Dystroglycanopathies; natural history and clinical observations

Katherine Mathews, MD
Disclosures

- Research funding: NIH, CDC, Friedreich Ataxia Research Alliance
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- Advisory board member: MDA, FSH Society, Serepta Therapeutics, aTyr Pharma, Marathon.
- No conflicts pertinent to today’s talk
Randomly chosen photos of my Wash U connections...

Trainee

Attending
Outline

• Introduce the dystroglycanopathies
• Two clinically important observations from the natural history study
• Preliminary discussion of outcome measures
Iowa Wellstone Muscular Dystrophy Cooperative Research Center

Kevin P. Campbell, PhD
• Professor and Roy J. Carver Biomedical Research Chair in Molecular Physiology and Biophysics
• Professor of Neurology and Internal Medicine
• Investigator, Howard Hughes Medical Institute

Steven A. Moore, MD, PhD
• Professor of Pathology

Senator Paul D. Wellstone

Cooperative Research Center
Iowa Wellstone Muscular Dystrophy Center
Wellstone Medical Student Fellows

Jamie Eskuri (2010-2011)
Pediatric Neurology Resident
Boston Children’s Hospital

Steve McGaughey (2011-2012)
Pediatric Hospitalist
Washington University, St. Louis

Katie Lutz (2012-2013)
Pediatric Neurology Resident
University of Iowa

Cameron Crockett (2013-2014)
Pediatric Neurology Resident
Washington University, St. Louis

CCOM medical student, M3

Brianna Brun (2015-2016)
CCOM medical student, M3

Courtney Carlson (2016-2017)
Current Fellow

Julia Collison
CCOM Medical student, M1
The Myopathies

Myopathies

- Congenital myopathies
- Muscular Dystrophies
- Inflammatory myopathies
- Toxic myopathies
- Systemic disease myopathies
- Metabolic myopathies
- Myotonic disorders

Classification systems
- Inheritance
- Clinical phenotype
- Pathophysiology
Muscular Dystrophies
Pathophysiologic categories

- All Myopathies
- Muscular Dystrophies
  - Membrane-related dystrophies
  - Dystroglycanopathies
Muscular dystrophies due to disorder of membrane structure/stability or repair

Calcium, etc

Satellite cells

Inflammatory cells

Mismatch between injury and repair

CK
Dystrophin Glyprotein Complex.
Muscular Dystrophies due to cell membrane abnormality

Limb-Girdle Muscular Dystrophies (LGMD)

Duchenne/Becker Muscular Dystrophy (DMD/BMD)

Sarcoglycan Complex

Dystroglycan Complex

Laminin-α2 (merosin)

Dystrophin

Glycosylation of α Dystroglycan

Limb-Girdle Muscular Dystrophy

(Courtesy of Kevin Campbell laboratory)
Dystroglycanopathies
What are they?

- Clinically heterogeneous group of muscular dystrophies that result from hypoglycosylation of $\alpha$-dystroglycan
- All are autosomal recessive
- Often referred to as secondary dystroglycanopathies
  - Abnormality is not typically in the dystroglycan gene
α and β Dystroglycan

- *DAG1* (chr 3p21); single propeptide cleaved to
  — α (extracellular)
  — β dystroglycan (transmembrane)
- α dystroglycan requires extensive glycosylation for binding to components of extracellular matrix (ECM)
Muscular Dystrophy and $\alpha$ DG Glycosylation

Abnormal glycosylation of $\alpha$-dystroglycan

- Disruption of the link between alpha-DG and ligands
  - Muscular dystrophy
  - +/- Developmental defect in brain
  - +/- Developmental defect in eye

Laminin-2 (merosin) and other ligands

LGMD and CMD

Dystroglycan Complex

Dystrophin
Genes involved in O-glycosylation of α-dystroglycan

- B3GALNT2
- GMPPB
- B3GNT1 (B4GAT1)
- ISPD
- DAG1
- LARGE
- DOLK
- POMGNT1
- DPM1
- POMGNT2 (GTDC2)
- DPM2
- POMK (SGK196)
- DPM3
- POMT1
- FKRP
- POMT2
- FKTN
- TMEM5

Alphabet soup!!
Glycosylation of $\alpha$-dystroglycan

Glycosylation takes place along the secretory pathway
Modulates protein stability, conformation, and function

