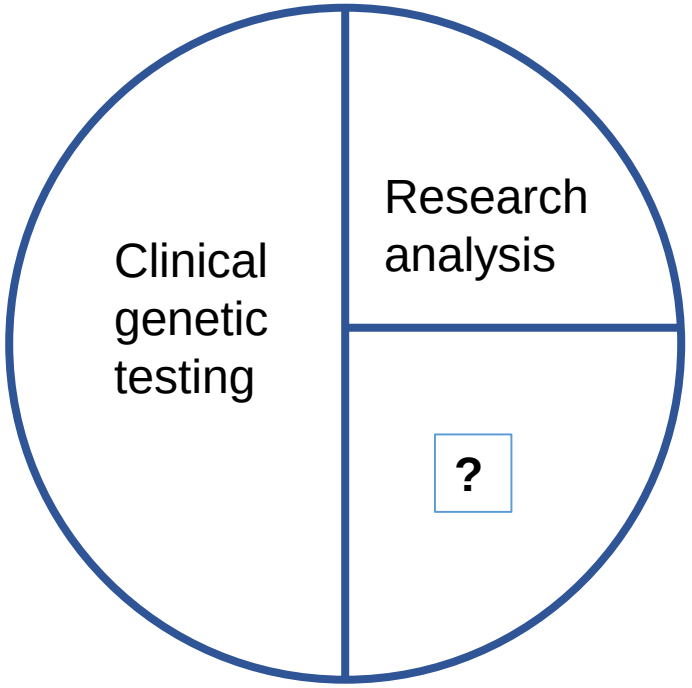
- LGMDD = dominant
  - LGMDD1 = *DNAJB6*
  - LGMDD2 = *TNP03*
  - etc
- LGMDR = recessive
  - LGMDR1 = *CAPN3*
  - LGMDR2 = *DYSF*
  - etc

Table 1
Comparison of the previous LGMD nomenclature to the proposed classification system after the definition has been applied to the current list of LGMD. Conditions which are no longer considered LGMDs are highlighted in grey with a reason for their exclusion given.

Old name	Gene	Proposed new nomenclature	Reason for exclusion
LGMD 1A	*Myot*	Myofibrillar myopathy	Distal weakness
LGMD 1B	*LMNA*	Emery–Dreifuss muscular dystrophy (EDMD)	High risk of cardiac arrhythmias; EDMD phenotype
LGMD 1C	*CAV3*	Rippling muscle disease	Main clinical features rippling muscle disease and myalgia
LGMD 1D	*DNAJB6*	LGMD D1 DNAJB6-related	
LGMD 1E	*DES*	Myofibrillar myopathy	Primarily false linkage; distal weakness and cardiomyopathy
LGMD 1F	*TNP03*	LGMD D2 TNP03-related	
LGMD 1G	*HNRNPDL*	LGMD D3 HNRNPDL-related	
LGMD 1H	?	Not confirmed	False linkage
LGMD 1I	*CAPN*	LGMD D4 calpain3-related	
LGMD 2A	*CAPN*	LGMD R1 calpain3-related	
LGMD 2B	*DYSF*	LGMD R2 dysferlin-related	
LGMD 2C	*SGCG*	LGMD R5 γ-sarcoglycan-related[a]	
LGMD 2D	*SGCA*	LGMD R3 α-sarcoglycan-related	
LGMD 2E	*SGCB*	LGMD R4 β-sarcoglycan-related	
LGMD 2F	*SGCD*	LGMD R6 δ-sarcoglycan-related	
LGMD 2G	*TCAP*	LGMD R7 telethonin-related	
LGMD 2H	*TRIM32*	LGMD R8 TRIM 32-related	
LGMD 2I	*FKRP*	LGMD R9 FKRP-related	
LGMD 2J	*TTN*	LGMD R10 titin-related	
LGMD 2K	*POMT1*	LGMD R11 POMT1-related	
LGMD 2L	*ANO5*	LGMD R12 anoctamin5-related	
LGMD 2M	*FKTN*	LGMD R13 Fukutin-related	
LGMD 2N	*POMT2*	LGMD R14 POMT2-related	
LGMD 2O	*POMGnT1*	LGMD R15 POMGnT1-related	
LGMD 2P	*DAG1*	LGMD R16 α-dystroglycan-related	
LGMD 2Q	*PLEC*	LGMD R17 plectin-related	
LGMD 2R	*DES*	myofibrillar myopathy	Distal weakness
LGMD 2S	*TRAPPC11*	LGMD R18 TRAPPC11-related	
LGMD 2T	*GMPPB*	LGMD R19 GMPPB-related	
LGMD 2U	*ISPD*	LGMD R20 ISPD-related	
LGMD 2V	*GAA*	Pompe disease	Known disease entity, histological changes
LGMD 2W	*PINCH2*	PINCH-2 related myopathy	Reported in one family
LGMD 2X	*BVES*	BVES related myopathy	Reported in one family
LGMD 2Y	*TOR1AIP1*	TOR1AIP1 related myopathy	Reported in one family
LGMD 2Z	*POGLUT1*	LGMD R21 POGLUT1-related	
Bethlem myopathy recessive	*COL6A1, COL6A2, COL6A3*	LGMD R22 collagen 6-related	
Bethlem myopathy dominant	*COL6A1, COL6A2, COL6A3*	LGMD D5 collagen 6-related	
Laminin α2-related muscular dystrophy	*LAMA2*	LGMD R23 laminin α2-related	
POMGNT2-related muscular dystrophy	*POMGNT2*	LGMD R24 POMGNT2-related	

