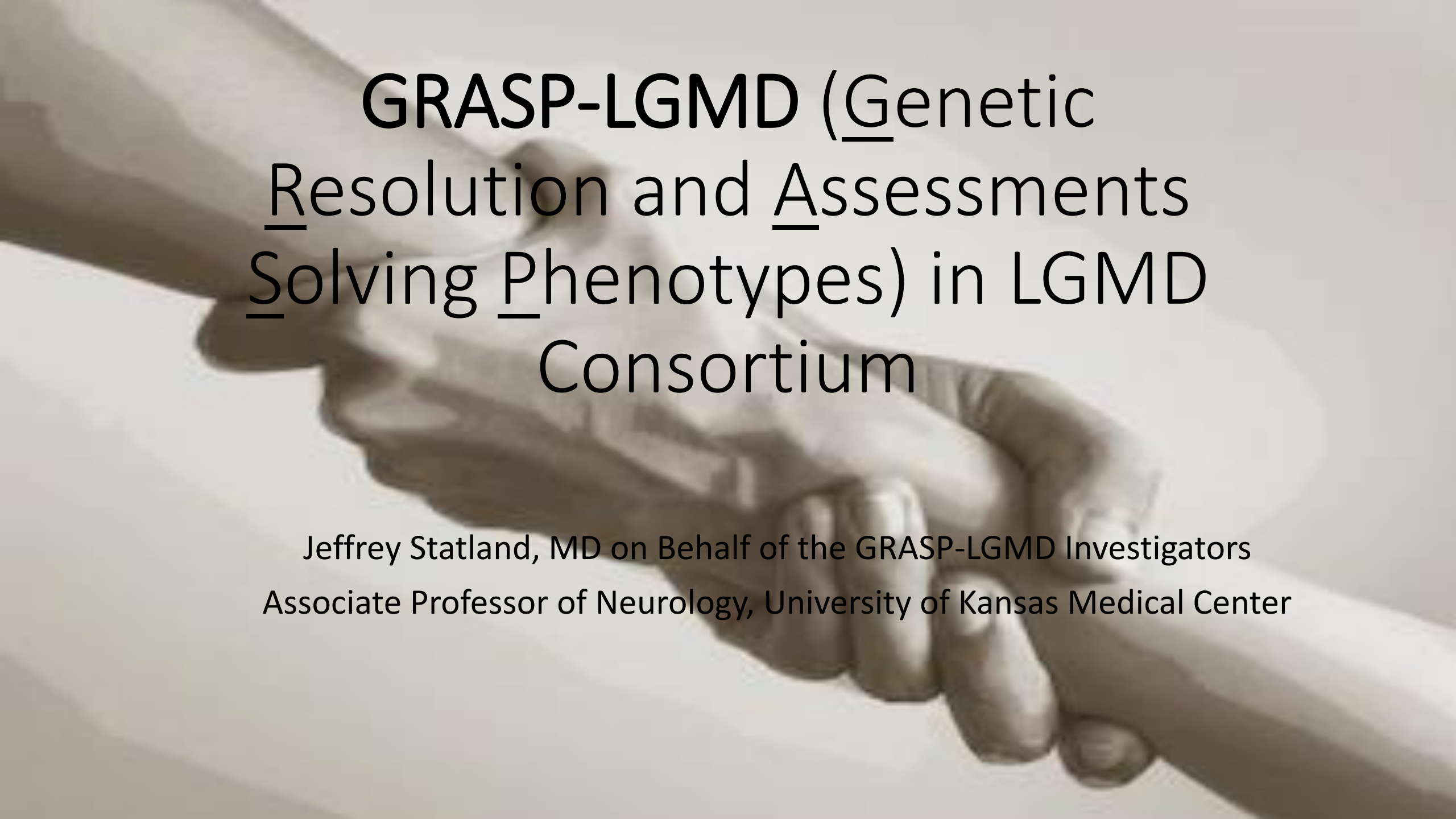


GRASP-LGMD (Genetic Resolution and Assessments Solving Phenotypes) in LGMD Consortium

Jeffrey Statland, MD on Behalf of the GRASP-LGMD Investigators

Associate Professor of Neurology, University of Kansas Medical Center

GRASP LGMD is a Group Effort

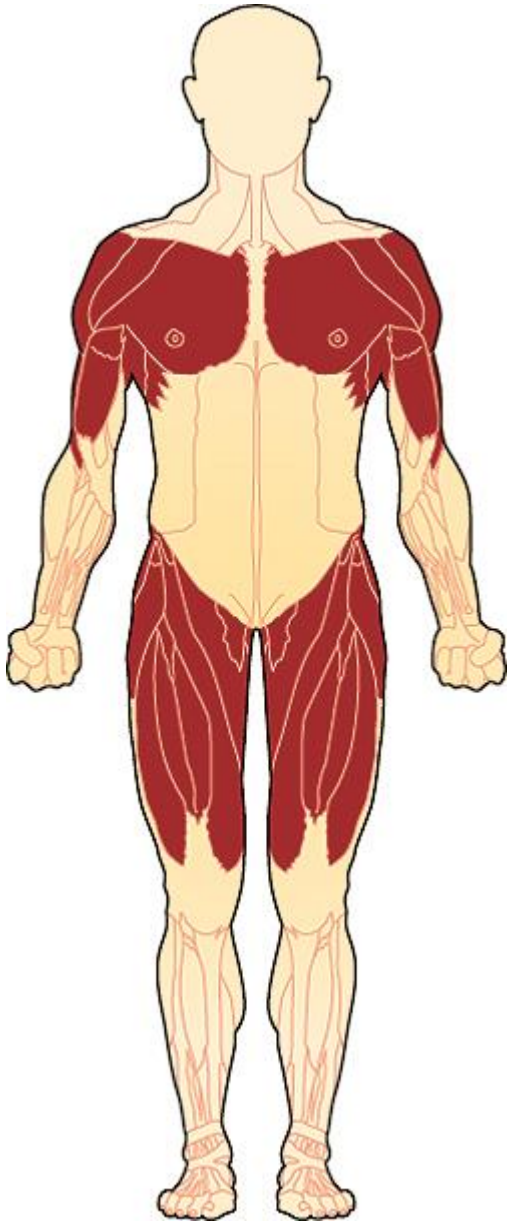
- Nicholas Johnson (VCU) – overall lead
- Jeff Statland (KU) and Nick Johnson – lead on clinical outcome assessment
- Chris Weihl (Wash U) and Monkol Lek (Yale) –lead on genetic resolution and gene discovery
- Matthew Wicklund (U of CO) – advocacy outreach and education
- All the site PIs (Tahseen Mozaffar - UCI!)
- All the PMs and coordinators
- Advocacy partners

