GENETIC DISCOVERY AND PATHOMECHANISM IN HEREDITARY MYOPATHIES

University of Kansas Medical Center
Neurology Grand Rounds

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Department of Neurology
Neuromuscular Division
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Disclosures

- Dr. Weihl has no relevant financial interests to disclose
Genetic discovery and pathomechanism in hereditary myopathies

Clinical Care → Patient Phenotyping

Model system development → Genetic discovery

Pathogenic mechanism → Therapeutics
Patient 1

- Patient is a 63 year old man with weakness
- Weakness began at age 33 with difficulty climbing stairs and rising from a chair
- Frequent falls and now unable to get up off the ground without assistance
- He uses a power wheelchair but can still walk and transfer
Physical Exam

- No facial weakness
- No neck weakness
- Symmetric, proximal upper and lower extremity weakness with minimal strength in the biceps, iliopsoas and quadriceps.
- Relatively preserved distal strength
Testing

- CK 632
- EMG was myopathic in the quadriceps and deltoids.
Muscle biopsy at age of 40
Rimmed Vacuoles
Protein aggregates
Family 1

- He has 8 siblings of which 3 have weakness
Family 1

- Autosomal dominantly inherited limb girdle muscular dystrophy with aggregate and vacuolar myopathology
Discussion Forum

Recent Activity

- Stem-Cell transplant in CIDP — Started by: John Sladky, MD
- Job opps for PA in Hawaii? — Started by: Dan Larrivee, MD, JD, FAAN
- Neuromuscular MD in Melbourne — Started by: Ari Breiner, MD
- Global Conference on Myositis — Started by: Todd Levine, MD
- Pregnant with Pompe’s — Started by: Daniel Jacobs, MD
- Sensory Ataxia secondary to sensory neuronopathy — Started by: Abbas Jowkar, MD, FAAN

Popular Topics

Categories

Most Active Members

#1 - Douglas Pavlak, MD, FAAPMR
#2 - Georgios Manousakis, MD
#3 - Richard A Lewis, MD

Read All Topics

Watch, Listen & Learn

Todd Levine, MD
When is Demyelination Not CIDP - And what to think when patients don’t

Todd Levine, MD
Diagnosing CIDP
Posted Nov 2016 • Views 2

Ken Gorson, M.D.
Recognizing CIDP
Posted Nov 2016 • Views 2

Ken Gorson, M.D.
Misdiagnosis of CIDP
Posted Nov 2016 • Views 3

Leadership

Founded September 29, 2011

Richard Barohn, MD
Professor & Chairman, University of Kansas Medical Center, Dept of Neurology, Kansas City, KS

Jon Katz, MD
Director Neuromuscular Research and ALS Clinic, Norris Center

Todd Levine, MD
MD, Phoenix Neurological Associates, Phoenix, AZ

New Members

Zoe Woodward, MBChB
Neurology Trainee, Christchurch Hospital

Pavani Gunat, MD
Neurologist, Cedars-Sinai Medical Group, CA
# LIMB-GIRDLE MUSCULAR DYSTROPHY (LGMD) SYNDROMES

