Patient skin fibroblasts:
A versatile tool for identification of novel muscular dystrophy disease genes

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The Dystroglycanopathies:
A Patient and Family Conference
08-18-2012
Dystrophin Glycoprotein Complex
Loss of $\alpha$-Dystroglycan functional glycosylation results in congenital muscular dystrophy

Normal Walker-Warburg syndrome (WWS)
Muscle-eye-brain disease (MEB)
Fukuyama congenital muscular dystrophy (FCMD)
MDC1C/1D
Limb girdle muscular dystrophy (LGMD)2I/2K/2M/2N
$Large^{myd}$ mouse
A proposed mechanism for the basal-lamina-mediated prevention of membrane damage during lengthening contractions.

Han R et al. PNAS 2009;106:12573-12579
Glycosylation of α-dystroglycan

6 genes know to be involved in α-dystroglycan glycosylation

**Endoplasmic reticulum**
- POMT1
- POMT2

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

Glycosylation happens during secretion along the secretory pathway

The assembly line - a simple model for α-dystroglycan glycosylation

Each glycosyltransferase / worker has a very specific skill set to perform only one specific task.
Dystroglycanopathy candidate genes

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)
- **LARGE1** (22q12.3)

Currently only 50% of dystroglycanopathy patients can be genetically diagnosed
Patient Analysis

Clinical diagnosis

Blood

Skin biopsy

Muscle biopsy

DNA

Fibroblast

Fibroblast/Myoblast

Histology

Immunofluorescence

Lymphocyte

DNA/RNA

Sequencing

Sequencing/PCR

Protein

Western Blot

DNA/RNA

Sequencing/PCR

Protein

Western Blot
α-DG glycosylation defect in dystroglycanopathy patient skin fibroblasts

Complementation assay

**Adenovirus**

Candidate genes

**Western Blot**

- WWS
- Complementation

Return of Glycosylation?
Ability of LARGE to hyperglycosylate α-dystroglycan correlates with the severity of the clinical phenotype

LARGE can bypass $\alpha$-DG glycosylation defects in CMD patients

The "LARGE" effect

$\alpha$-Dystroglycan
Inamori et al., Science (2012)
Bypass of α-DG glycosylation defects by LARGE correlates with residual activity of impaired CMD gene

Genetic and Phenotypic distribution of cells analyzed by On-Cell Western Blot complementation (n=63)

<table>
<thead>
<tr>
<th>Genetic defect</th>
<th>WWS</th>
<th>MEB/FCMD</th>
<th>CMD</th>
<th>total</th>
<th>total %</th>
</tr>
</thead>
<tbody>
<tr>
<td>POMT1</td>
<td>8</td>
<td></td>
<td>5</td>
<td>13</td>
<td>21%</td>
</tr>
<tr>
<td>POMT2</td>
<td>1</td>
<td>6</td>
<td>5</td>
<td>12</td>
<td>19%</td>
</tr>
<tr>
<td>POMGnT1</td>
<td>1</td>
<td>9</td>
<td>3</td>
<td>13</td>
<td>21%</td>
</tr>
<tr>
<td>FKTN</td>
<td>1</td>
<td>4</td>
<td>3</td>
<td>8</td>
<td>13%</td>
</tr>
<tr>
<td>FKRP</td>
<td>1</td>
<td></td>
<td>4</td>
<td>5</td>
<td>8%</td>
</tr>
<tr>
<td>LARGE</td>
<td></td>
<td></td>
<td>1</td>
<td>1</td>
<td>1%</td>
</tr>
<tr>
<td>n.d.</td>
<td>11</td>
<td></td>
<td></td>
<td>11</td>
<td>17%</td>
</tr>
<tr>
<td>total</td>
<td>23</td>
<td>19</td>
<td>21</td>
<td>63</td>
<td>100</td>
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<tr>
<td>total %</td>
<td>37%</td>
<td>30%</td>
<td>33%</td>
<td>100</td>
<td></td>
</tr>
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</table>