Disclosures

- Grant support: FSH Society, NINDS, MDA, Friends of FSH Research
- Consultant/Advisory Board: Acceleron Pharma, Fulcrum Therapeutics, Sarepta, and Strongbridge

Background LGMD

- Group of heterogenous muscular dystrophies which share a common phenotype: shoulder and pelvic girdle weakness
- Originally coined to distinguish from x-linked muscular dystrophy (DMD) and myotonic dystrophy
- Can affect men and women
- Age of onset from childhood to adulthood
- Prevalence taken together worldwide
  - 3/100,000 or ~10,000 in the US
- In general slowly progressive
  - Morbidity: ambulation or maintain a job
  - Some: cardiac and respiratory complications



Deenen JC, et al. J Neuromuscul Dis. 2015;2(1):73-85. PubMed PMID: 28198707
Liewluck T, Milone M. Muscle Nerve. 2018 Aug;58(2):167-177. PMID: 29350766

LGMD Genetics: What's in a name

~90% LGMD

>30 LGMD

Features	LGMD1	LGMD2
Inheritance	Autosomal dominant	Autosomal recessive
Subtypes	1A–1H	2A–2Z
Typical age at onset	Adolescent to late adulthood except LGMD1B–1D may present with childhood onset	Childhood to young adulthood
Limb weakness	Mild	Moderate to severe
Creatine kinase (CK) levels	Normal to mildly elevated except for LGMD1C that typically have significantly elevated CK levels	Mildly to highly elevated
Exercise intolerance or rhabdomyolysis	1C	2A, 2B–2E, 2I, 2L, and 2T

Liewluck T, Milone M. Muscle Nerve. 2018 Aug;58(2):167-177. PMID: 29350766.

LGMD Mutations

- Many in structural muscle proteins (dystrophin-associated glycoprotein complex)
  - Genes like sacroglycans role in protecting against damage during contractions
  - Genes like anoctamin 5, dysferlin role in membrane repair