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<tr>
<th>LGMD: General features</th>
<th>Limb girdle dystrophies: X-linked</th>
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<td><strong>Dominant</strong></td>
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<td><em>Connective tissue</em></td>
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<td>IA: Myotilin; 5q31: Dysamin</td>
<td>Becker: Dystrophin; Xp21</td>
<td><em>Dystrophin &amp; associated proteins</em></td>
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<td>IB: Lamin C; 1q21: Cardiac</td>
<td>Duchenne: Dystrophin; Xp21</td>
<td><em>Intermediate filaments</em></td>
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<td>IC: Caveolin-3; 3p25: Child onset</td>
<td>Emery-Dreifuss: Emerin; Xq28</td>
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<td>ID: Tq</td>
<td>McLeod Syndrome: XK; Xp21.1</td>
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<td>Dilated Cardiomyopathy (Q1E): 6q23</td>
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<td>IE: 7q32</td>
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<td>LG: 4p21</td>
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<td>Mental retardation &amp; Cardiomyopathy</td>
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<td>Bethlem: Collagen VT: 21q22 &amp; 2q37</td>
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<td>Central core: Ryuridine receptor (19q13)</td>
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<td>Cytoplasmic body: 2q24; 2q21 + Other</td>
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<td>Myofbriller (Dermatomyositis)</td>
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<td>Desmin: 2q35; AD or AR.</td>
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<td>αβ-crystallin: 11q12</td>
<td>Basal lamina</td>
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<td>LGMD1A: Myotilin; 5q31</td>
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<td>Congenital: SEFN1; 1p36</td>
<td>Agrin</td>
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<td>ZASP myopathy: 10q22</td>
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<td>Other</td>
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<td>Myotonic (DM1): DMPK, 19q13</td>
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<td>Myotonic (DM2): ZNF9; 3q21</td>
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<td>OCTOpharyngeal: PABP2; 14q11</td>
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<td>Paget disease: Myopathy: VCP, 9p13</td>
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<td>Spheroid body</td>
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<td>Limb girdle dystrophies: Recessive</td>
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<tr>
<td>2A: Calpain-3: 15q15</td>
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<td>2C: γ-Sarcoglycan: 13q12</td>
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<td>2F: δ-Sarcoglycan: 3q33</td>
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<td>2G: Telethonin: 17q11-12</td>
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<td>2H: TRIM32; 9q21-9q33</td>
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<td>2I: FKRP; 19q13.3</td>
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<td>2J: Titin; 2q31</td>
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<td>2K: POMT1; 9q34</td>
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<tr>
<td>Myostatin (Laminin α2)</td>
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<tr>
<td>Absent: 6q2</td>
<td>Glycogen</td>
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<td>Reduced: 6q2</td>
<td>Lipid</td>
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<tr>
<td>Abnormal: LGMD 2I</td>
<td>Mitochondrial</td>
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<tr>
<td>Caveolin-2 mutation (Gly558ec)</td>
<td>Myopathy + PK: 17p13; Recessive</td>
<td></td>
</tr>
</tbody>
</table>

**Other inherited myopathies**
- Bannano’s myopathy
- Cardiomyopathy
- Cardiomyopathy-associated myopathy
- Cardiomyopathy (LGMD1B)
- Dilated Cardiomyopathy: 6q23
- Congenital
- Myopathies: Late-onset
- Muscular dystrophies
- Cytoplasmic body myopathies
- Distal myopathies
- Excessive autophagy: Xq28
- Fatal myohereditary myopathy
- FSH dystrophy: 4q35
- Hereditary IBM syndromes
- IBM1: Dominant
- IBM2: GNE, 9q12: Recessive
- IBM3: MyHC-III, 17p13: Dominant
- IBM: Paget disease: 9p13: Dominant
- Metabolic myopathies
- Glycogen
- Lipid
- Mitochondrial
- Myopathy + PK: 17p13: Recessive
- Myotonic dystrophy
- Other dystrophies
- Respiratory failure
- Scapuloperoneal syndromes
- Skeletal Myopathy
- Diphosphoglycerase: TGFβ1: 19q13
- Epidermal dysplasia: COL9A3: 20q13
- Tubular aggregates
Genetic discovery in hereditary muscle disease – exome sequencing

- Utilization of next generation sequencing strategies to sequence all coding exons
- ~19,000 genes and 230,000 exons in the human genome
Genetic discovery in hereditary muscle disease – exome sequencing

- 22 novel coding variants were shared across 3 affected family members

Limb girdle dystrophies: **Dominant**
- 1A: Myotilin; 5q31; Dysarthria
- 1B: Lamin A/C; 1q21; + Cardiac
- 1C: Caveolin-3; 3p25; Child onset
- **1D**: 7q

Dilated Cardiomyopathy (?1E): 6q23
- 1F: 7q32
- 1G: 4p21

Four new Finnish families with LGMD1D; refinement of the clinical phenotype and the linked 7q36 locus

Peter Hackman a,1,*, Satu Sandell b,d,1, Jaakko Sarparanta a, Helena Luque a, Sanna Huovinen c, Johanna Palmio b, Anders Paetau e, Hannu Kalimo e,f, Ibrahim Mahjneh g, Bjarne Udd a,b,c,h

