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

Tobias Willer

The Dystroglycanopathies:
A Patient and Family Conference
08-18-2012

LGMD2i Fund
Limb Girdle Muscular Dystrophy 2I Research Fund

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UNIVERSITY of IOWA CARVER COLLEGE OF MEDICINE

Project 2:
Clinical Studies

Project 1:
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Iowa

Wellstone Muscular Dystrophy Research Center

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Muscle Disease Neuropathology Conference

Core A:
Administration

Core B:
Muscle Tissue and Cell Culture Repository

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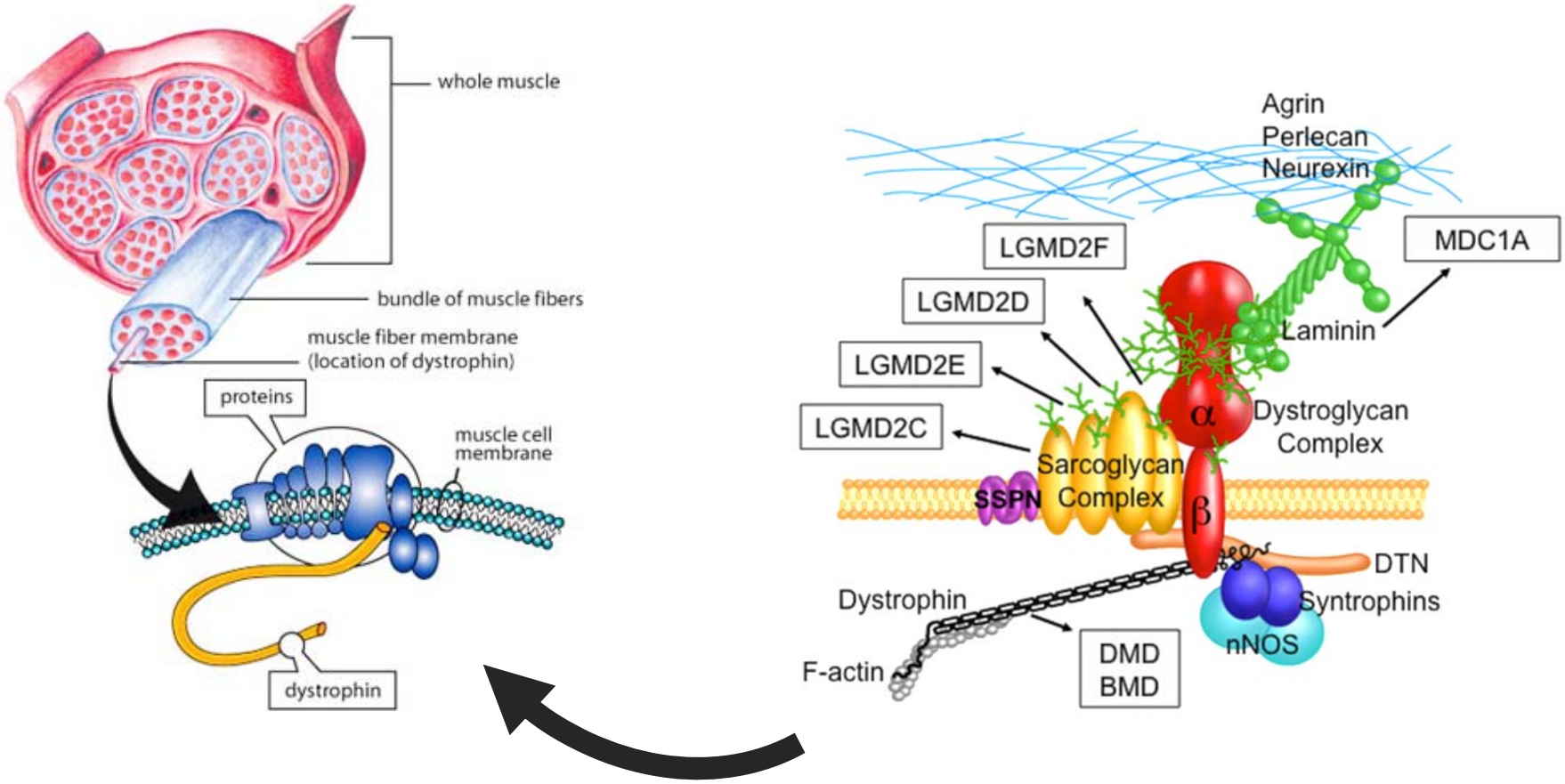
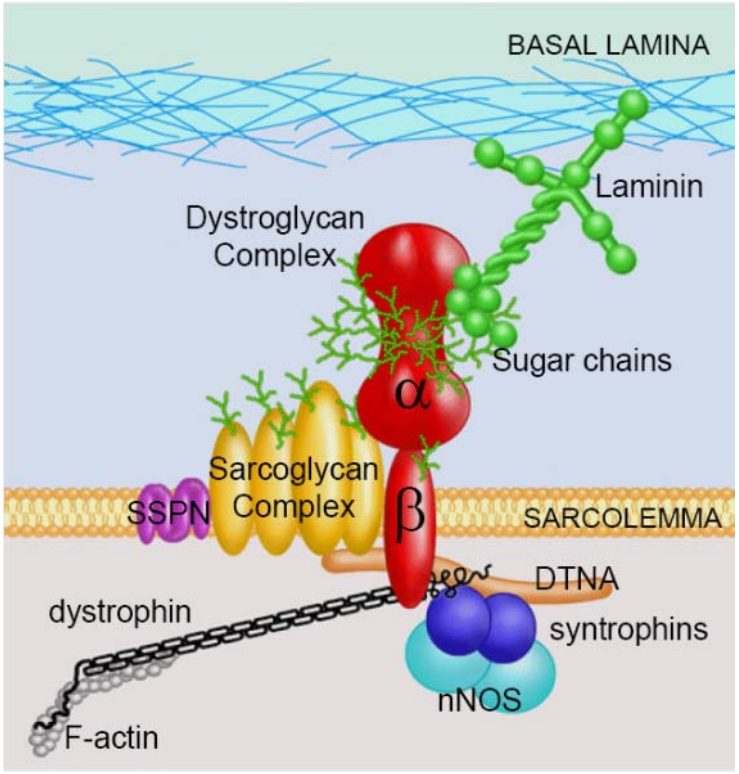
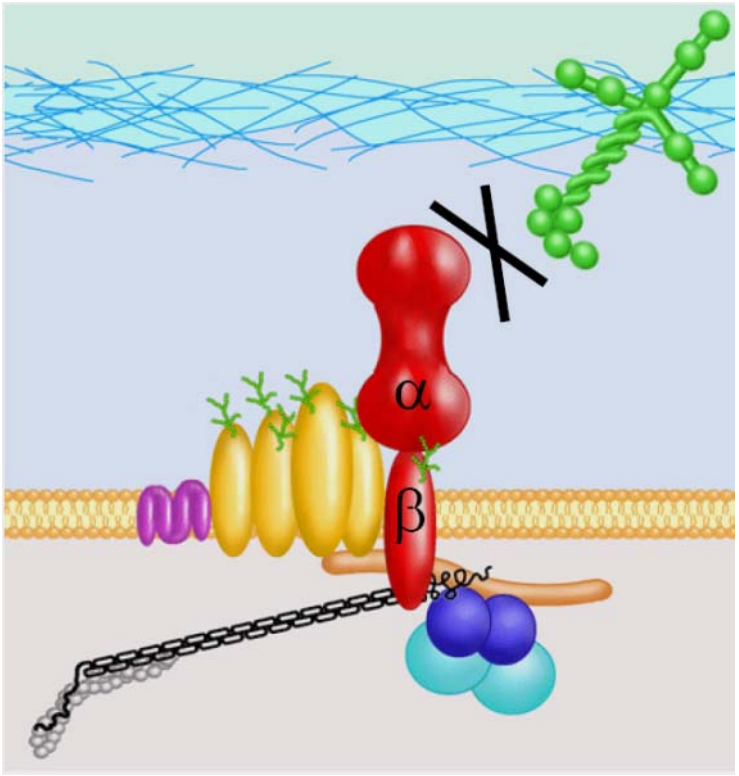
Dystrophin Glycoprotein Complex