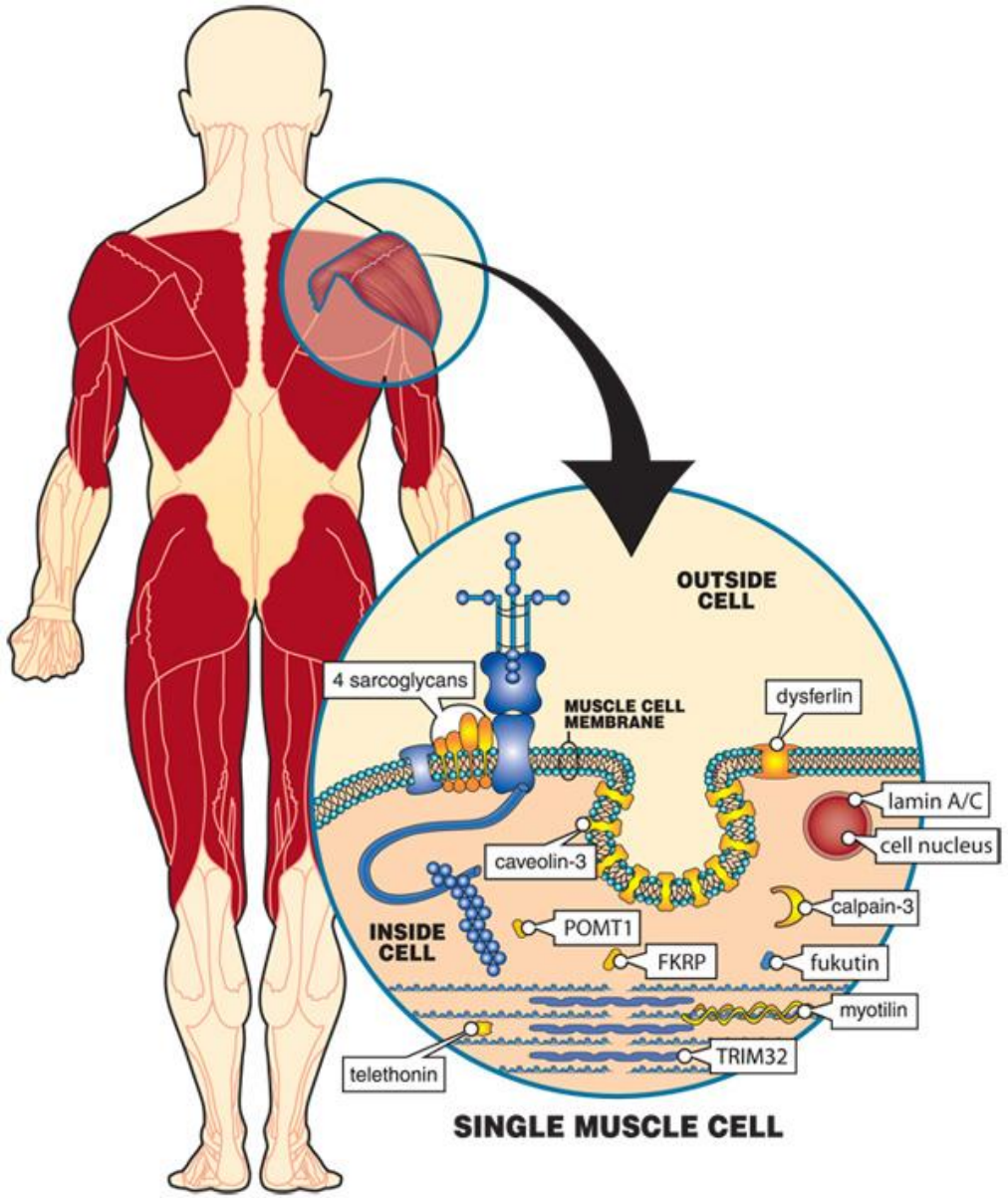
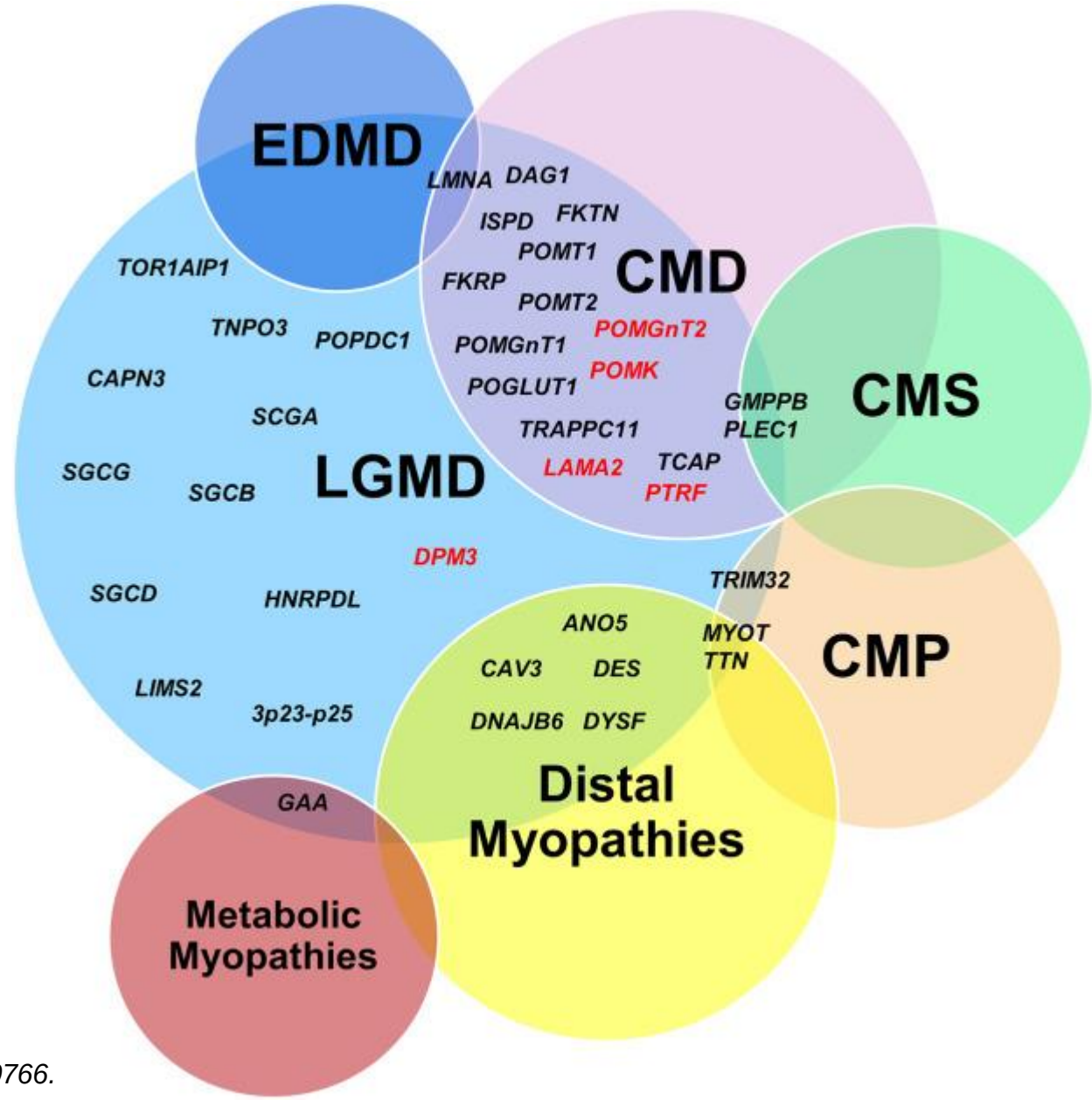


Image from MDA website

Allelic Disorders

- Some overlap between LGMD and other myopathies



Liewluck T, Milone M. Muscle Nerve. 2018 Aug;58(2):167-177. PMID: 29350766.

LGMD Genetics: what's in a name?

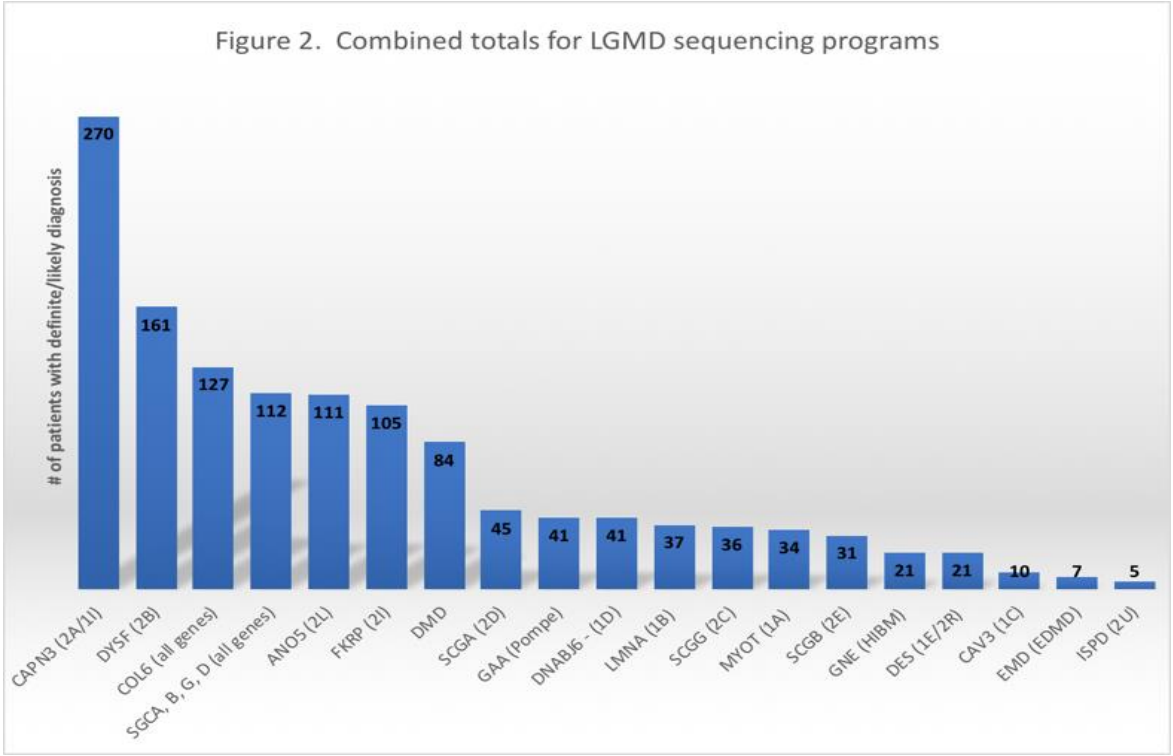
- More recent ENMC workshop on naming
- Only included disorders in > 2 families
- Primary limb-girdle presentation
- Left out:
  - Type 1A-C: Myot, LMNA, CAV3
  - Type 2: GAA, DES
- Changed nomenclature
  - D=dominant, R=recessive
  - Number according to order gene discovered

