Clinical Introduction to the Dystroglycanopathies

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Welcome to Iowa City!
Overview

• Where do the dystroglycanopathies fit in among neuromuscular diseases?
• What is the clinical features of the dystroglycanopathies?
• How are the dystroglycanopathies managed?
Dystroglycanopathies

All Muscle Diseases

Muscular Dystrophies

Membrane-related dystrophies

Disorders of alpha dystroglycan glycosylation
What are muscular dystrophies?

• Inherited diseases of muscle
• Degeneration and regeneration

• Muscular Dystrophies--clinical categories
  – Dystrophinopathies (Duchenne/Becker)
  – Limb girdle Muscular Dystrophies (18 types)
  – Congenital Muscular Dystrophies (14 types)
  – Facioscapulohumeral Muscular Dystrophy
  – Emery-Dreifuss Muscular Dystrophy (XL and AD)
  – Oculopharyngeal Muscular Dystrophy
  – Scapuloperoneal Muscular Dystrophy
  – Distal Muscular Dystrophies
What disorders are included among the dystroglycanopathies?

- NOTE: nomenclature is evolving

- Congenital muscular dystrophies
  - Walker Warburg syndrome
  - Muscle Eye Brain disease
  - Fukuyama Muscular Dystrophy
  - Congenital muscular dystrophy types 1C and 1D

- Limb Girdle muscular dystrophy
  - Types 2I, K, M, N, and O
Dystrophin-Glycoprotein Complex and Associated Muscular Dystrophies

- Duchenne/Becker Muscular Dystrophy (DMD/BMD)
- Congenital Muscular Dystrophy (CMD)
- Limb-Girdle Muscular Dystrophies (LGMD)

Glycosylation of dystroglycan

Dystrophin-Glycoprotein Complex and Associated Muscular Dystrophies (Courtesy of Kevin Campbell laboratory)
Genes Known to Result in a Dystroglycanopathy

- **POMT1** (Protein O-mannosyltransferase 1)
- **POMT2** (Protein O-mannosyltransferase 2)
- **POMGnT1** (protein O-mannose beta-1,2-N-acetylglucosaminyItransferase)
- **Fukutin**
- **FKRP** (Fukutin related protein)
- **LARGE**
- **DAG1** (dystrophin-associated glycoprotein; dystroglycan)
The Dystroglycanopathies--Clinical Spectrum

Walker-Warburg
Muscle-Eye-Brain
Fukuyama
MDC-1C

CMDs

LGMDs

Clinical Severity/Age at onset

POMT1
POMT2
FKTN

POMGnT1
FKRP
LGMD 2I

- Caused by mutations in FKRP
- Most common cause of LGMD in northern European population
- Wide range of clinical severity
- Common mutation (c. 826C>A)
  - 2 copies of common mutation = milder disease
  - 1 copy of common mutation + 1 copy of some other mutation = often more severe disease
<table>
<thead>
<tr>
<th>Category</th>
<th>Range (years)</th>
<th>Mean age (S.D)</th>
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<tbody>
<tr>
<td>All patients</td>
<td>birth-28</td>
<td>7.4 (7 )</td>
</tr>
<tr>
<td>Homozygous (826 C&gt;A)</td>
<td>2-28</td>
<td>10.1 (7.7)</td>
</tr>
<tr>
<td>Heterozygous (826 C&gt;A + unique)</td>
<td>0.25 -12</td>
<td>4.3 (4)</td>
</tr>
<tr>
<td>Heterozygous (2 unique mutations; 1 patient)</td>
<td>Birth</td>
<td>--</td>
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Congenital Muscular Dystrophies

- Onset of weakness before age 2
- Range of involvement
  - Muscle weakness present in all
    - Abnormal development of brain and eye
    - Cognitive impairment with minor abnormality of brain formation
    - Cognitive impairment with normal brain structure on MRI
    - Normal intelligence and normal eyes
DGs are Autosomal Recessive

- Both parents are carriers
  - Carriers have no symptoms or weakness
- With each pregnancy, 1 in 4 (25%) chance that a child will be affected.
- Non affected siblings
  - 2/3 chance of being a carrier
- Carrier rate in Iowa 1/315
Organ system involvement in Dystroglycanopathies

- Muscles
- Breathing
- Heart
- Bones and joints
- Eyes
- Brain
Muscles get weaker over time

Mismatch between muscle injury and repair

Satellite cells
Calcium
Inflammatory cells

Muscles get weaker over time
Exercise

- Too little exercise causes muscles to atrophy and become weak
- Normal response to exercise:
  - Membrane breaks
  - CK release
  - Repair of membrane
  - Protein synthesis
  - Fiber hypertrophy
Exercise
General Recommendations

• Maintain active lifestyle, as possible
  – Swimming is an excellent activity
• Pay attention to your body—don’t overdo
• Avoid muscle-building exercise, zealous training, overly aggressive PE teachers
• Do regular stretching
Breathing in Dystroglycanopathy

Lung tissue is healthy

Symptoms:
- Morning headache
- Excessive fatigue
- Daytime sleepiness
- Recurrent pneumonia