[a] Sarcoglycan-related LGMDs rationalised based on order of gene discovery.

Straub et al, Neuromuscul Disord 2018;702-710



Classic/common LGMD genes

• LGMD R1 = LGMD2A	*CAPN3*
• LGMD R2 = LGMD2B	*DYSF*
• LGMD R3 = LGMD2D	*SGCA*
• LGMD R4 = LGMD2E	*SGCB*
• LGMD R5 = LGMD2C	*SGCG*
• LGMD R6 = LGMD2F	*SGCD*
• LGMD R9 = LGMD2i	*FKRP*
• LGMD R12 = LGMD2L	*ANO5*

Classic/common CMD genes

- Collagenopathies: no structural or functional CNS
  - *COL6A1*, *COL6A2*, *COL6A3*
- Merosinopathies: white matter lesions
  - *LAMA2*
- Dystroglycanopathies: structural and functional CNS
  - *FKRP*, *FKTN*, *ISPD*, *LARGE*, *POMGNT1*, *POMGNT2*, *POMT1*, *POMT2*

Other muscular dystrophy genes

- Facioscapulohumeral muscular dystrophy (FSHD)
  - FSHD1: D4Z4 macrosatellite contraction + permissive allele
  - FSHD2: *SMCHD1* variant + permissive allele
- Emery-Dreifuss muscular dystrophy (EDMD)
  - *EMD*, *LMNA*, others
- Myotonic dystrophy (DM)
  - DM1: *DMPK*
  - DM2: *CNBP* (*ZNF9*)
- Distal myopathy / distal muscular dystrophy
  - Complicated
- Oculopharyngeal muscular dystrophy (OPMD)
  - *PABPN1*

FDA-approved therapies

3 FDA-approved therapies for SMA

- 2016: nusinersen (antisense oligonucleotide)
  - Intrathecal (via lumbar puncture)
  - Requires ongoing dosing
- 2019: onasemnogene abeparvovec (AAV9-based gene therapy)
  - Single intravenous dose
  - Requires significant pre- and post-treatment monitoring
- 2020: risdiplam (small molecule)
  - Oral
  - Requires ongoing dosing

TABLE. DISEASE-MODIFYING TREATMENTS FOR SPINAL MUSCULAR ATROPHy			
Medication	**Nusinersen**	**Onasemnogene abeparvovec**	**Risdiplam**
Route of delivery	Intrathecal	Intravenous	Oral
Dosing intervals	4 loading doses in 4 months, then maintenance dosing every 4 months	1-time	Once daily
Common side effects	Thrombocytopenia, renal toxicity, coagulation abnormalities	Elevated liver transaminases	Thrombocytopenia, renal toxicity, coagulation abnormalities
Indications	All patients with SMA	SMA ≤2 years old	SMA ≥2 months old
Year approved	2016	2019	2020
Abbreviation: SMA, spinal muscular atrophy.			

Cook MK & Kang PB. Spinal muscular atrophy. *Practical Neurology* 2022

2 FDA-approved therapies for Pompe disease

- 2006: alglucosidase alfa (enzyme replacement therapy for all Pompe disease cases)
  - Limited uptake in skeletal muscles, necessitating high doses
- 2021: avalglucosidase alfa-ngpt (enzyme replacement therapy indicated for patients 1 year and older with late onset Pompe disease
  - Conjugated with multiple synthetic bis-mannose-6-phosphate-tetra-mannose glycans
  - Has enhanced binding to mannose-6-phosphate receptor
  - Improved clearance of glycogen

1 FDA-approved therapy for periodic paralysis

- 2015: dichlorphenamide – carbonic anhydrase inhibitor, oral medication used for hypokalemic and hyperkalemic periodic paralysis
- Other medications are used off label

1 FDA-approved therapy for Friedreich ataxia

- 2023: omavexalone – oral medication for those ages 16-40 that activates Nrf2 and augments mitochondrial function