Gene	Old nomenclature	Proposed nomenclature
CAPN	LGMD2A	LGMDR1 calpain3-related
DSYF	LGMD2B	LGMDR2 dysferlin-related
SGCA	LGMD2D	LGMDR3 α-sarcoglycan-related
SGCB	LGMD2E	LGMDR4 β-sarcoglycan-related
SGCG	LGMD2C	LGMDR5 γ-sarcoglycan-related
SGCD	LGMD2F	LGMDR6 δ-sarcoglycan-related
FKRP	LGMD2I	LGMDR9 FKRP-related
ANO5	LGMD2L	LGMDR12 anoctamin-5-related
DNAJB6	LGMD1D	LGMDD1 DNAJB6-related
TNP03	LGMD1F	LGMDD2 TNP03-related

Straub V, Murphy A, Udd B; LGMD workshop study group. Neuromuscul Disord. 2018 Aug;28(8):702-710. PMID: 30055862.

US prevalence of LGMD subtypes

- Older study 2006:
  - 18% DYSF (LGMD2B), 15% SGCA (LGMD2C-F), 15% FKRP (LGMD2I), 12% CAPN3 (LGMD2A), and 1.5% CAV3 (LGMD1C)
- More recent free testing programs
- Meta analysis 2018 using large WES/WGS data bases
- But common to all the most frequent include:
  - CAPN3, DYSF, SGCA (group), ANO5, FKRP
  - DNAJB6



Courtesy of MDA and Jain Foundations

Moore SA, Shilling CJ, Westra S, et al. J Neuropathol Exp Neurol 2006;65(10):995–1003.
Liu W, et al. Genet Med. 2019 May 20. doi: 10.1038/s41436-019-0544-8. [Epub ahead of print] PubMed PMID: 31105274.

Clinical Features of LGMD

- In theory each subtype has its own trajectory
- Disease progression = variable
- Age of onset = variable
- Incidence in both males and females
- Progressive weakness and wasting in limb musculature
- CK on average higher in recessive LGMD
- If cardiac (arrythmia, cardiomyopathy) think: sarcoglycan, CAV3, LMNA, DES, FKRP
- If pulmonary think: sarcoglycan,
  - In most respiratory follows limb weakness – late feature

Pegoraro E, Hoffman EP. LGMD GeneReviews. www.ncbi.nlm.nih.gov/books/NBK1408/. Accessed March 30, 2019.
Liewluck T, Milone M. *Muscle Nerve.* 2018;58(2):167-177.

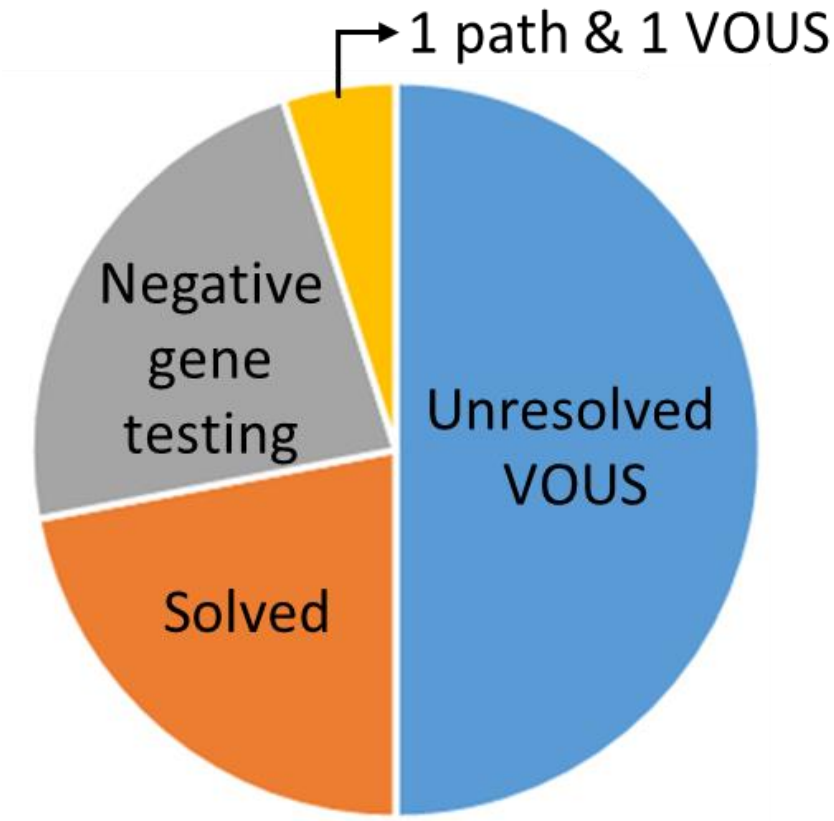
Management of LGMD: Supportive Care

- Multi-disciplinary care
- PT/OT/orthotics – assistive devices / mobility aids
- Pulmonary / RT -respiratory aids if required
- Cardiology – if affected
- Orthopedics/pain – for some
- Management approaches aim to improve quality of life and prolong survival
- Genetic counseling

➢No FDA approved medications

WHY NOW?