Neuromuscular Disorders 21 (2011) 338–344
Exome sequencing

1 variant resided in an interval previously ascribed to LGMD1D at 7q36

Linkage studies confirmed this chromosomal region in family 1

Subsequent sequencing of another family identified a mutation in the same gene
Exome Sequencing Reveals DNAJB6 Mutations in Dominantly-Inherited Myopathy

Matthew B. Harms, MD, R. Brian Sommerville, MD, Peggy Allred, DPT, Shaughn Bell, BA, Duanduan Ma, PhD, Paul Cooper, BA, Glenn Lopate, MD, Alan Pestronk, MD, Conrad C. Weihl, MD, PhD, and Robert H. Baloh, MD, PhD

Mutations affecting the cytoplasmic functions of the co-chaperone DNAJB6 cause limb-girdle muscular dystrophy

Jaakko Sarpantaa, Per Harald Jonsson, Christelle Golzio, Satu Sandell, Helena Luque, Mark Screen, Kristin McDonald, Jeffrey M Stajich, Ibrahim Mahjneh, Anna Vihola, Olayinka Raheem, Sini Penttilä, Sara Lehtinen, Sanna Huovinen, Johanna Palmio, Giorgio Tasca, Enzo Ricci, Peter Hackman, Michael Hauser, Nicholas Katsanis & Bjarne Udd
## Limb-Girdle Muscular Dystrophy (LGMD) Syndromes

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<td>1G: 4p21</td>
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<td>Dystrophic myopathies:</td>
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<td>IBM2; GNE: 9p12; Recrecessive</td>
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<td>2B: Dysferin; 2p15.1</td>
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<td>2C: α-Sarcoglycan; 13q12</td>
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<td>2F: Γ-Sarcoglycan; 5q23</td>
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<td>2G: Telethonin; 17q11-12</td>
<td>Diaphragm dystrophy: TGFβ1; 19q13</td>
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<tr>
<td>LGMD</td>
<td>Tubular aggregates</td>
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**Muscle Proteins**

- Connective tissue
- Dystrophin & associated proteins
- Intermediate filaments
- Neuromuscular junction
- Structural & Contractile
# Limb-Girdle Muscular Dystrophy (LGMD) Syndromes