Glycosylation of alpha-dystroglycan

**ER**
- **Man-P**
- **GMPPB**
- **GDP-Man**
- **DPM1**
- **DPM2**
- **DPM3**

**GalNAc**
- **B3GALNT2**
- **GlcNAc**
- **POMGNT2**
- **Dol-P-Man**
- **POMT1/2**
- **POMK**
- **Ser/Thr**

**α-dystroglycan**

**Golgi**
- **FKTN**
- **FKRP**
- **TMEM5**
- **B4GAT1**
- **LARGE**

- **xylose**
- **glucuronate**

**laminin binding glycan domain**

**CDP**
- **CTP**
- **CDP-ribitol**
- **phospho-ribitol**

**ISPD**

**slide modified from original by T. Willer, courtesy S. Moore**
Approach to diagnosis of a dystroglycanopathy

• Recognize the phenotype
• Genetic testing
• Confirmatory or suggestive testing
  – Muscle biopsy with immunostaining for glycosylated α-DG
  – Fibroblast complementation studies (for selected DG’s)
    • Can suggest a gene to be tested or confirm that mutations are pathogenic
Dystroglycanopathy clinical classification
(basis for OMIM classification)

- Walker Warburg Syndrome (and WWS-like)
- Muscle Eye Brain/Fukuyama CMD-like
- CMD with cerebellar involvement (cysts, hypoplasia, dysplasia)
- CMD with mental retardation (normal brain structure)
- CMD with no mental retardation

-----------------------------------------------

- LGMD (onset of weakness after starting to walk) with mental retardation
- LGMD (onset of weakness after starting to walk) with no mental retardation

Tools in Diagnosis
Case 1. Congenital muscular dystrophy

• Female infant
• Normal at birth, hypotonic by a few months
  – first seen at 13 months
  – progressive weakness, hyptonia, delayed motor milestones, large calves, and CK 16,000
• Non-weight bearing at 15 months
CMD (CA-C; continued)

• Best motor function: walking with gait trainer
  – never walked independently
  – slowly progressive motor weakness
• Normal cognitive function
• Scoliosis surgery at 12 yo
• Nocturnal BiPAP started at 14 yo
• Echocardiogram normal
• Death at age 18 years (several years ago)
  – Genetic diagnosis unknown
CMD (continued)

• At 13 months old, muscle biopsy → dystrophic (necrotizing myopathy)

• 4yo - biopsy re-evaluation → interpreted as partial merosin deficiency
  – *LAMA2* (merosin) mutation analysis normal

• 2014 – re-evaluation of muscle biopsy with current antibody panel →
  dystroglycanopathy
Courtesy of S. Moore
CMD (continued)

– Dystroglycanopathy panel sequencing → heterozygous ISPD mutation in exon 8
  • c.1114_1116delGTT, p.V372del

– Complementation assay in fibroblasts shows rescue with ISPD
Fibroblast on-cell western confirms pathogenic mutations

Healthy control

Disease control ISPD-WWS

Adenoviral delivery of ISPD restores glycosylation

Courtesy of S. Moore
CMD (continued)

- Further analysis of exome sequencing data shows heterozygous deletion of ISPD exon 2
- Information provided to the family, useful for closure and genetic counseling

- Muscle biopsy can suggest the diagnosis of a dystroglycanopathy
- For some dystroglycanopathy genes, fibroblast complementation can confirm or suggest a specific gene is involved in the disease
1. Dystroglycanopathies encompass a huge phenotypic spectrum

Walker-Warburg
Muscle-Eye-Brain
Fukuyama
Congenital MD

All Dystroglycanopathy Genes

Clinical Severity

Walker-Warburg
Muscle-Eye-Brain
Fukuyama
Congenital MD

CMDs

LGMDs

All Dystroglycanopathy Genes

POMT1
POMT2
POMGnT1
FKRP
Gene doesn’t predict phenotype; illustrated by *GMPPB* cases

- **GMPPB**: Guanosine diphosphate mannose pyrophosphorylase B
- Catalyzes the formation of GDP-mannose from GTP and mannose-1-phosphate
- Functions very early in the α-dystroglycan glycosylation pathway
CASE 2, Male