Loss of α-Dystroglycan functional glycosylation results in congenital muscular dystrophy



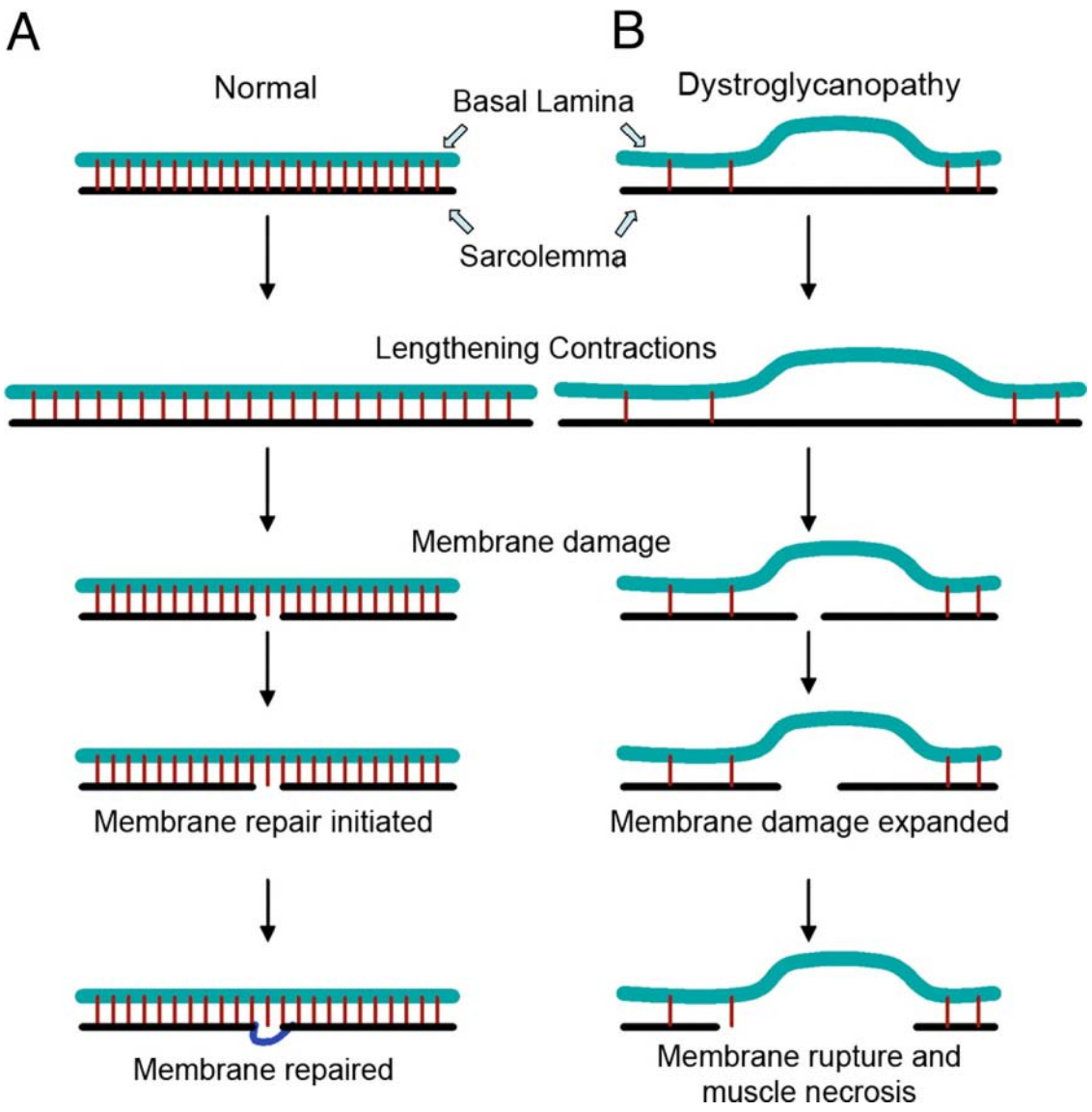
Normal



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

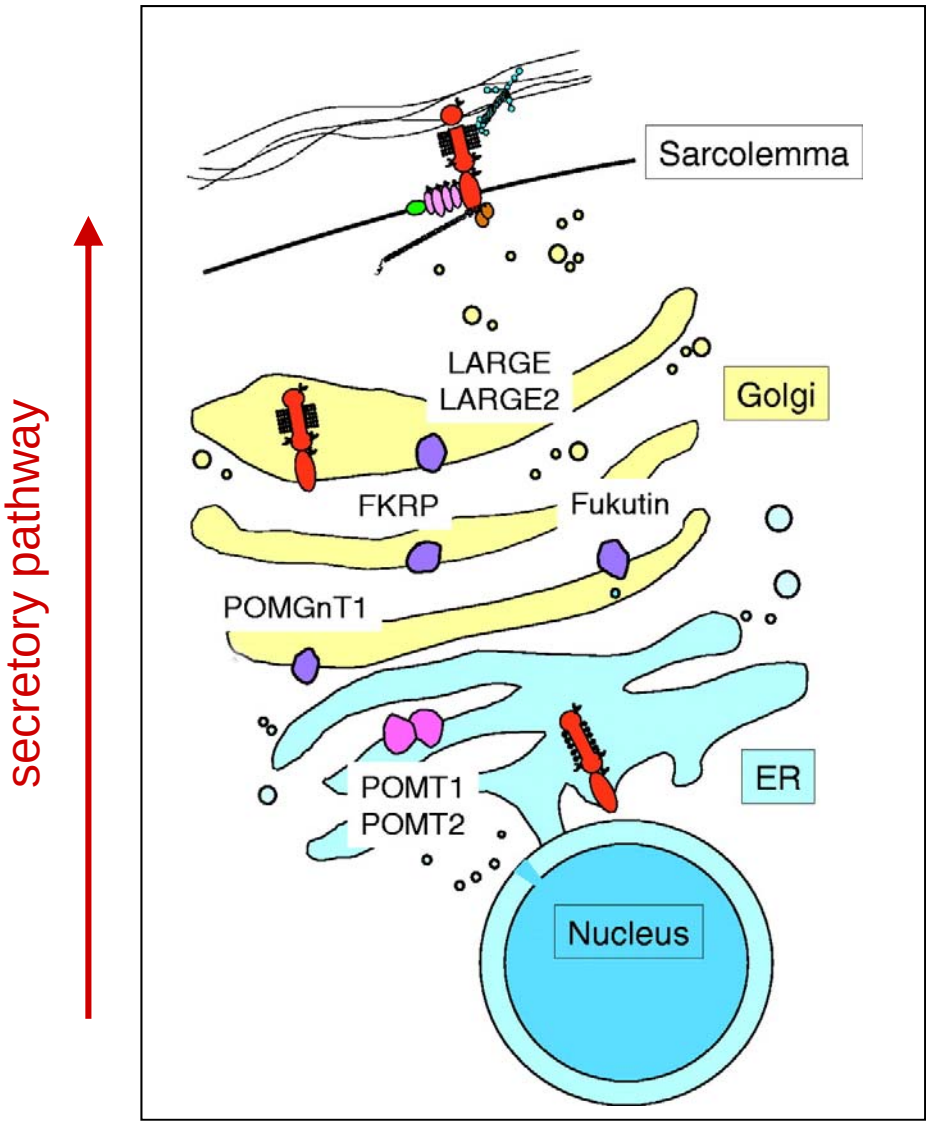
Large^{myd} mouse