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<th>Limb Girdle Dystrophies</th>
<th>Other Inherited Myopathy Syndromes</th>
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<td><strong>α-Dystroglycan disorders</strong> (MDDGC)</td>
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<tr>
<td>1A: Myotilin; 5q31; D</td>
<td><strong>AFECED, AIRE; 21q22; Recessive</strong></td>
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<tr>
<td>1B: Lamin A/C; 1q21; +Cardiaria</td>
<td><strong>Autophagy</strong></td>
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<td>1C: Caveolin-3; 3p25; Child onset</td>
<td><strong>Excessive; VMA21; Xq28</strong></td>
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<td>1D: Desmin; 2q35</td>
<td><strong>Multiple; CLN3; 16p11; Recessive</strong></td>
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<td>1F: TNPO3; 7q22</td>
<td><strong>Other</strong></td>
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<td>1G: HNPPD; 4q21</td>
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<td>1H: 3p23</td>
<td><strong>Dentral &amp; Rapp; 7q31; Dominant</strong></td>
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<tr>
<td><strong>Recessive</strong></td>
<td><strong>IBM1; Desmin; 7q35; Dominant</strong></td>
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<td>1B: Lamin A/C; 1q21; +Cardiaria</td>
<td><strong>IBM2; GNE; 5p12; Recessive</strong></td>
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<tr>
<td>2A: Calpain-3; 15q15</td>
<td><strong>IBM3; MYH2; 17p13; Dominant</strong></td>
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<td>2B: Dysferlin; 2p13</td>
<td><strong>IBM4: 7q22; Dominant</strong></td>
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<td>2C: γ-Sarcoglycan; 13q12</td>
<td><strong>LGMD1D; DNAJB6; 7q36; Dominant</strong></td>
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<td>2D: α-Sarcoglycan; 17q21</td>
<td><strong>IBM + Paget</strong></td>
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<td>2E: β-Sarcoglycan; 4q12</td>
<td><strong>HNSFP; Titin; 2q24; Dominant</strong></td>
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<td>2F: α-Sarcoglycan; 5q33</td>
<td><strong>Lipid</strong></td>
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<td>2G: Telethonin; 17q12</td>
<td><strong>Mitochondrial</strong></td>
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<tr>
<td>2H: TRIM32; 9q33</td>
<td><strong>Myotonic dystrophy</strong></td>
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<tr>
<td>2I: MDDGC5; FKRP; 19q13</td>
<td><strong>Ophthalmooplagia; MYH2; 17p13; Recessive</strong></td>
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<td>2J: Titin; 2q24</td>
<td><strong>Other dystrophies</strong></td>
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<td>2K: MDDGC1; POMT1; 9q34</td>
<td><strong>Proteinopathy; CASQ1; 1q23; Dominant</strong></td>
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<td>2L: MDDGC2; POMT2; 14q24</td>
<td><strong>Reducing body</strong></td>
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<td>2M: MDDGC4; Fukutin; 9q31</td>
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<td>2N: MDDGC5; FKRP; 19q13</td>
<td><strong>Scapulopelvic syndromes</strong></td>
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<td>2O: MDDGC6; POMT1; 9q34</td>
<td><strong>Skeletal – Myopathy; Dominant</strong></td>
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<td>2P: SEDN1; 1p11</td>
<td><strong>Bone fragility; MTAP; 9p21</strong></td>
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<td>3A: Fukutin; 9q31</td>
<td><strong>Paget (VCP; HNRNPA2B1; HNRNPA1)</strong></td>
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<td>3B: Dysferlin; 2p13</td>
<td><strong>Dysplasia</strong></td>
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<td>3C: Dystrophin; 2X</td>
<td><strong>Diaphyseal; TGF1; 19q13</strong></td>
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<td>3D: Sarcoglycan; 4q12</td>
<td><strong>Emphyseal (COL9A3; COL9A2; COMP)</strong></td>
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<td>3E: α-Sarcoglycan; 17q21</td>
<td><strong>Spheroid body (Myotilin)</strong></td>
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<td>3F: α-Sarcoglycan; 5q33</td>
<td><strong>Tubular aggregates</strong></td>
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<tr>
<td>3G: Telethonin; 17q12</td>
<td><strong>Tubular arrays</strong></td>
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**Limb Girdle Dystrophies:**

- **Dominant**
  - 1A: Myotilin; 5q31; D
  - 1B: Lamin A/C; 1q21; + Cardiaria
  - 1C: Caveolin-3; 3p25; Child onset

- **Recessive**
  - 2A: Calpain-3; 15q15
  - 2B: Dysferlin; 2p13
  - 2C: γ-Sarcoglycan; 13q12
  - 2D: α-Sarcoglycan; 17q21
  - 2E: β-Sarcoglycan; 4q12
  - 2F: α-Sarcoglycan; 5q33
  - 2G: Telethonin; 17q11-12
  - 2H: TRIM32; 9q33
  - 2I: MDDGC5; FKRP; 19q13
  - 2J: Titin; 2q24
  - 2K: MDDGC1; POMT1; 9q34
  - 2L: MDDGC2; POMT2; 14q24
  - 2M: MDDGC4; Fukutin; 9q31
  - 2N: MDDGC5; FKRP; 19q13
  - 2O: MDDGC6; POMT1; 9q34

**Other Inherited Myopathy Syndromes:**

- **α-Dystroglycan disorders** (MDDGC)
- **AFECED, AIRE; 21q22; Recessive**
- **Autophagy**
- **Excessive; VMA21; Xq28**
- **Multiple; CLN3; 16p11; Recessive**
Genetic discovery and pathomechanism in hereditary myopathies

Clinical Care → Patient Phenotyping

Model system development → Genetic discovery

Pathogenic mechanism
Genetic discovery leads to pathomechanism

- DNAJB6 is an HSP40 co-chaperone
- Potent mediator of protein aggregation in models of neurodegeneration
Protein aggregate diseases

- Aβ
- TDP-43
- huntingtin
- α-synuclein
- Tau
- Ubiquitin
Protein aggregation in skeletal muscle