- Excellent general health and development
- 8th grade: legs were sore and tight after exercise
- 9th grade: reddish brown urine associated with the muscle soreness and fatigue
- Urologic evaluation normal
- 17 yo: evaluation for worsening exercise intolerance and fatigue
- 27 yo: works full time, normal strength on MMT
- CK 52,010 during acute pain episode, later 7,250 IU/l
muscle biopsy at age 18

\(\alpha\)-dystroglycan
(IIH6)
\(\alpha\)-dystroglycan
(VIA4-1)
\(\beta\)-dystroglycan

GMPPB mutations: c.79G>C (p.D27H); c.1069G>A (p.V357I)
CASE 3, Female

• Normal pregnancy and perinatal course, did well initially
• Myoclonic seizures started around 3 months, evolved into infantile spasms
• Initial exam at 8 months
  – Acquired microcephaly—OFC normal at birth but <3rd %tile at 8 month
  – Irritable
  – Hypotonic with poor head control and frog leg posture, non weight-bearing
MRI, 4 yo

Microcephaly, mild hypoplasia of cerebellar vermis
Contrast with Walker Warburg Syndrome Brain MRI
Case 3, cont

- 4 yo: able to crawl, improving sitting control
- Seizures adequately controlled on 3 drugs
- 4.5 yo: viral illness $\rightarrow$ loss of crawling, worse head control, worse sitting
- CK 556, repeat 752, repeat 2789
Case 3, cont

• Muscle biopsy: dystroglycanopathy
• 10 yo: Spinal fusion for scoliosis
• 13 yo:
  – Rare seizures on 2 anticonvulsants
  – Non-verbal, but communicates wishes
  – Understands some spoken language
  – Eats by mouth but supplements with g-tube
  – Non weight bearing
• GMPPB sequencing:
  – c.859C>T, p. R287W
  – c.860G>A, p. R287Q
## Summary of GMPPB cases
(Wellstone cases, 4 additional known to us, literature)

<table>
<thead>
<tr>
<th>Study</th>
<th>Age at Onset</th>
<th>Achieved Ambulation</th>
<th>Intellectual Disability</th>
<th>Epilepsy</th>
<th>Rhabdo/cramps</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wellstone cases</td>
<td>Birth-17 yo</td>
<td>2 (50%)</td>
<td>2 (50%)</td>
<td>2 (50%)</td>
<td>1 (25%)</td>
</tr>
<tr>
<td>(4 cases, 4 families)</td>
<td></td>
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</tr>
<tr>
<td>Collaborator cases</td>
<td>Infancy-17 yo</td>
<td>4 (100%)</td>
<td>3 (75%)</td>
<td>1 (25%)</td>
<td>0</td>
</tr>
<tr>
<td>(4 cases, 4 families)</td>
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<tr>
<td>Carss, 2013</td>
<td>Birth-4 yo</td>
<td>6 (75%)</td>
<td>7 (88%)</td>
<td>4 (50%)</td>
<td>0</td>
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<tr>
<td>(8 cases, 8 families)</td>
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<tr>
<td>Raphael, 2014</td>
<td>1-2 yo?</td>
<td>2 (100%)</td>
<td>2 (100%)</td>
<td>1 (50%)</td>
<td>0</td>
</tr>
<tr>
<td>(2 cases, 1 family)</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Cabrera-Serrano, 2015</td>
<td>15-35 yo</td>
<td>8 (100%)</td>
<td>2 (25%)</td>
<td>1 (13%)</td>
<td>4 (50%)</td>
</tr>
<tr>
<td>(8 cases, 5 families)</td>
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<td></td>
</tr>
<tr>
<td>Bharucha-Goebel, 2015</td>
<td>12 yo</td>
<td>3 (100%)</td>
<td>3 (100%)</td>
<td>1 (33%)</td>
<td>0</td>
</tr>
<tr>
<td>(3 cases, 1 family)</td>
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<tr>
<td><strong>Total</strong></td>
<td>Birth-35 years</td>
<td><strong>25 (84%)</strong></td>
<td><strong>19 (66%)</strong></td>
<td><strong>10 (34%)</strong></td>
<td><strong>5 (17%)</strong></td>
</tr>
<tr>
<td>(29 cases, 23 families)</td>
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</tbody>
</table>