- Our ability to diagnose LGMD has improved
  - Advocacy groups/Genzyme supporting free genetic testing programs ~ 6K tested
  - The cost of commercial testing has lowered (~$250)
- This has increased the prevalence of LGMD in the US
  - But also increasing the number of individuals with variants of unknown significance or negative testing = great deal of uncertainty, often no clear path forward



Courtesy of the Jain Foundation and MDA

WHY NOW?

U.S. FOOD & DRUG ADMINISTRATION

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/ FDA approves innovative gene therapy to treat pediatric patients with spinal muscular atrophy, a rare disease and leading genetic cause of infant mortality

FDA NEWS RELEASE

FDA approves innovative gene therapy to treat pediatric patients with spinal muscular atrophy, a rare disease and leading genetic cause of infant mortality

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More Press Announcements

For Immediate Release: May 24, 2019

Press Announcements

The U.S. Food and Drug Administration today approved Zolgensma (onasemnogene abeparvovec-xioi), the first gene therapy approved to treat children less than two years of age with spinal muscular atrophy (SMA), the most severe form of SMA and a leading

Content current as of:
05/24/2019

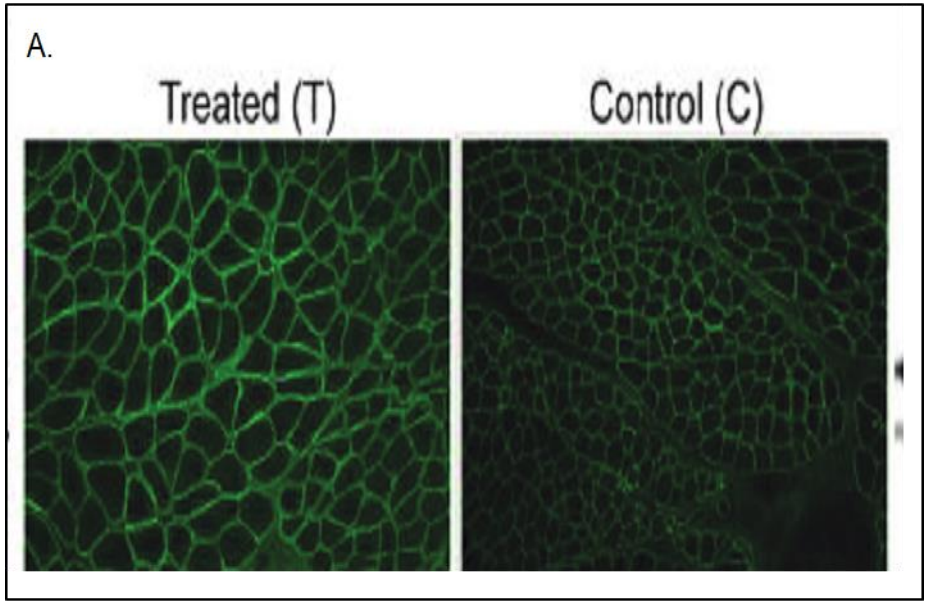
Follow FDA

- At the same time, advances in gene therapy technology have
  - Made systemic AAV-delivered gene replacement therapy a reality (FDA approved Zolgensma for SMA)
  - Virtually all recessive LGMDs could be amenable to gene replacement (dominant LGMD to other approaches, RNAi)– making them targets for future drug development
    - Industry involved: Myonexus/Sarepta, Genethon, etc

WHY NOW?

- Gene replacement therapy is happening now for sarcoglycanopathies (LGMD2C-F)
- Proof of concept in single muscles
- Press Releases from Sarepta's LGMD2E program
  - 3 individuals dosed and 2 month biopsy results released
  - 51% sarcoglycan positive fibers by immunohistochemistry / +Western Blot
  - 90% drop in serum CK

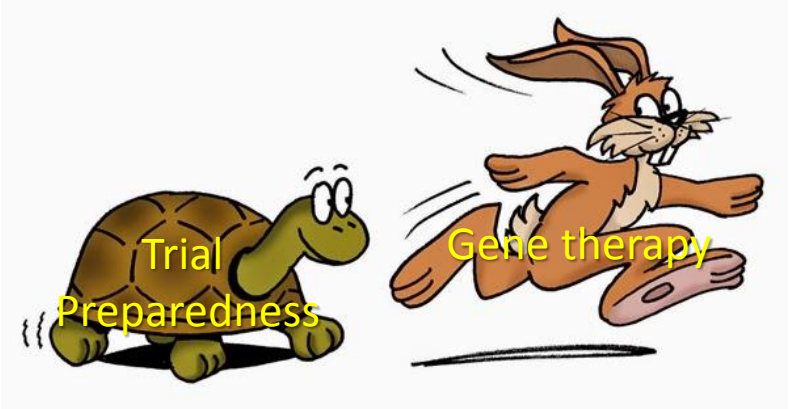
Limb-girdle muscular dystrophy type 2D



Mendell et al. Ann Neurol. 2009 Sep;66(3):290-7.

http://investorrelations.sarepta.com/news-releases/news-release-details/sarepta-therapeutics-announces-positive-and-robust-expression

WHY NOW?