- **SMI31**
- **Aβ**
- **VCP**
- **Ubiquitin**
- **TDP-43**
- **αB-crystallin**
DNAJB6 accumulates with other aggregates in LGMD1D

LGMD1D

DNAJB6

normal

DNAJB6

LGMD1D

DNAJB6 + TDP-43

sIBM

DNAJB6
Why does a protein aggregate?
1% of the genome encodes chaperone proteins
~3 HSP70s
>40 DNAJ proteins dictate chaperone-client specificity
DNAJB6/LGMD1D

- Chaperonopathies
  - Myofibrillar myopathies (*BAG3*, αB-crystallin)
  - Inclusion body myopathies (*VCP*)
  - Limb-girdle muscular dystrophy 1D (*DNAJB6*)
  - Distal myopathies (*HSPB8*)
  - Congenital myopathies (*SIL1*)
  - Sporadic Inclusion body myositis (sIBM)
Pathomechanism in a chaperonopathy (DNAJB6)

- DNAJB6 is ubiquitously expressed
- Yet; DNAJB6 mutations cause a myopathy

1) DNAJB6 mutations alter global chaperone function for all proteins and skeletal muscle is exquisitely susceptible to accumulation of protein inclusions

Or

2) DNAJB6 affects specific client proteins in skeletal muscle that dictate disease pathogenesis.
Hypothesis

- Loss of DNAJB6 function will lead to the selective accumulation of proteins or “clients” that are misfolded leading to toxicity.
DNAJB6 knockout myoblasts

- Of the 5500 proteins identified, 9 were increased by >2X in KO myoblasts
- 2 chaperones (HSPB1, HSPB5)
- 5 Z-disc proteins
  - Desmin
  - α-actinin
  - Four-half LIM domains protein 1 (FHL1)
Sarcomere and Z-disc

[Diagram showing muscle sarcomere and Z-disc components with annotations such as A-band, M-line, I-band, Z-disc, and various proteins and structures labeled.]
Z Disc proteins
Hypothesis

- DNAJB6 will localize to the Z-disk
- LGMD1D mutations in DNAJB6 will disrupt Z-disk proteins leading to their aggregation
LGMD1D mutant DNAJB6 transgenic mice
DNAJB6 localizes to the Z-disc
LGMD1D mutant DNAJB6 expression causes weakness

C. Wire screen holding test

Mean Holding Impulse (N sec)

- **P<0.00005
- * P<0.0005

- Control
- hDNAJB6b-WT
- hDNAJB6b-F93L

D. C57

- hDNAJB6b-WT
- hDNAJB6b-F93L
LGMD1D mutant DNAJB6 expression causes a myopathy
LGMD1D mutant DNAJB6 myopathy muscle has desmin inclusions
Z-disc proteins accumulate in LGMD1D mutant muscle
Summary

- DNAJB6 localizes to the sarcomere at the Z-disc
- Z-disc proteins accumulate in DNAJB6 knockout myoblasts and LGMD1D mutant expressing mice
- Z-disc elements may be DNAJB6 client proteins
Genetic discovery and pathomechanism in hereditary myopathies

Clinical Care → Patient Phenotyping

Model system development ← Genetic discovery

Pathogenic mechanism → Novel molecular mechanism
How do LGMD1D mutations disrupt DNAJB6 function?
DNAJB6 mutations in LGMD1D

<table>
<thead>
<tr>
<th>DNAJB6</th>
<th>GGSGGHFDSPF---EFGFTRN---PDVVREFFGGGRDPFSF</th>
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<td>SGPSGSGGGGANGTS-FSYTFHGDPHAMFAEFFGGGRNPFDT</td>
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<tr>
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<td>GSGGGGGMDE----------------------DIFSHIFGGGLFGFM</td>
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</tr>
<tr>
<td>DNAJA3</td>
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<tr>
<td>DNAJA4</td>
<td>GGSGPSFSSSPM----------------------DIFDMFFGGGG--</td>
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Mutations highlighted in red: F89L, F93L, P96R, F91L, N95L, F91I
G/F domain in DNAJ proteins

Sis1  |  GGFPGGAGGFGSFGHAFSNEDAFNIFSQFFGG  |  121
Ydj1  |  GGFPGGGFGFGD-------------------DIFSQFFGA  |  101
DNAJB6 |  GGGGGSHFDSPFEFGFTFRNPDDVFREFFGG  |  106

F91  N95
F89  F93  P96

Specificity of Class II Hsp40 Sis1 in Maintenance of Yeast Prion [RNQ⁺]

Nelson Lopez,*† Rebecca Aron,‡§ and Elizabeth A. Craig*†
Yeast prions

- Stably inherited protein aggregates/epigenetic elements
- Prion propagation requires chaperone activity
- Yeast prions can support different protein aggregate conformations or “strains”.