6 FDA-approved therapies for DMD

- 2016: eteplirsen (antisense oligonucleotides that induces exon 51 skipping)
  - [Mendell JR et al, *Ann Neurol* 2016;79:257-271]
- 2017: deflazacort (corticosteroid)
  - [Griggs RC et al, *Neurology* 2016;87:2123-2131]
- 2020: golodirsen (antisense oligonucleotide that induces exon 53 skipping)
  - [Frank DE et al, *Neurology* 2020;94:e2270-e2282]
- 2020: viltolarsen (antisense oligonucleotide that induces exon 53 skipping)
  - [Clemens PR et al, *JAMA Neurol* 2020;77:982-991]
- 2021: casimersen (antisense oligonucleotide that induces exon 45 skipping)
  - [Wagner KR et al, *Muscle Nerve* 2021;64:285-292]
- 2023: delandistrogene moxeparvovec (gene therapy)
  - [Mendell JR et al, *Muscle Nerve* 2023;epub August 14]

Antisense oligonucleotide therapy

- Studies suggested that antisense oligonucleotide therapy is effective in boys with DMD amenable to exon 51 skipping
  - Mendell et al, Ann Neurol 2013;74:637-647
  - Voit et al, Lancet Neurol 2014;13:987-996
  - Mendell et al, Ann Neurol 2016;79:257-271
- FDA approval for eteplirsen granted in 2016
- Weekly intravenous infusions required
- Other antisense oligonucleotide compounds have now been approved

FDA approvals for AAV gene therapy

- Voretigene neparvovec rzyl (2017) – inherited retinal disease (biallelic RPE65 mutation-associated retinal dystrophy – Leber congenital amaurosis)
- Onasemnogene abeparvovec xioi (2019) – spinal muscular atrophy (SMA)
- Etranacogene dezaparvovec drlb (2022) – hemophilia B
- Valoctocogene roxaparvovec rvox (2023) – hemophilia A
- Delandistrogene moxeparvovec rokl (2023) – Duchenne muscular dystrophy (DMD)

Therapies in development

More gene therapy

- DMD: multiple other AAV therapies are undergoing human clinical trials
- LGMD R2/2B (DYSF): SRP-6004
- LGMD R3/2D (SGCA): SRP-9004
- LGMD R4/2E (SGCB): SRP-9003
- LGMD R5/2C (SGCG): Multicenter Phase 1b study of ATA-200, clinical trial application filed in Europe
- LGMD R9/2i (FKRP): LION-101 Phase 1/2 study
- Pompe disease: ACT-101

Gene Delivery Vehicles

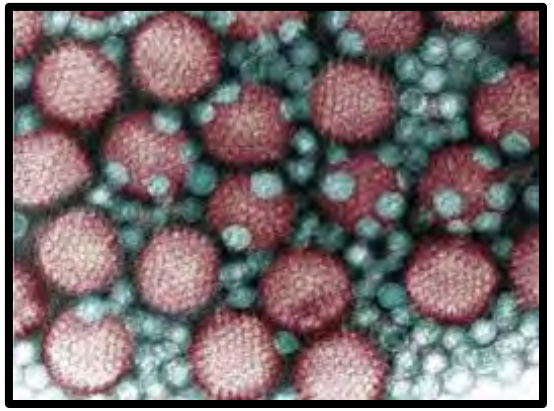
Many exist (plasmid delivery systems, retroviruses, lentiviruses, adenoviruses, adeno-associated viruses, extracellular vesicles) and all have strengths and limitations

Adeno-associated virus (AAV)

Courtesy Christina Pacak

Adeno-Associated Virus (AAV) for gene therapy

- Discovered as a contaminant in adenovirus preparations – requires help for replication



- Recombinant AAV (rAAV) used for gene therapy lacks elements necessary for replication

Wild-Type AAV (wtAAV)

4,680 bp

Recombinant AAV (rAAV)

Up to ~5,000 bp

Humoral Immunity to AAV Vectors in Gene Therapy: Challenges and Potential Solutions
June 27, 2013. Elisa Masat, Giulia Pavani, Federico Mingozzi

Courtesy Christina Pacak

Control of AAV Expression Increases Patient Safety – Many Layers of Optimization

- Capsid serotype
- Vector sequence design
- Dose
- Delivery route

Courtesy Christina Pacak

Small molecules

- DMD/BMD
  - EDG-5506 – inhibitor of fast skeletal muscle myosin protects muscle from hypercontractile stress
  - Ataluren – stop codon readthrough
- LGMD R9/2i (FKRP)
  - EDG-5506 – protects muscle from hypercontractile stress
  - BBP-418 – naturally occurring sugar that is a substrate for the FKRP enzyme
- All of these investigational compounds are orally stable

Nitric oxide agonists

- PDE5 inhibitors ameliorated the muscular dystrophy phenotype in mouse models
- Human trials of sildenafil have been disappointing
  - Leung et al, Ann Neurol 2014;76:541-549
  - Witting et al, Ann Neurol 2014;76:550-557
- A human trial of tadalafil has been disappointing
  - Victor RG et al, Neurology 2017;89:1811-1820