- ***The pace of therapeutic development has outpaced our efforts to prepare for clinical trials***
- Leaving several gaps which need to be addressed:
  - Gap1: In how we diagnose people with LGMD
  - Gap 2: In the tools we will use to measure a response to therapy in a clinical trial
  - Gap 3: In our understanding of the patient experience of living with LGMD
- Barriers: rarity, heterogeneity, and broad age range affected

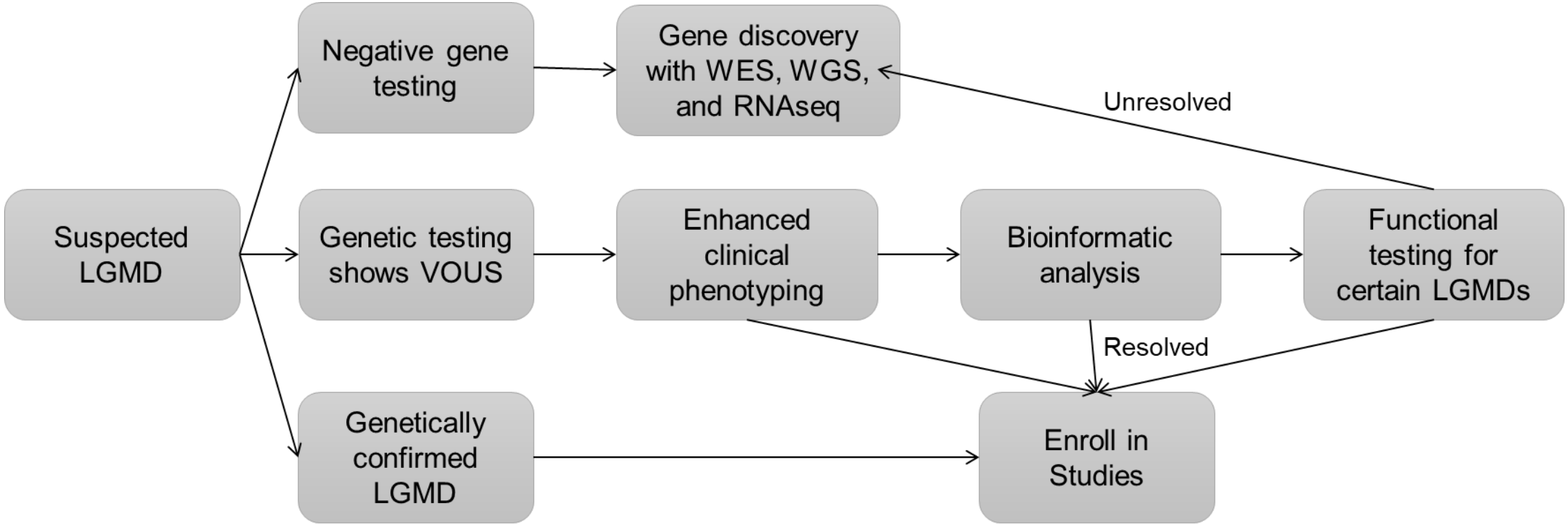
Genetic Resolution and Assessments Solving Phenotypes in LGMD (GRASP-LGMD)

- Funding for a smaller network of sites from the MDA and industry funds = growing quickly from 6 to 11 sites
- Close relationship with advocacy group partners who are tremendous help (Jain Foundation, Coalition to Cure Calpain, LGMD1D, LGMD2L, Team Titin)
  - First ever combined LGMD patient and family day: Aug30-Sep2, 2019, Chicago, IL
- The Goal: hasten therapeutic development for LGMD by:
  - Improving diagnostics (Gap1)
  - Developing outcome measures (Gap2)
  - Understanding the patient reported impact (Gap3)
  - Training the next generation of LGMD researchers
- Common network efficiencies
  - Streamlined regulatory oversite
  - Data management
  - Training sites and patient engagement

Gap1: Improved diagnostics

- Chris Weihl (Wash U) and Monkol Lek (Yale) – lead
- Proposal to:
- **Aim 1:** Identify patients with an LGMD phenotype and negative or indeterminate genetic testing with panel based sequencing. Using national MDA clinics, and ability to reach out to previously sequenced individuals in Jain/MDA programs if available
- **Aim 2:** Resolve LGMD gene variants and establish LGMD diagnoses. Workflows for variant resolution
- **Aim 3:** Identify new genes associated with LGMD. WES/WGS/RNA seq

Proposed workflow



Example

- Collected clinical information and existing muscle tissue
- Informatics
  - DYSF in general pop; CAPN3 not
  - CAPN3 VOUS near splice site junction
- Clinical: progressive LG weakness, no prior family history
- Functional study
  - Normal DYSF staining, reduction in CAPN3 by immunoblot
  - Consider RT PCR for cryptic splicing
- Result: compound heterozygous LGMD2A

Example courtesy of Chris Weihl, Washington University, St Louis, MO

Example 1: **Patient with one pathogenic and one VOUS in LGMD2A/CAPN3 gene.**

Gene	MIM#	Disease (Inheritance)	Exon	Nucleotide change	Amino acid change	Zygosity	Type
CAPN3	114240	Limb girdle muscular dystrophy 2A (AR)	Ex3	c.402delC		Heterozygous	Pathogenic[a]
			Ex11	c.1524G>A	p.E508=	Heterozygous	VOUS*
*DYSF**	603009	Miyoshi muscular dystrophy 1 (AR); LGMD2B (AR); Distal myopathy with anterior tibial onset (AR)	Ex24	c.2423G>A	p.R808Q	Heterozygous	VOUS[b]

Abbreviations used in this table: AR - autosomal recessive; VOUS - Variant of unknown significance, **DYSF* transcript number - NM_003494.3

Provided history: 34-year-old man with LGMD of unknown genetic cause. LGMD panel identifies one previously reported pathogenic mutation and one synonymous VOUS in CAPN3 as well as a single VOUS in DYSF.

Steps for variant resolution: 1) Perform database/literature search. 2) Obtain clinical history and muscle tissue. 3) Consider functional testing.

Database search: *DYSF* p.R808Q VOUS has a minor allele frequency (MAF) of .03%; ClinVar "uncertain significance;" no reports in Pubmed.

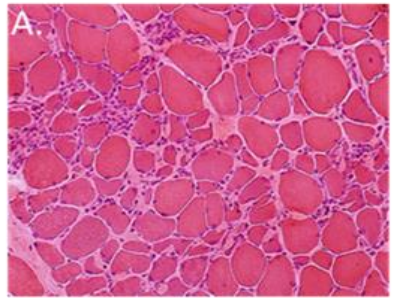
CAPN3 p.E508= VOUS not reported in Gnomad and Exac Browsers; ClinVar "uncertain significance"; Pubmed search reveals report of two Italian patients homozygous for the c.1524G>A; p.E508= variant with LGMD and absent CAPN3 on western blot due to cryptic splice site.