Stably inherited protein aggregates/epigenetic elements

Prion propagation requires chaperone activity

Yeast prions can support different protein aggregate conformations or “strains”.

Yeast prions
Prion strains may explain the phenotypic diversity of prion diseases, tauopathies and synucleinopathies.
LGMD1D mutations affect propagation of select prion aggregate conformers in yeast
IBM mutations affect propagation of select Rnq1 conformers

**Diagram:**
- **Sis1**
  - J: Green/Fluorescent (G/F), G/M, CTD
  - 84, 123, 171, 352
- **DNAJB6b**
  - J: Green/Fluorescent (G/F), CTD
  - 11, 108, 241
- **SDSS**
  - J: Green/Fluorescent (G/F), G/M, CTD

**Images:**
- **A**
  - [rnq-]: GFP, DIC
  - WT SDSS
- **E**
  - s.d. med [RNQ+]: GFP, DIC
  - WT SDSS
  - F89I
LGMD1D mutations may abrogate recognition of distinct aggregate conformers
CHAPERONOPATHY
Desmin filament

Stress

Destabilizing variant
Sarcomere and Z-disc

Muscle Sarcomere

Z-disc  Sarcomere  Z-disc

Z-Disc

Troponin  Tropomyosin  Actin  Myopalladin  S100  CapZ  Desmin

Myosin  Non-muscle myosin II  Titin  Nebulette  gamma-Filamin  Calsarcin

Myopodin  ALP  MinK

alpha-Actinin  Ankyrin  Obscurin  MLP  PKC  Cypher  T-Cap  Calcineurin

43 nm
LGMD1D pathogenesis
Genetic discovery and pathomechanism in hereditary myopathies

Clinical Care → Patient Phenotyping

Model system development → Genetic discovery

Pathogenic mechanism → Therapeutics
What about genetic discovery and pathomechanism in **sporadic** muscle disease

Clinical Care \[\rightarrow\] Patient Phenotyping

Model system development \[\leftarrow\] Genetic discovery

Pathogenic mechanism \[\rightarrow\] Therapeutics
Sporadic Inclusion Body Myositis (sIBM)

- Rare myopathy affecting ~5/1,00,000
- Onset is >50 in 80% of patients
- ~60% of patients are men
- Causes insidious asymmetric proximal and distal weakness
  - preferentially affecting the knee extensors and forearm flexors
- Sporadic disorder (i.e. no clear family history of weakness)
Whole exome sequencing

- 62 pts all with ENMC 2013 Clinico-pathologically defined IBM and biopsy material on 54/62 patients
- 38/62 (61%) male: 24/62 (37%) female
- Average age 65+/−10 years
Rare variant analysis

- Coding variant (disrupts the normal amino acid sequence of the protein)
- Rare (the variant has an allele frequency [MAF] of ≤1-0.1%)
- Shared (in more than one individual)
- Statistically over represented in sIBM patients vs. controls
- Depending upon ethnicity, individuals carry 300-500 rare coding variants
Rare variant analysis

**TREM2 Variants in Alzheimer’s Disease**

Rita Guerreiro, Ph.D., Aleksandra Wojtas, M.S., Jose Bras, Ph.D., Minerva Carraquillo, Ph.D., Ekaterina Rogaeva, Ph.D., Elisa Majounie, Ph.D., Carlos Cruchaga, Ph.D., Celeste Sassi, M.D., John S.K. Kauwe, Ph.D., Steven Younkin, M.D., Ph.D., Lilinaz Hazrati, M.D., Ph.D., John Collinge, M.D., Jennifer Pocock, Ph.D., Tammaryn Lashley, Ph.D., Julie Williams, Ph.D., Jean-Charles Lambert, Ph.D., Philippe Amouyel, M.D., Ph.D., Alison Goate, Ph.D., Rosa Rademakers, Ph.D., Kevin Morgan, Ph.D., John Powell, Ph.D., Peter St. George-Hyslop, M.D., Andrew Singleton, Ph.D., and John Hardy, Ph.D., for the Alzheimer Genetic Analysis Group*