Patient fibroblast complementation assay: unknowns

Yeast mating complementation cell fusion

unknown mutation

n.d.WWS 1
n.d.WWS 2
n.d.WWS 3
n.d.WWS 4
n.d.WWS 5
n.d.WWS 6

○ = lI1H6 positive / complementation
Hypothesis:
Fusion between co-cultured cells harboring recessive mutations with independent genetic defects would result in successful rescue.
Cell fusion of independent patient fibroblasts restores α-DG glycosylation defect

PEG induced cell fusion assay

<table>
<thead>
<tr>
<th>Condition</th>
<th>glyco α-DG</th>
<th>glyco α-DG + DAPI</th>
</tr>
</thead>
<tbody>
<tr>
<td>POMT1-WWS only</td>
<td></td>
<td></td>
</tr>
<tr>
<td>FKTN-WWS only</td>
<td></td>
<td></td>
</tr>
<tr>
<td>POMT1-WWS + FKTN-WWS</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Cell fusion of independent patient fibroblasts restores α-DG glycosylation defect

Linkage analysis of inbred samples in WWS #1 group

Inbred samples in WWS #1 complementation group have overlapping linkage at Chr. 7p21 and share mutations in *ISPД*

*ISPД*: Isoprenoid synthase domain containing

Validation of pathogenic \textit{ISPD} mutations with fibroblast complementation

Willer et al., \textit{Nature Genetics} 44, 575–580 (2012)
ISPD-WWS patient P1: clinical presentation

Brain MRI

Muscle biopsy

Knockdown of zebrafish *ispd* recapitulates pathological defects of human WWS

Where does ISPD fit into the α-dystroglycan glycosylation assembly line?

- What is the function of ISPD?
- How do ISPD defects affect α-DG glycosylation?
- What step in the sugar synthesis is affected by ISPD defects?
How do *ISPD* defects affect $\alpha$-DG glycosylation? What step in the sugar synthesis is affected by ISPD defects?

In vitro assay available
**ISPD mutations impair protein O-mannosylation**

*Willer et al., Nature Genetics 44, 575–580 (2012)*
ISPD mutations impair protein O-mannosylation
Summary

• Patient fibroblasts can be used to study $\alpha$-dystroglycan glycosylation and complementation assay can be used to diagnose/validate genetic defect

• Identification of 5 novel WWS complementation groups representing 5 new WWS candidate genes

• Identification of ISPD gene defects as common cause in muscular dystrophies associated with $\alpha$-dystroglycan glycosylation defect

• ISPD mutations lead to impaired $\alpha$-dystroglycan O-mannosylation, establishing a new pathway and mechanism for disease in WWS.
New dystroglycanopathy gene discovery:

Identify genetic defect in the remaining unidentified 4 WWS complementation groups

Screen for therapeutic compounds:

test in cell culture and mouse model systems
Acknowledgement

Kevin Campbell
Steve Moore
Kathy Mathews
Takako Yoshida-Moriguchi
Daniel Beltran
David Venzke
Andrew Crimmins
Greg Morgensen
Jamie Eskuri
Alex Dietz
Dan Michele

Campbell lab
University of Iowa

Harry Schachter
Jiri Vajsar
University of Toronto, Canada

Hans v. Bokhoven
Radboud University Nijmegen, Netherland

Francesco Muntoni / Sebahattin Cirak
Imperial College London, UK

Thomas Voit
Institute of Myology, Paris, France

Andrea Loder
White-Wilson Medical Center, Ft. Walton Beach, Florida

Tom Winder
PreventionGenetics, Marshfield, WI, USA

Pascale Guicheney / Nigel Clarke
INSERM, Paris, France

Hane Lee / Stanley Nelson
University of California, Los Angeles, CA, USA

Mark Lommel / Sabine Strahl
University of Heidelberg, Germany

Adeno vector generation
The University of Iowa Center for Gene Therapy (NIDDK P30 DK54759

Funding:
- Paul D. Wellstone Muscular Dystrophy Cooperative Research Center Grant (1U54NS053672)
- ARRA Go Grant (1 RC2 NS069521-01)