Myostatin inhibition

- Report of a muscular boy with mutation in myostatin triggered great interest in this approach
  - Schuelke et al, N Engl J Med 2004;350:2682-2688
- Initial human trials of antibody mediated approaches disappointing
  - Wagner et al, Ann Neurol 2008;63:561-571 (no efficacy)
  - Campbell C et al, Muscle Nerve 2017;55:458-464 (toxicity for ACE-031)
- Further trials in process

CRISPR/Cas9

- Gene editing approaches
  - Zinc finger mutagenesis
  - Transcription activator-like effector nuclease (TALEN)
  - CRISPR/Cas9 – adaptation of bacterial system to protect against viruses
- Gene editing can potentially alter DNA sequences in a variety of ways
- CRISPR/Cas9 showing immense promise in preclinical studies
  - Nelson et al, *Science* 2016;351:403-407 (excising exon 23 from *mdx* mouse)
  - El Refaey et al, *Circ Res* 2017;121:923-929 (excising exon 23)
  - Amoasii et al, *Sci Transl Med* 2017;9:eaan8081 (exon 51 skipping)
  - Nelson et al, *Nat Med* 2019;25:427-432 (excising exon 23 from *mdx* mouse)
- A human clinical trial of CRISPR/Cas9 therapy in DMD was associated with a fatality in 2022

Stem cell therapy

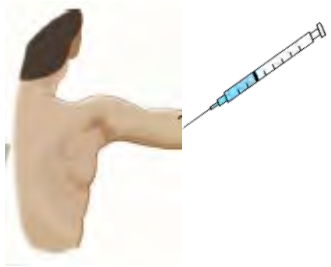
- Early human trials in early 1990s disappointing
- Experiments in murine and canine models have suggested therapeutic potential for many years
- There is a major effort here at the University of Minnesota to develop an iPSC-derived cell therapy, first for DMD, then potentially other dystrophies