- Coding variant with <1% MAF in TREM2
- 60/1091 (5.5%) AD patients vs 38/1105 (3.4%) control patients
- R47H variant was present in 22/1091 (2%) AD patients vs 5/1105 (0.45%) control patients
Rare variant analysis

TREM2 Variant p.R47H as a Risk Factor for Sporadic Amyotrophic Lateral Sclerosis

Janet Cady, BS; Erica D. Koval, BA; Bruno A. Benitez, MD; Craig Zaidman, MD; Jennifer Jockel-Balsarotti, BS; Peggy Allred, DPT; Robert H. Baloh, MD, PhD; John Ravits, MD; Ericka Simpson, MD; Stanley H. Appel, MD; Alan Pestrunk, MD; Alison M. Goate, PhD; Timothy M. Miller, MD, PhD; Carlos Cruchaga, PhD; Matthew B. Harms, MD

- 10/920 (1.09%) ALS vs 3/1848 (0.16%) controls
TBK1 missense variants MAF<1%
46/4161 ALS (1.1%) vs 17/8776 controls (0.194%)
TBK1 unifies a “pathogenic” pathway in ALS
Can the same approach be used in our sIBM study?

- sIBM genetic studies are powered with 100s of patients and not 1000s of patients

- Our study currently has 62 sIBM patient exomes
Gene enrichment approach

- Laser microdissection and label free quantitative mass spectrometry for proteins that accumulate in rimmed vacuoles of sIBM patients

Collaboration with Matthias Vorgerd, Katrin Marcus and Rudi Kley; Bochum Germany
Identification of proteins enriched within rimmed vacuoles

- Using 18 control samples and 18 sIBM samples we identified 2084 proteins
- 213 proteins were enriched by 1.5X specifically in rimmed vacuoles
- These proteins included proteins previously reported to accumulate including VCP, SQSTM1, TDP-43, hnRNPA2/B1
intermediate filaments
DES, NES, LMNA, VIM, SYNC

eextracellular matrix/ basal lamina
COL6A3, COL6A1, FBN1, COL6A2, HSPG2, COL4A2, COL1A1, LUM, DCN, CILP, COL15A1, CMA1

chaperone/stress response/autophagy
CRYAB, HSPA5, VCP, HSPA9, ANXA9, CTSD, FYCO1, SQSTM1, UNC45B, PSAP, HSP90B1, USP5, CALR, GBP2, CCT8, HSPB3, PDIA6, STIP1, CCT4, CCT6A, TALDO1, PSMA1, TCP1, PPIB, LAMP2, SYNGR2, PSMD2, PSMB10, BAG2

sarcosomal proteins
AHNAK, DYF4, LAMB2, LAMA2, DMD, LAMC1, SLMAP, SPTAN1, SPTB, SGCA, SPTBN1, SGCD

sarcomere
NRAP, XIRP2, PGM5, NEXN

immune response
IGKC, IGHG1, FTH1, SERPINA1, PDIA3, C3, MSM, A2M, CLU, FGB, PSMA4, LCP1, FGG, HNRNPA0, ITGB1, MLF2, RAB35

actin dynamics
GSN, VCL, MAP4, PACSIN3, FLNA, MURC, MYL4, MYH14, LMOD2, ARPC4, CAPG, MYH7B, COBL, LASP1, ARPC5L, ARPC1B

nucleus/DNA
HISTH4A, HNRNPA2B1, PBXIP1, TARDBP, HNRNPK, NCL, H2AFY, KPNB1, HNRNPU, LMO7, ATP1B4, PA2G4, HNRNPD, BHLHE41

cytoplasmic vesicle
HSD17B4, ANXA1, ANXA11, DYNC1H1, PLIN3, DYSPL2, CLTC, ASAH1, CPA3, EHD2, AHSG, SCARB2, VAPA, TXLNB, GSTK1, TOM1, SXN3, TPP1, EXOC3L4, SEC22B, LAMTOR1