Jensen, et al. Hum Mutat. 2015 Dec;36(12)
Genotype-phenotype observations

- c.79G>C--mild
  - Case 2
  - 13 known individuals, 8 families
  - Onset >10 yo, rare epilepsy (1/13), normal or near normal intellectual function (10/13)

- c.860G>A--more severe
  - Case 3
  - 9 individuals, 8 families
  - Onset in infancy, intellectual disability (8/9), epilepsy (6/9), never achieved walking (6/9)

Jensen, et al. Hum Mutat. 2015 Dec;36(12)
FKRP genotypes in our cohort; c.826C>A founder mutation
**FKRP c.826A>C homozygotes are older at onset of symptoms (p=0.006)**

### Age at first symptom in LGMD2I

<table>
<thead>
<tr>
<th></th>
<th>Range (years)</th>
<th>Mean age (S.D)</th>
</tr>
</thead>
<tbody>
<tr>
<td>All patients</td>
<td>0.25-28</td>
<td>9.4 (8.4)</td>
</tr>
<tr>
<td>Homozygous c.826 C&gt;A</td>
<td>2-28</td>
<td>13.6 (8.5)</td>
</tr>
<tr>
<td>Heterozygous (c.826 C&gt;A + unique)</td>
<td>0.25 -12</td>
<td>4.3 (3.5)</td>
</tr>
</tbody>
</table>
**FKRP** c.826A>C homozygotes are older at use of full time wheelchair (small numbers)

<table>
<thead>
<tr>
<th>Age at FT w/c</th>
<th>Allele 1</th>
<th>Allele 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>15</td>
<td>c.826C&gt;A</td>
<td>c.469G&gt;C</td>
</tr>
<tr>
<td>11</td>
<td>c.826C&gt;A</td>
<td>c.661_662insA</td>
</tr>
<tr>
<td>18</td>
<td>c.826C&gt;A</td>
<td>c.266C&gt;T</td>
</tr>
<tr>
<td>37</td>
<td>c.826C&gt;A</td>
<td>c.826C&gt;A</td>
</tr>
<tr>
<td>41</td>
<td>c.826C&gt;A</td>
<td>c.826C&gt;A</td>
</tr>
</tbody>
</table>
4 stair climb, *FKRP* homozygotes faster in the pediatric population

Significant difference between groups (p=0.019).
Summary of this section

• $\alpha$-DG undergoes extensive post-translational glycosylation that is required for binding to components of the extracellular matrix

• The dystroglycanopathies encompass a wide range of genotypes
  – Confirmatory testing with fibroblast complementation assay for some genes

• The specific gene involved doesn’t predict phenotype
  – Some recurring mutations may have phenotypic implications
Clinical observations
13 mo twins

Thank you to patients and family for providing this video
Acute weakness associated with viral illness

- Reported previously in patients with FKTN mutations (Fukuyama congenital muscular dystrophy)
- We found 16/94 (17%) patients reported sudden onset of weakness within one week following illness
- 5 genes
  - FKRP: 10
  - ISPD: 1
  - FKTN: 2
  - POMT1: 1
  - POMT2: 2
- Median age at episode: 2.5 yo (range 3 mo-27 yrs)
  - 81% were < 7 yo

Courtney Carlson, S. McGaughey, in preparation
Acute weakness associated with viral illness

• Mean CK >20,000
• 8/16 hospitalized, one intubated
• Clear loss of motor function
  – loss of head control, inability to sit unassisted, and inability to walk
• 15/16 reported full recovery to baseline
  – Usually within 1 week
• Dx of muscular dystrophy AFTER episode in 9/16
“Metabolic” presentation of DGs

• Patients with dystroglycanopathy may experience a syndrome of acute weakness less than a week after a febrile illness
  – Patients are usually less than 7 years old
  – Very high CK that doesn’t recover to normal

• Exercise-induced myoglobinuria is seen in ~25% of our DG cohort

• ~30% report muscle pain as an early symptom
  • Mathews, et al. Neurology 2011;76;194
Cardiac involvement in the DGs
Cardiomyopathy in the DGs, N=58