Translational Muscle Working Group

Rita Perlingeiro

John Wagner

Michael Kyba



UMN – Center for Translational Medicine

David McKenna

Robert Schumacher

UMN - Molecular & Cellular Therapeutics Facility

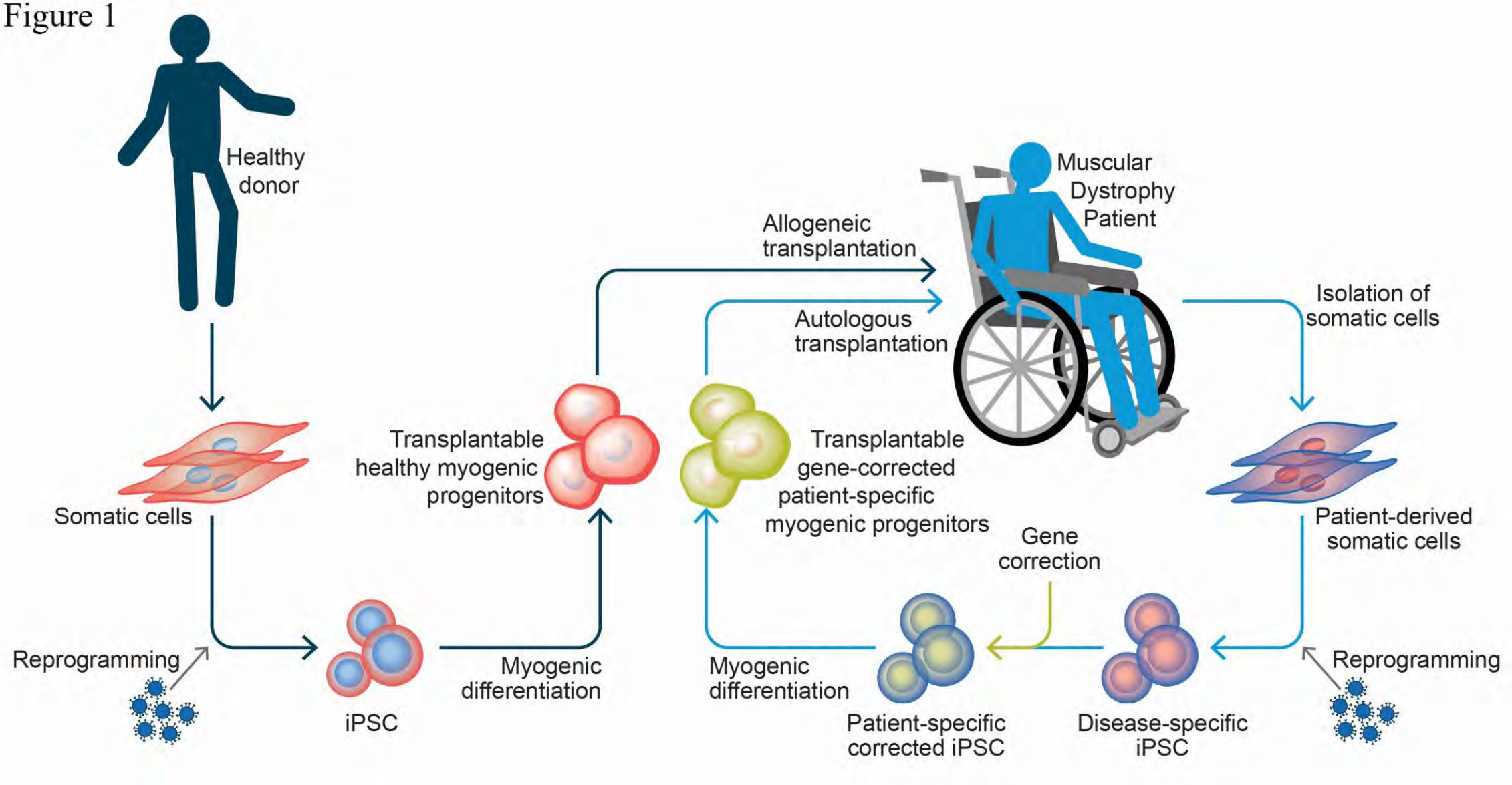
Peter Karachunski

Peter Kang

Edward Cheng

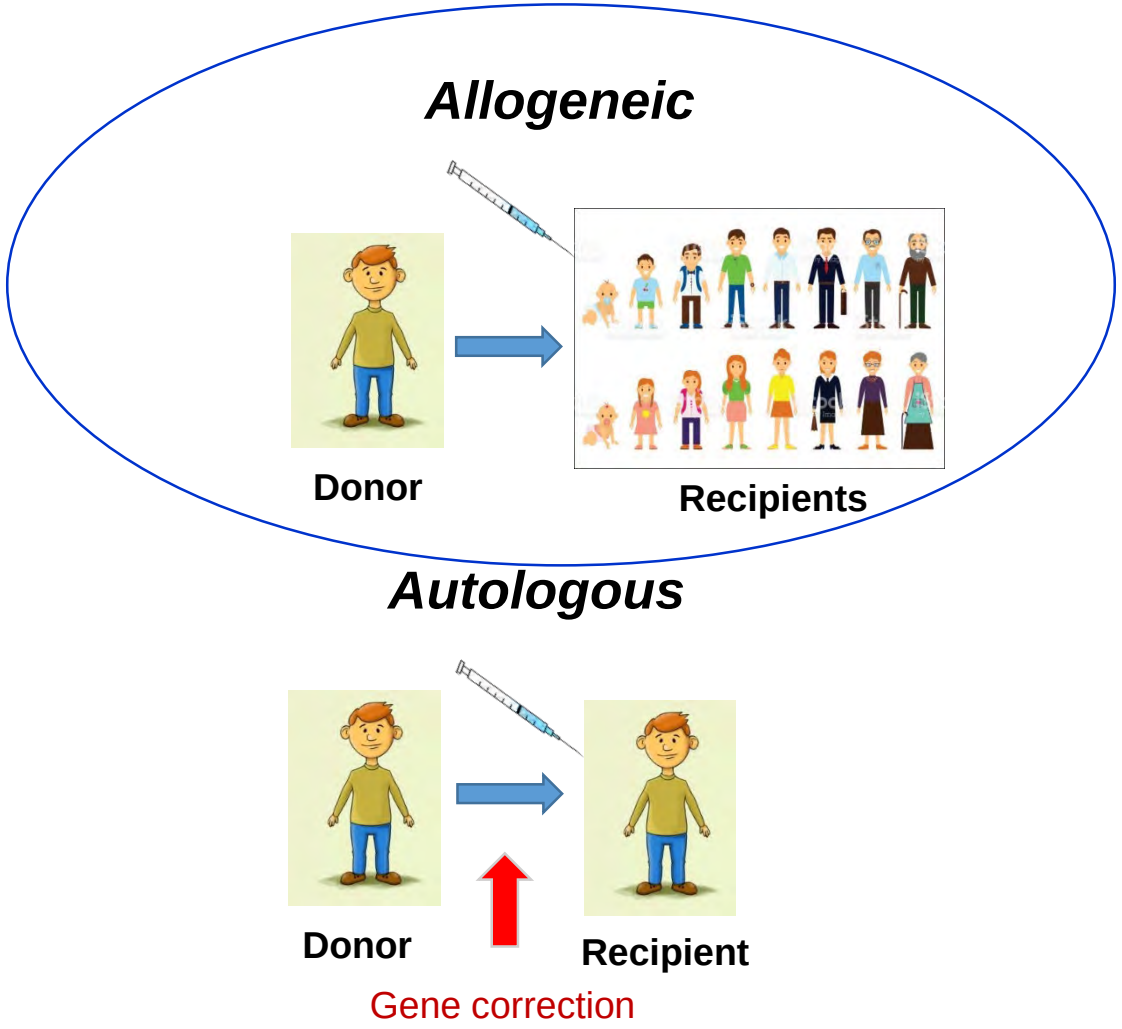
Courtesy Rita Perlingeiro

Figure 1



Selvaraj S, Kyba M, Perlingeiro RCR. *Trends in Mol. Med*. 2019;25:803

Allogeneic versus Autologous



Courtesy Rita Perlingeiro

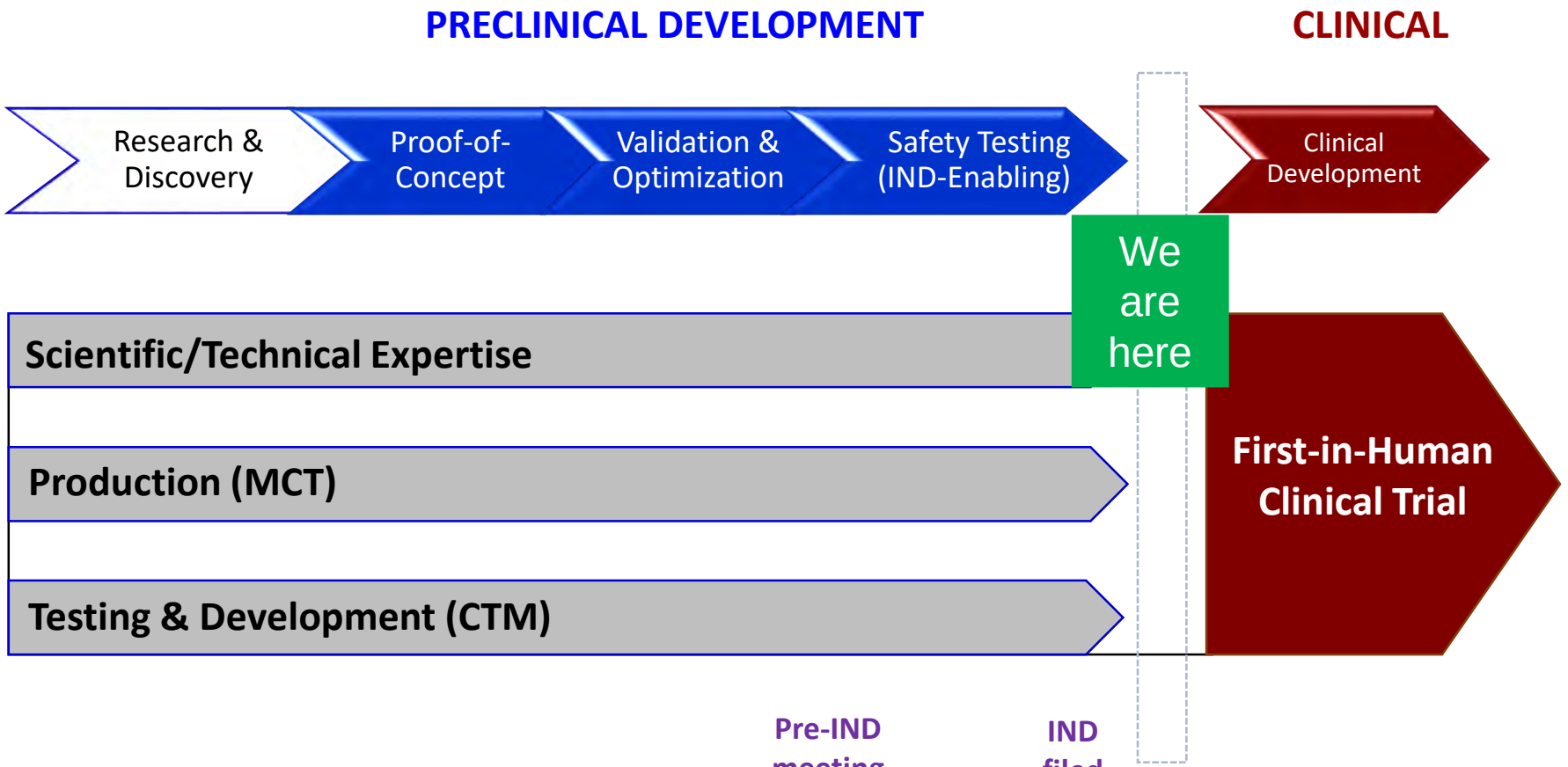
Roadmap for Clinical Translation

- √ **1- Methodology to generate cell product**
  - Transgene-free methods failed to generate cells with *in vivo* regenerative potential (Kim et al; Stem Cell Reports, 9:12, 2017)
  - Controlled expression of PAX7 is required – switched to third generation LV vectors (Magli, Incitti et al; Cell Reports, 19:2867, 2017)
- √ **2- Purification of cell product** (α9β1, **CD54** and SDC2 identify PAX7+ PSC-derived myogenic progenitors; Magli, Incitti et al; Cell Reports, 19:2867, 2017)
- √ **3- Generate GMP-compliant cell product** (Eliminate and/or replace components that are not compatible with clinical trials)
- √ **4- Scalability with GMP-compliant method** (To determine ideal conditions to maximize expansion)
- √ **5- Characterization of cell product (*in vitro* and *in vivo*)**
- √ **6- Technology transfer to clinical grade facility (cGMP production)**
- √ **7- Clinical grade lentiviral vectors**
- √ **8- Production clinical grade cell product**
- √ **9- GLP preclinical studies (IND-enabling)**
- √ **10- IND filling with the FDA**