Muscles that move the air can become weak

Poor cough

Risk of pneumonia

Shallow breaths in sleep
Breathing in Dystroglycanopathy

- Not a problem early in disease
- Monitor strength of muscles involved in breathing, cough
- Lots of options for management
  - Suction Machine
  - BiPAP (night-time only, fulltime)
  - Cough Assist
BiPAP results
Patients with progressive neuromuscular weakness

- Prolonged survival (DMD, ALS, SMA)
- Fewer hospitalizations/fewer days in ICU
  - 85% reduction in hosp days compared to the year prior to BiPAP
- Improved measures of respiratory function by sleep study, ABGs
- Improved quality of life
Cardiomyopathy (Heart Disease) in Dystroglycanopathy

- The heart muscle can also become weak
  - 60% in one series of 23 LGMD2I patients

- Limited data is available
  - Highly variable, even within families
  - In patients with FKRP mutations, no apparent relationship between skeletal muscle weakness and cardiomyopathy
    - Cardiomyopathy can occur before weakness
  - Cardiomyopathy generally affects adults
Cardiomyopathy Management

• Monitoring
  – Echocardiogram every 1-2 years and if symptoms

• Consider prevention treatment (Enalapril, Losartan)
  – No data
  – Discuss pros/cons with cardiologist
Osteoporosis

- **Risk factors**
  - Abnormal forces on bone
  - Non weight bearing
  - Medications?
  - Lack of sun exposure

- **Result:** Frequent fractures
  - Fracture → permanent loss of walking

- **Monitoring:** DEXAs

- **Treatment**
  - Calcium, Vitamin D
  - Prolong walking, weight bearing
  - Fosamax or other bisphosphonate
Scoliosis

• ~1-2 years after wheelchair in growing children

• Reasons for surgery
  – Cosmetic
  – Relief of pain
  – Improved respiratory function late in disease

• Against surgery
  – Major operation; pain, complications
  – Trouble eating
  – Trouble fitting into van
Abnormal Eye Development in DGs

• Reported only in patients with congenital muscular dystrophies

• Wide range of abnormalities
  – Microphthalmia (abnormally small eyes), cataracts, glaucoma, severe myopia, and others

• Everyone with CMD (vs LGMD) should have eye exam
Brain in Dystroglycanopathies

- (Dr. Moore will discuss animal research)
- DG plays a role in brain development
- With some abnormalities of DG, brain structure and/or function are affected
Brain in Dystroglycanopathies

• Cognitive impairment ranging from mild to severe
• Seizures
  – More likely in those with cognitive impairment
• LGMD 2I
  – Normal intelligence
  – Small study suggested mild deficits in planning and organization
    • Needs to be repeated with more patients
Many Other Aspects of Management!

- School modifications
- Emotional adjustments
  - Patient
  - Parents
- Home modifications
- Financial and insurance issues
- Transitions to independent adulthood
Management of Dystroglycanopathy is Multifaceted

- Personalize the management team for each patient
- Optimal treatment of each system can affect outcome in other systems.

Maintain walking for as long as possible

- Improved bone density
- Less scoliosis
- Fewer compression fractures
- Better cardiac function?
- Better pulmonary function
Muscle Pathology in the Dystroglycanopathies

Welcome to Iowa!

Steven A. Moore, M.D., Ph.D.
The University of Iowa
Department of Pathology
and
Wellstone Muscular Dystrophy Cooperative Research Center
Dystroglycanopathy Muscle Pathology – the basics

- Skeletal muscle structure
- Muscle biopsy evaluation
- What looks different in muscular dystrophy
- How to distinguish dystroglycanopathy from similar muscular dystrophies
muscle structure

http://cyhsanatomy1.wikispaces.com/file/detail/Skeletal_Muscles-1.gif
muscle structure

- A muscle
- A group of cells
- A single cell
- Contractile proteins inside a single cell

web image – source unknown
muscle structure
muscle biopsy in a clamp

~1.5 cm

~0.5 cm
mount muscle on cork for cross sections

1) cut biopsy from clamp

2) mount on cork
3) freeze in isopentane

~1.0 cm
Cool isopentane inside a metal cup by suspending the cup in liquid nitrogen. The optimum freezing temperature is about -160°C.

freeze muscle in **very** cold isopentane
normal muscle - frozen section H&E
What is muscular dystrophy?