- Requested films and reports on all enrollees in natural history study
- 18/58 (31%) had at least one abnormal echocardiogram
  - $FKRP = 14/39$ (36%)
    - Homozygous (c.826C>A, c.826C>A) = 4/15 (27%)
    - Other $FKRP = 10/24$ (42%)
  - All other genes = 4/19 (21%)
    - $FKTN = 2/4$
    - $POMT2 = 1/3$
    - $ISPD = 1/1$
    - $GMPPB = 0/4$
    - $POMGnT1 = 0/4$
    - $POMT1 = 0/1$
    - Unknown DG = 0/2

Julia Collison, Katie Lutz, work prepared for submission
Median age at onset of cardiomyopathy (whole cohort): 36 yrs

Males/females equally affected
No significant difference based on gene involved \( p = 0.133 \)

- Median age at cardiomyopathy in \( FKRP \) cohort = 36 years
- Median age all other genes (4 subjects with cardiomyopathy) = 15 yrs
*FKRP* cohort:

Non-c. 826C>A homozygotes significantly higher rate of cardiomyopathy

- Cox hazard ratio 8.92 (95% CI: 1.88, 42.40; Wald test p=0.006)
- **Homozygous** c.826C>A Median age at onset: 48 yrs
- **Heterozygous** c.826C>A/other Median age at onset: 29 yrs
Our results are gratifyingly similar to previous series of similar size


Median age at onset of cardiomyopathy; *FKRP* cohort

<table>
<thead>
<tr>
<th></th>
<th>Homozygotes</th>
<th>other</th>
</tr>
</thead>
<tbody>
<tr>
<td>Iowa</td>
<td>48</td>
<td>29</td>
</tr>
<tr>
<td>Poppe</td>
<td>51</td>
<td>20</td>
</tr>
</tbody>
</table>
Cardiomyopathy monitoring

• High awareness of possibility of cardiomyopathy (assess symptoms)
• Echocardiogram at diagnosis
• Children <18 yo: Echocardiogram/cardiac MRI at least every 2 years
• Adults homozygous for the common mutation (c.826C>A) and with normal imaging—repeat every 3-5 years.
• Adults with other genotypes: Echocardiogram/cardiac MRI every 2 years.
Outcome measures, preliminary

- Limited to subjects with \textit{FKRP} mutations
- Functional measures
10 m walk speed, steps/second, *FKRP* cohort, All values
Approach

• Nonlinear progression across ages
  • Pediatric 6 to ≥ 18yo
    – Adult >18 yo

• Variation in years of follow up
  – Included only patients who had more than one data point (generally annual follow-up)
  – Determined rate of progression for each individual
  – Averaged those rates for the group

• Converted outcomes to speed when needed to avoid ceiling/floor effect
Speed 10m walk, meter/sec
(Age 6-18 yrs)

There was a significant decline in 10m walk speed in FKRP subjects, with a mean decline per year of **-0.15** (95% CI: -0.08, -0.22; p=0.0003).

Start at 10 seconds to walk 10 meters= 1m/sec

One year later, 0.85m/second or 11.8 seconds

Start at a speed of 3m/sec (3.3 seconds/10 m)

One year later, 2.85 m/second or 3.5 seconds to walk 10 m
Speed 10m walk, meter/sec (Age >18 yrs)

There was a mean decline per year in 10m walk speed in FKRP subjects of -0.076 (95% CI: -0.153, 0.002; $p=0.052$).
Significant (but very small) annual change in most measures

<table>
<thead>
<tr>
<th>&lt;18 yo</th>
<th>p value</th>
<th>Measure</th>
<th>&gt;18 yo</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>-0.11</td>
<td>0.047</td>
<td>Average MRC</td>
<td>-0.10</td>
<td>0.005</td>
</tr>
<tr>
<td>-0.15</td>
<td>0.000</td>
<td>10 m walk, meters/sec</td>
<td>-0.08</td>
<td>0.052</td>
</tr>
<tr>
<td>-0.12</td>
<td>0.002</td>
<td>4 stair climb, stairs/sec</td>
<td>-0.07</td>
<td>0.024</td>
</tr>
<tr>
<td>-3.89</td>
<td>0.615</td>
<td>6 min walk distance</td>
<td>-10.80</td>
<td>0.041</td>
</tr>
<tr>
<td>-1.50</td>
<td>0.000</td>
<td>FVC, % pred</td>
<td>-1.17</td>
<td>0.000</td>
</tr>
<tr>
<td>-13.4%</td>
<td>0.002</td>
<td>Ave steps/day (SAM)</td>
<td></td>
<td></td>
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</table>
## Power calculations, pediatric patients