ribosome
RPL8, RPSA, RPL4, RPL6, RPL7, RPL7A, RPL18, NPM1, RPL23A, RPS18, RPS6, RPS8, RPS4X, RPL12, RPL27, RPS11, RPS9, RPS3A, RPL10A, RPL17, RPS2, RPL18A, RPS19, RPL35A, RPL30, RPS12

mitochondrion/ ER
CYB5R3, HK1, ALDH1B1, ALDH3A2, USMG5, HSD17B10, AFG3L2, SSBP1, IDH3B, DIABLO, SLC25A5

cytoskeleton
TUBA8, EZR, CAP1, TUBB2A, TPP3, MAPRE3, SNTB1

β-amyloid metabolic process
APEH, APCS, TTR

others
ALB, TF, CKB, PTRF, EPHX1, F13A1, HRC, CDH13, ESYT1, CDC42, PGD, JSRP1, LRRC47, EEF1B2, FARSB, MGST2, FBLN5, TGFB1, YWHAQ, EIF4H, RTL, CNTNAP4, CD36, CNDP2, NCF2, S100A11, DHRS7, TAGLN2, KCTD12, PGLS, S100A13, AKR1D1, EIF2S1, FXYD1, ME1
Proteomic/genomic overlay

Proteins enriched in rimmed vacuoles >1.5X
N=215

Genes with rare (<0.1% MAF) missense variants in ≥2 sIBM patients
N=17

Allele frequency statistically similar from 680 sporadic ALS patients
N=16

FYCO1
FYCO1 variants

- 7/62 (11.3%) sIBM and 18/680 sALS patients (MAF <0.1%) missense coding variant in FYCO1

- p value = 0.0029 with a relative risk of 4.2
Rare FYCO1 variants in the 1000 genomes

- 1000 genomes database (actually 2504 genomes)
- 153/2504 (6.1%) with rare missense FYCO1 variant compared to 7/62 sIBM p=0.1055

However; if only include 503 patients of European ancestry (Finnish, US, Spain, Italy, UK) then 17/503 vs 7/62 sIBM and p=0.011
FYCO1 is an autophagic adaptor protein

**Diagram:**

- **Cargo recruitment**
  - Phagophore

- **Autophagosome**
  - ULK1–ULK2 complex
  - LC3-PE (LC3-II)
  - Mitophagy receptor
  - Mitochondria

- **Maturation**
  - Autophagosome
    - SLRs (p62, NBR1, NDP52, OPTN)
    - Ubiquitylated misfolded protein
    - ATG8–LIR-motif interaction
  - Transport
    - Microtubule
  - Autolysosome

**Key:**
- ULK1–ULK2 complex
- LC3-PE (LC3-II)
- Mitophagy receptor
- Mitochondria
- SLRs (p62, NBR1, NDP52, OPTN)
- Ubiquitylated misfolded protein
- ATG8–LIR-motif interaction
- ATG4
- TBC1D5, TBC1D25
- DOR and TP53INP1
- FYCO1
FYCO1 and autophagic proteins co-localize at RVs
FYCO1 variants are associated with BMAR congenital cataracts

<table>
<thead>
<tr>
<th>Variant</th>
<th>Protein variant</th>
<th>Minor allele frequency (MAF)</th>
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<td>c.1157A&gt;G</td>
<td>p.K386R</td>
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<td>c.2514C&gt;G</td>
<td>p.N838K</td>
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<td>c.1971G&gt;C</td>
<td>p.Q657H</td>
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<tr>
<td>c.452T&gt;C</td>
<td>p.V151A</td>
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<td>c.3905C&gt;T</td>
<td>p.P1302L</td>
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<td>p.T1270A</td>
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<td>c.3234C&gt;A</td>
<td>p.D1078E</td>
<td>0.001005</td>
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sIBM variants are within the LC₃ interacting region (LIR)

FYCO₁ and LC₃ co-localize in muscle
Acknowledgements

- Weihl lab
  - Sara Pittman
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