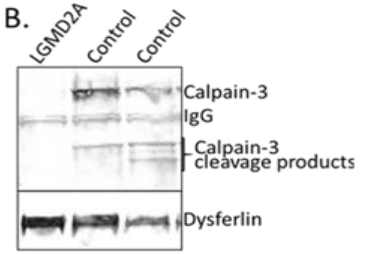
Bioinformatics Analysis: NNSplice identifies that VOUS is 3 bp from splice site junction and is predicted "donor lost".

Clinical history: progressive proximal weakness beginning at age 25, exam with proximal weakness and scapular winging. No family history of weakness. A) Muscle biopsy: chronic myopathy.

Functional study: Washington University Clinical Neuromuscular lab obtained muscle tissue; performed immunohistochemistry for dystrophy related proteins including dysferlin and performed immunoblot for calpain-3. B) Result normal dystrophy stains including dysferlin but a significant reduction of normal of calpain-3. Consider RT-PCR to evaluate for cryptic splicing.

Diagnosis: Compound heterozygous LGMD2A with pathogenic variant and resolved synonymous VOUS leading to cryptic splicing – SOLVED enroll in Project 1.

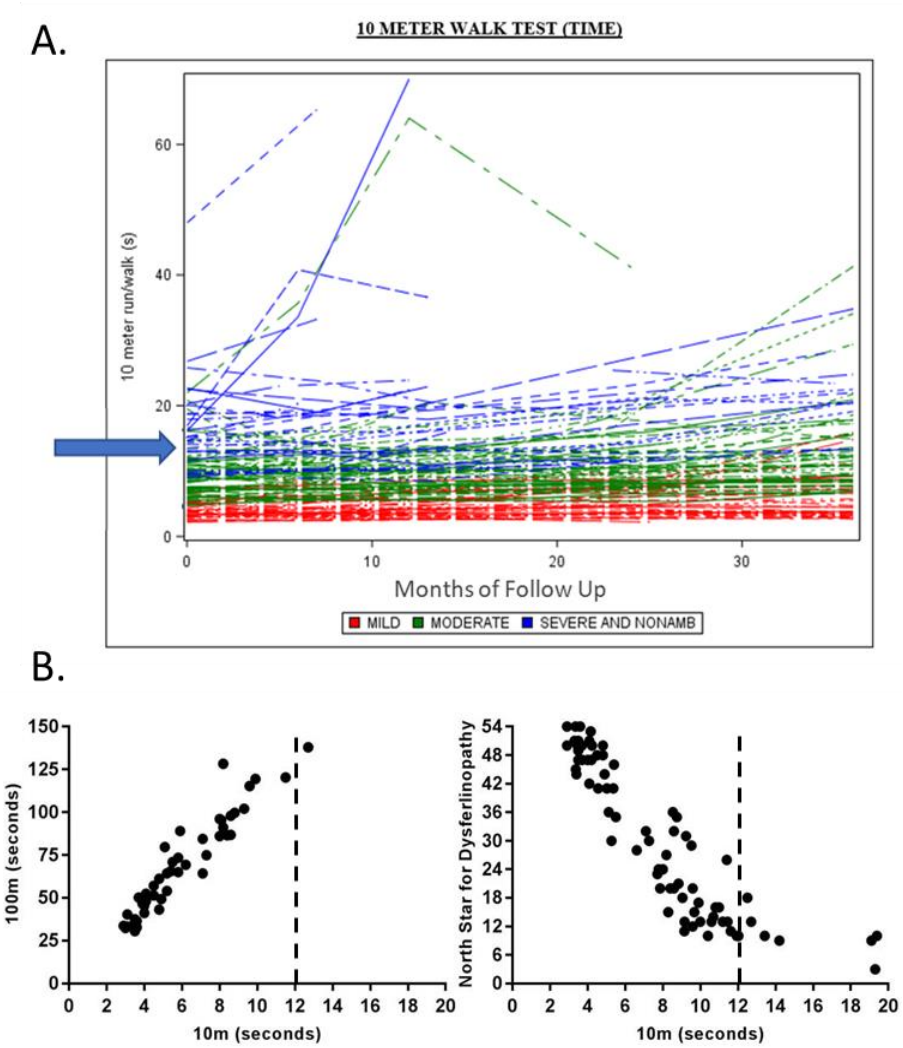


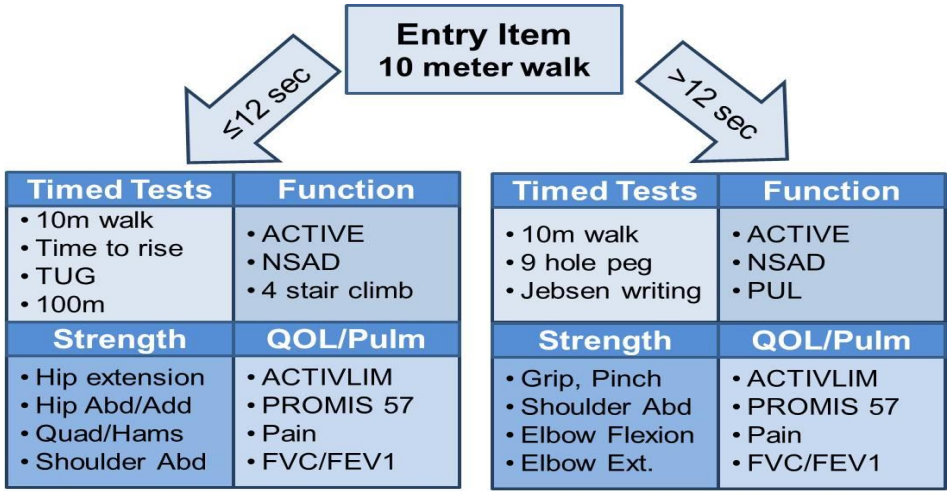


Gap 2 - Development of COAs for LGMDs

- Nicholas Johnson (VCU) and Jeff Statland (KUMC) - lead
- Common protocol (platform study) regardless of LGMD mutation
- COAs chosen by experienced PT leads at Nationwide Children's (Lindsay Alfano, PT, DPT, PCS, and Linda Lowes, PT, PhD)
- Proposal for 12-24 months study:
  - To define a common set of pelvic and shoulder girdle COAs, and COAs useful for capturing phenotypic diversity. To establish **multi-site training**, **test-retest reliability**, **cross sectional associations**
  - To determine the **sensitivity** to longitudinal disease progression. We will use statistical and anchoring methods to determine what will be a **minimally important clinical difference**. We will use factor analysis to identify which COAs capture variation in disease severity across the cohort.
  - To **refine clinical trial strategies** (i.e. inclusion criteria) based on baseline and longitudinal phenotypic characteristics. We will determine which baseline characteristics, **mutation, age, gender, and baseline functional status** are most likely to predict **progression**, using regression trees to assess subgroups likely to progress.
- Data useful for understanding natural history and trial planning

Two pathways: LEXT v UEXT