<table>
<thead>
<tr>
<th>Variable</th>
<th>mean chg/yr</th>
<th>SD (1 yr chg)</th>
<th>detectable diff</th>
<th>n/group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Speed 10m walk</td>
<td>-0.15</td>
<td>0.31</td>
<td>0.075 (50% reduction)</td>
<td>269</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.12 (80% reduction)</td>
<td>106</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.14 (93% reduction)</td>
<td>78</td>
</tr>
<tr>
<td>Stairs steps/sec</td>
<td>-0.12</td>
<td>0.36</td>
<td>0.06 (50% reduction)</td>
<td>566</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.09 (75% reduction)</td>
<td>252</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.11 (92% reduction)</td>
<td>169</td>
</tr>
<tr>
<td>FVC</td>
<td>-1.50</td>
<td>7.52</td>
<td>1.40 (93% reduction)</td>
<td>454</td>
</tr>
<tr>
<td>Ave MRC</td>
<td>-0.114</td>
<td>0.59</td>
<td>0.10 (88% reduction)</td>
<td>547</td>
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# Power calculations, adult patients

<table>
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<th>Variable</th>
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<th>SD (1 yr chg)</th>
<th>detectable diff</th>
<th>n/group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Speed 10m walk</td>
<td>-0.076</td>
<td>0.179</td>
<td>0.038 (50% reduction)</td>
<td>349</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.061 (80% reduction)</td>
<td>136</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.07 (92% reduction)</td>
<td>104</td>
</tr>
<tr>
<td>Stairs steps/sec</td>
<td>-0.07</td>
<td>0.28</td>
<td>0.035 (50% reduction)</td>
<td>566</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.05 (71% reduction)</td>
<td>493</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.065 (93% reduction)</td>
<td>292</td>
</tr>
<tr>
<td>FVC</td>
<td>-1.17</td>
<td>5.25</td>
<td>1.11 (95% reduction)</td>
<td>352</td>
</tr>
<tr>
<td>Ave MRC</td>
<td>-0.104</td>
<td>0.627</td>
<td>0.099 (95% reduction)</td>
<td>631</td>
</tr>
</tbody>
</table>
Conclusions from standard outcome measures in FKRP cohort

• Current data suggest we will need:
  – Better/more sensitive outcomes
  – Relatively large and/or long trials ($$$)
  – Alternative study design for small effect, rare disease
  – Very effective treatment that will improve function

• The caveats
  – Data collected over many years
  – Participants are competitive
  – Participants in a natural history study don’t represent the total population
  – Still relatively small numbers
Dystroglycanopathies

• Clinically and genetically heterogeneous group of muscular dystrophies that cause a lack of membrane stability
• Consider this group of diseases in setting of what appears to be metabolic myopathy
• Monitoring for cardiomyopathy is required
• Clinical trials will have some challenges
2016 Dystroglycanopathy Family Conference

Thank you to the families who have participated.
Mutations in GMPPB cause congenital myasthenic syndrome and bridge myasthenic disorders with dystroglycanopathies

Katsiaryna Belaya,1 Pedro M. Rodríguez Cruz,1,2 Wei Wei Liu,1 Susan Maxwell,1 Simon McGowan,3 Maria E. Farrugia,4 Richard Petty,4 Timothy J. Walls,5 Maryam Sedghi,6 Keivan Basiri,7 Wyatt W. Yue,8 Anna Sarkozy,9,10 Marta Bertoli,9 Matthew Pitt,11 Robin Kennett,2 Andrew Schaefer,5 Kate Bushby,9 Matt Parton,10 Hanns Lochmüller,9 Jacqueline Palace,2 Francesco Muntoni12 and David Beeson1