11- Clinical Trial

Courtesy Rita Perlingeiro

Roadmap to the Clinic



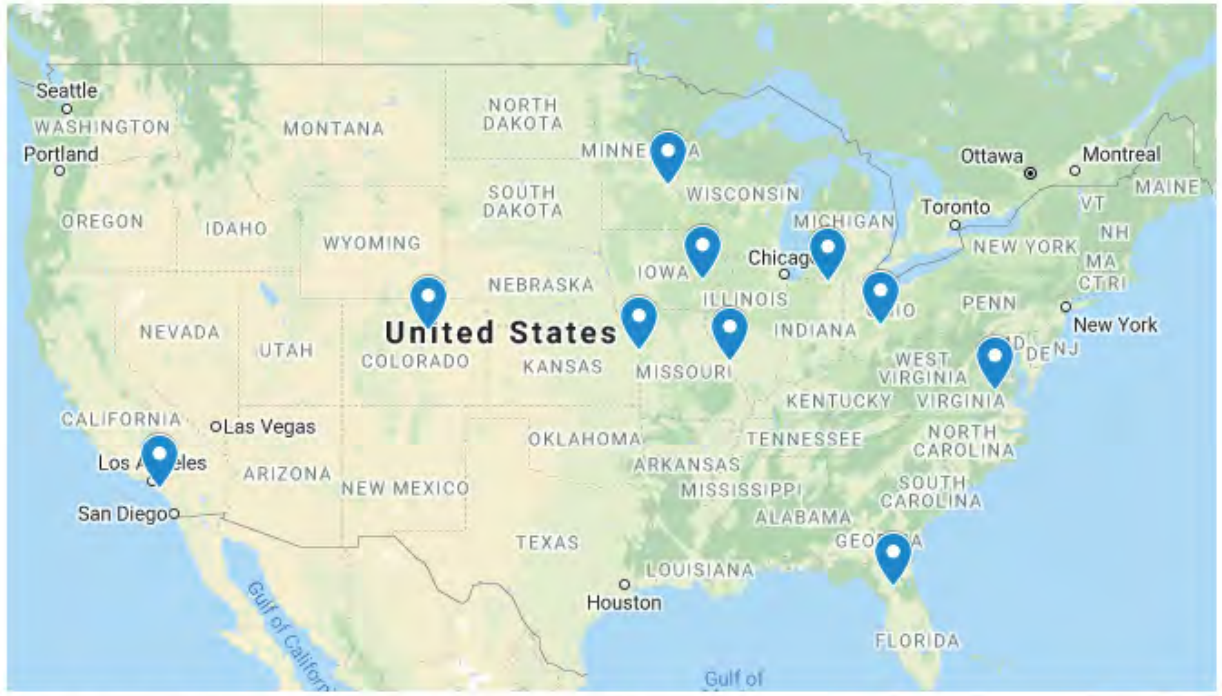
*Includes IND-enabling (GLP) safety and toxicology studies required for FDA approval and IND development & filing

Courtesy Rita Perlingeiro

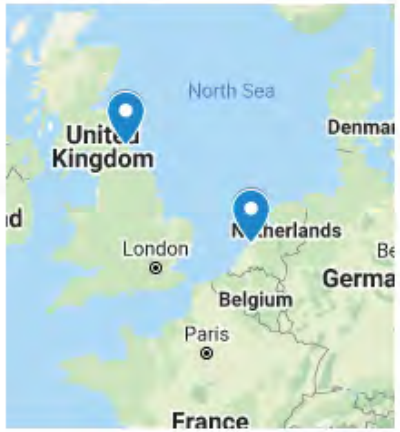
GRASP LGMD Background

- Genetic Resolution and Assessments Solving Phenotypes in LGMD (GRASP-LGMD) Consortium
- Overall PI: Nicholas Johnson, MD
- Coordinating Center: Virginia Commonwealth University
- Foundation, corporate, and NIH funding

GRASP LGMD Sites



- University of Kansas Medical Center
- University of Iowa
- Nationwide Children's Hospital
- University of California Irvine
- Washington University
- University of Minnesota
- University of Florida
- University of Colorado Anschutz Medical Center
- Community Health Clinic
- VCU Neurology Department
- Leiden University
- Newcastle University



Goals include:

1. Gathering natural history data
2. Optimizing outcome measures
3. Providing a platform for multi-center clinical trials

Courtesy Nicholas Johnson, MD

GRASP-LGMD Studies

- A platform natural history study
- Develop outcome measures for upcoming clinical trials
- Studies are 12 months in duration
- Visits include:
  - Surveys
  - Muscle strength and function testing
  - Blood draw
  - May include muscle biopsy or MRI

Current GRASP Natural History Studies		
LGMD Subtype	Gene	Sponsor
R1/2A	CAPN3	NIH, MDA, C3
R2/2B	DYSF	MDA
R3/2D R4/2E R5/2C R6/2F	SGCA SGCB SGCG SGCD	Sarepta
R9/2I	FKRP	ML Bio
R12/2L	ANO5	MDA
D1	DNAJB6	MDA

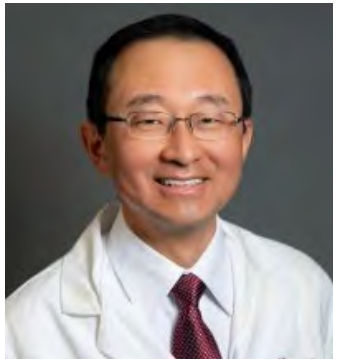
Courtesy Nicholas Johnson, MD

Paul & Sheila Wellstone Muscular Dystrophy Center

University of Minnesota

Driven to Discover℠

The MD Center Team



Peter Kang, MD, FAAN, FAAP

MD Center Director

Dr. Kang is a pediatric neuromuscular neurologist and physician-scientist whose laboratory studies the genetics of muscular dystrophy and mechanisms of rare muscle diseases, with the goal of discovering new therapeutic targets for these diseases.

Peter Karachunski, MD

MD Center Clinical Director

Dr. Karachunski is board certified neurologist with special qualification in pediatric neurology. He specializies n neuromuscular medicine for both adult and pediatric patients. Dr. Karachunski is a director of comprehensive multidisciplinary Muscular Dystrophy Association Care Center.

James Ervasti, PHD

MD Center Research Director

Dr. Ervasti and his lab aim to fully define the function of dystrophin in striated muscle to understand how its absence or abnormality leads to the pathologies observed in Duchenne and Becker muscular dystrophies.His unique approach integrates biochemical and biophysical analyses of the very large dystrophin protein with in vivo assessments of its function in transgenic mouse models of muscular dystrophy.

Paul & Sheila Wellstone Muscular Dystrophy Center

UNIVERSITY OF MINNESOTA
Driven to Discover℠

The MD Center Team

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Administrative Director

- **Andrew Thesing,**

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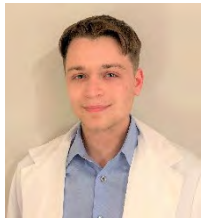

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- **Allison Johnson,**

Research Coordinator

- **Erin Aguero,**

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- **Sarah Hilbert,**

Research Manager

- **Molly Stark,**

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Research Coordinator

- **Ellen Poppy,**

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- **Samantha Cozine,**

Administrative Specialist

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MD Center & MDA Clinic

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- Jenny Myhre
- Sarah Hilbert
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- Seth Stafki
- Andrew Thesing
- Erin Aguero
- John Martone
- Allison Johnston

MDA Clinic

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- Nathan Rodgers
- Helena Molero
- John Fox
- Molly Stark
- Jacie Ihinger
- Kelly Sichmeller
- Jayne Earhart
- Delaney Kennedy

Laboratory Members & Collaborators

Current Laboratory Members (alphabetical)

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- Gloriana Campos
- Audrey Daugherty
- Mekala Gunasekaran
- Khanhlinh Lambuu
- Hannah Littel
- Seth Stafki (also MD Center)
- Johnnie Turner

Laboratory Alumni (chronological)

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- Kyungah Cho
- Madhurima Saha
- Hemakumar Mutra Reddy
- Skylar Rizzo
- Manashwi Ramanathan
- Chengcheng Li
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- Basil T. Darras
- Partha S. Ghosh
- Elicia A. Estrella

Tufts Medical Center

- Isabelle Draper

Broad Institute

- Anne O'Donnell Luria
- Vijay Ganesh
- Lynn Pais

University of Florida

- Carla D. Zingariello

Support

AMY and FRIENDS
COCKAYNE SYNDROME/TRICHOTHIODYSTROPHY/RELATED DISORDER SUPPORT
Registered Charity No' 1119746

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Muscular Dystrophy Center
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Neurological Disorders and Stroke
National Institutes of Health
Reducing the burden of neurological disease...

NIH National Institute of Arthritis and Musculoskeletal and Skin Diseases

NICS National Initiative for Cockayne Syndrome

Greg Marzolf Jr. Foundation
GMJF
Funding a tomorrow free of Muscular Dystrophy

Ferlita Family Fund

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Fighting Muscle Disease

MD STARnet