- NorthStar Ambulatory Assessment for Dysferlin
  - Combined NSAA + elements from MFM domain 1
- ACTIVE
  - Video game and stereographic camera to define workspace volume (i.e. reachable workspace)

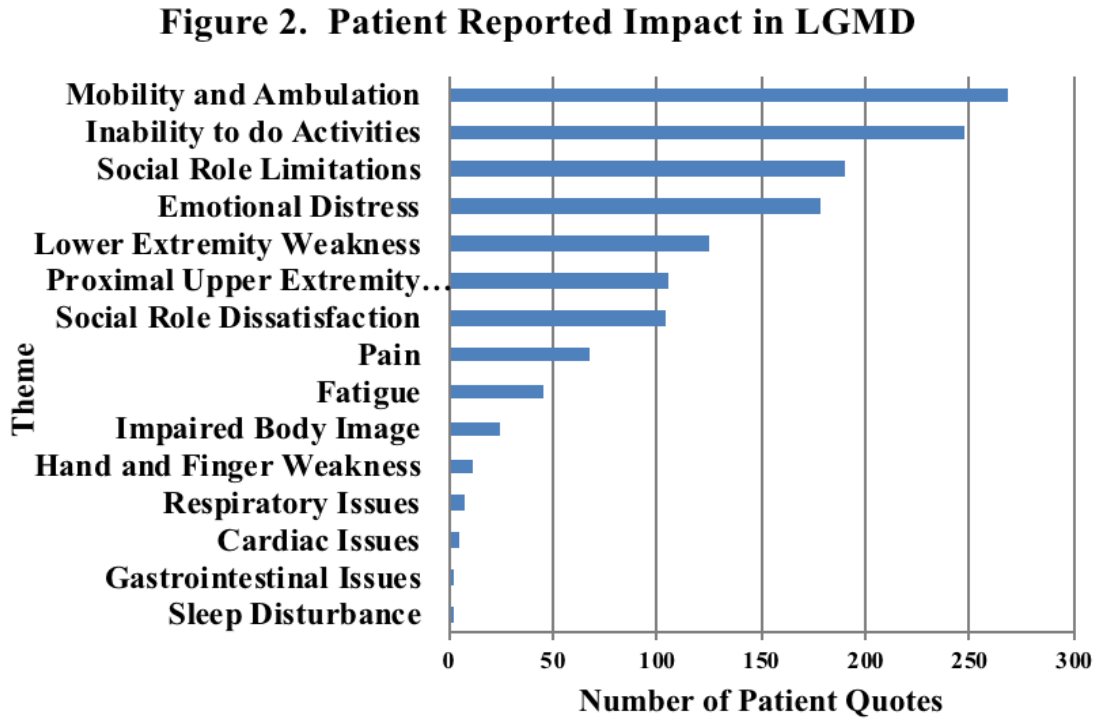
More: Next Talk Jay Han

Figure courtesy of COS1 Study, and Nationwide Children's

Moore U, et al. Neurology. 2019 Jan 9. PMID: 30626655; PMCID: PMC6369904.
Alfano LN, et al. Dev Med Child Neurol. 2019 Apr 8. PubMed PMID: 30963554.

Gap3: The patient perspective

- The choice of what you measure needs to match a persons perception of LGMD
- We are in the process of developing an LGMD-specific health inventory – a questionnaire for use in clinical trials
- Strategy outline in FDA Guidance to Industry for PROs:
  - Open ended interviews: themes we need to address
  - Other themes like respiratory issues, or cardiac issues may not apply to all LGMD, but will be important for people for whom it is an issue
- GRASP-LGMD:
  - The next step is a large (several 100s) **survey** to determine how **common** each symptom is and how **impactful** on someone's life
  - Then the instrument needs to be included in a **prospective study**



GRASP-LGMD Today and in the Future

- Funded pilot of n=20 for CAPN3, DYSF, ANO5, and DNAJB6 at 8 sites
- Industry funded study for sarcoglycans, n=100, 11 sites in US and UK
- Proposal out for larger CAPN3
- Proposal out for variant resolution and genetic discovery
- Anticipate 1st patient enrolled 2019
- Working on establishing training program
- Future projects:
  - MRI
  - Biomarkers
  - Additional LGMD subtypes

LGMD patients and family members!!

Organizations

- MDA
- Jain Foundation
- Coalition to Cure Calpain
- Other LGMD advocacy groups

Proposed GRASP-LGMD Sites:

University of Kansas Medical Center (Jeffrey Statland);

Virginia Commonwealth University (Nick Johnson);

Washington University (Conrad Weihl);

University of Colorado (Matthew Wicklund);

Kennedy Krieger Institute (Kathryn Wagner);

Brigham and Women's Hospital (Anthony Amato);

UC-Irvine (Tahseen Mozaffar)

University of Iowa (Katherine Matthews);

University of Florida (Peter Kang);

Yale University (Monkol Lek);

Newcastle, UK (Volker Straub)

Thank You!