Congenital myasthenic syndromes are inherited disorders that arise from impaired signal transmission at the neuromuscular junction. Mutations in at least 20 genes are known to lead to the onset of these conditions. Four of these, ALG2, ALG14, DPAGTI and GFPT1, are involved in glycosylation. Here we identify a fifth glycosylation gene, GMPPB, where mutations cause congenital myasthenic syndrome. First, we identified recessive mutations in seven cases from five kinships defined as congenital myasthenic syndrome using decrement of compound muscle action potentials on repetitive nerve stimulation on electromyography. The mutations were present through the length of the GMPPB, and segregation, in silico analysis, exon trapping, cell transfection followed by western blots and immunostaining were used to determine pathogenicity. GMPPB congenital myasthenic syndrome cases show clinical features characteristic of congenital myasthenic syndrome subtypes that are due to defective glycosylation, with variable weakness of proximal limb muscle groups while facial and eye muscles are largely spared. However, patients with GMPPB congenital myasthenic syndrome had more prominent myopathic features that were detectable on muscle biopsies, electromyography, muscle magnetic resonance imaging, and through elevated serum creatine kinase levels. Mutations in GMPPB have recently been reported to lead to the onset of muscular dystrophy dystroglycanopathy. Analysis of four additional GMPPB-associated muscular dystrophy dystroglycanopathy cases by electromyography found that a defective neuromuscular junction component is not always present. Thus, we find mutations in GMPPB can lead to a wide spectrum of clinical features where deficit in neuromuscular transmission is the major component in a subset of cases. Clinical recognition of GMPPB-associated congenital myasthenic syndrome may be complicated by the presence of myopathic features, but correct diagnosis is important because affected individuals can respond to appropriate treatments.
Repetitive stimulation in DG subjects

A total of 31 patients from 27 unrelated families were tested.

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>n=31</th>
<th>CMD</th>
<th>LGMD</th>
</tr>
</thead>
<tbody>
<tr>
<td>FKRP (n=25)</td>
<td>0</td>
<td>25</td>
<td></td>
</tr>
<tr>
<td>GMPPB (n=4)</td>
<td>2</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>POMT2 (n=1)</td>
<td>1</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>PMGnT1 (n=1)</td>
<td>1</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>

One subject with mild LGMD due to GMPPB mutations had >10% decrement on rep stim

Gonzalez Perez, in preparation
DG Natural history and outcome measures

• Inclusion
  – Anyone with a high probability of having dystroglycanopathy based on clinical phenotype plus muscle immunohistochemistry, genetic testing, or fibroblast complementation studies

• Protocol (has evolved over the past 10 years)
  – Medical history and intro surveys
  – Motor function (standard and exploratory)
  – QOL
  – Speech/language evaluation (selected patients)
  – Genetic testing if not completed
  – Tissue bank (fibroblasts)-Steve Moore

• Annual re-evaluation
4 stair climb, FKRP

Pediatric population
Significant difference between groups (p=0.019).

Adult population
No difference between groups (p=0.268)
Most measures are highly correlated with 10m walk speed

<table>
<thead>
<tr>
<th>Variable</th>
<th>&lt;18 yo, r=</th>
<th>P value</th>
<th>&gt;18 yo, r=</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Average MRC</td>
<td>0.87</td>
<td>&lt;0.0001</td>
<td>0.85</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Arise from Supine (1/time)</td>
<td>0.91</td>
<td>&lt;0.0001</td>
<td>0.81</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Climb Stairs (steps/sec)</td>
<td>0.94</td>
<td>&lt;0.0001</td>
<td>0.97</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>6 minute walk distance</td>
<td>0.84</td>
<td>&lt;0.0001</td>
<td>0.97</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Ave steps per day (ln)</td>
<td>0.48</td>
<td>0.025</td>
<td>-0.01</td>
<td>0.973</td>
</tr>
<tr>
<td>FVC (%Pred Sitting)</td>
<td>0.23</td>
<td>0.252</td>
<td>0.71</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>
Repetitive nerve stimulation (3Hz)

A) AN Coneus Muscle

**Baseline**

**Post-exercise**

**1 min post-exercise**

RNS on anconeus muscle is consistent with a postsynaptic dysfunction at the NMJ in this patient
Repetitive nerve stimulation (3Hz)

A) TRAPEZIUS MUSCLE

Control

Patient A.R.

Baseline

Post-exercise

1 min post-exercise

RNS on trapezius muscle is suggestive of mild postsynaptic dysfunction at the NMJ in this patient.
Aug 1, 2016: 89 living dystroglycanopathy participants

- 52 <21 years old
- 37 >21 years old