- inherited muscle disorder characterized by repeated cycles of muscle degeneration (necrosis) and regeneration
- patients present with weakness and a wide variety of other signs and symptoms
- classified by patterns of inheritance, distribution of muscle involvement, age of onset, the abnormal gene (protein)
muscular dystrophy - frozen section H&E
necrosis
necrosis

regeneration
muscular dystrophy
muscular dystrophy
Dystrophin-Glycoprotein Complex (DGC)

- LGMD2F
- LGMD2E
- LGMD2D
- LGMD2C

Laminin-α2 (merosin)
- FCMD, MEB, WWS
- MDC1C, MDC1D

Dystroglycan Complex
- LGMD2I, 2K, 2M, 2N, 2O

Sarcoglycan Complex
- Merosin-Deficient CMD (MDC1A)

Basal Lamina (Basement Membrane)

Dystrophin-Related Muscular Dystrophies

- Duchenne/Becker Muscular Dystrophy (DMD/BMD)
- Actin
laminin-α2 (merosin)

dystrophin
dystroglycans
sarcoglycans

contractile proteins

Figure modified from Pathology of Skeletal Muscle, Oxford, 2001.
immunostaining for the diagnosis of muscular dystrophy

<table>
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<th></th>
<th>normal</th>
<th>dystrophin</th>
<th>utrophin</th>
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Duchenne muscular dystrophy (DMD)
DMD dystrophin

control dystrophin

DMD dystrophin

control utrophin

Dystrophin stains normally in dystroglycanopathies.
Sarcoglycans stain normally in dystroglycanopathies.
adult onset LGMD – dystroglycanopathy
patchy, reduced staining for alpha-dystroglycan

α-dystroglycan antibody

LGMD 2I
LGMD 2I patient with cardiomyopathy

α-dystroglycan is missing “laminin binding glycosylation” in the dystroglycanopathies

Glycosylation

Glyco –
A Greek word that means sweet

Glycobiology-
The branch of science that studies the role of carbohydrates (sugar molecules) and their implication on health and disease.

Tobias Willer, Ph.D.
Assistant Research Scientist in Dr. Kevin Campbell’s lab
Department of Molecular Physiology and Biophysics
University of Iowa College of Medicine
All cells are coated with “glycans”

Glycoproteins are found on the outer surface of plasma membrane, in the extracellular matrix, in the blood, and in specific organelles, Golgi complexes, lysosomes, and secretory granules.
Roles of oligosaccharides in recognition and adhesion at the cell surface

Surface carbohydrates on cells serve as points of attachment for other cells, infectious bacteria, viruses, toxins, hormones and many other molecules.
Why glycoproteins? – The biological advantages of modifying proteins with sugars

- important for function
  sugars can be important for receptor function

- important for folding
  enhances stability of proteins

- important for targeting
  sugars can act as a ZIP code to direct proteins to a specific cellular compartment

“... while the function of DNA and proteins are generally known ... it is much less clear what carbohydrates do ...”

Ciba Foundation Symposium 1988
Schematic representation of common classes of glycoconjugates expressed in human cells

Glycoproteins

Proteoglycans

Glycolipids

Key:
- GlcNAc
- GalNAc
- HexNAc
- Gal
- Glc
- Man
- Fuc
- Xyl
- Sialic Acid
- GlcA

With growing interest in Glycobiology, these **essential sugars** and their complex carbohydrates are gaining increased recognition for their physiological importance in everyday life.

### The Eight Essential Biological Sugars

1. **Glucose**
   - (from table sugar)
   - Typically, only these two are found in our modern diets
2. **Galactose**
   - (from milk products)
3. **Mannose**
4. **Xylose**
5. **Fucose**
6. **N-Acetyl-glucosamine**
7. **N-Acetyl-galactosamine**
8. **N-Acetyl-neuraminic acid**
Next challenge: Decoding the glycome

Two different hexoses can combine in many different ways! What a vast number of different structures for recognition purposes.

> $10^{15}$ hexa-oligosaccharides with 20 different monosaccharides.

> $10^7$ (=$20^6$) hexapeptides with 20 amino acids.

~4000 (=$4^6$) hexanucleotides with 4 nucleotide subunits

By comparison “Glyco-Legos” can build more complex structures than amino acids and nucleotides combined.
Glycosylation and disease

- **Congenital disorders of glycosylation (CDG)**
  - defect in N-glycan synthesis, metabolic disease that affects the brain and many other organs.

- **Cancer**
  - glycans are used as marker for progressive tumors

- **Autoimmune disease**

- **Dystroglycanopathies**
  - $\alpha$-dystroglycan glycosylation defect => loss of receptor function
  - => loss of laminin binding

![Normal vs. Dystroglycanopathy](image-url)
Glycosylation of α-dystroglycan

6 genes known to be involved in α-dystroglycan glycosylation

**Endoplasmic reticulum**
- POMT1
- POMT2

**Golgi**
- POMGnT1
- FKRP
- Fukutin
- LARGE1

Glycosylation happens during secretion along the secretory pathway

primary dystroglycanopathy: dystroglycan (DAG1) defect, 1 patient identified

secondary dystroglycanopathy:  6 known/putative genes causing

- POMT1 (9q34.1)
- POMT2 (14q24.3)
- POMGnT1 (1p34.1)
- FKRP (19q13.32)
- Fukutin (9q31)
- LARGE (22q12.3)

Currently only 50% of dystroglycanopathy patients can be explained with known genes and can be provided with genetic diagnosis. Preliminary linkage data suggest ~ 5 additional candidate genes that still remain unidentified.

Outlook: α-Dystroglycan glycosylation .... there is still a lot to discover