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



Barresi, R. et al. J Cell Sci 2006;119:199-207

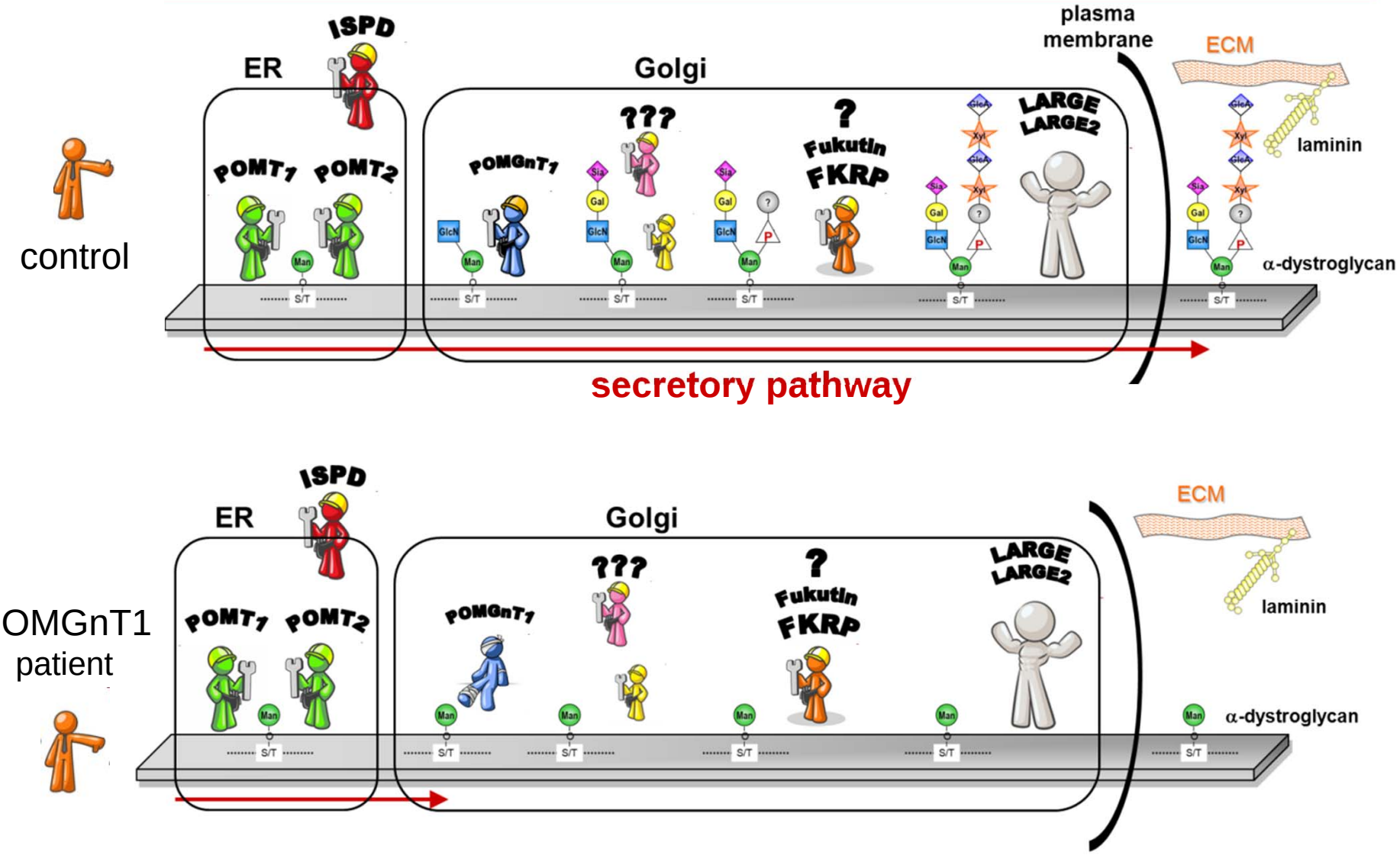
6 genes know to be involved in α-dystroglycan glycosylation

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



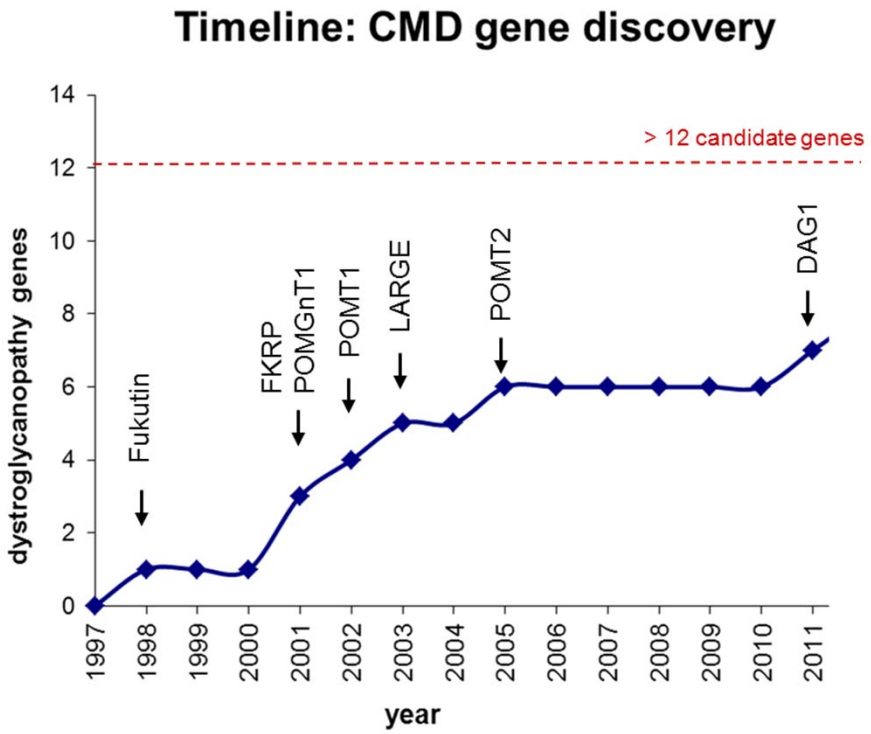
each glycosyltransferase / worker has a very specific skill set to perform only one specific task

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

Lymphocyte

Histology
Immunofluorescence

DNA

Fibroblast

Fibroblast/Myoblast

DNA/RNA

Protein

DNA/RNA

Protein

Sequencing

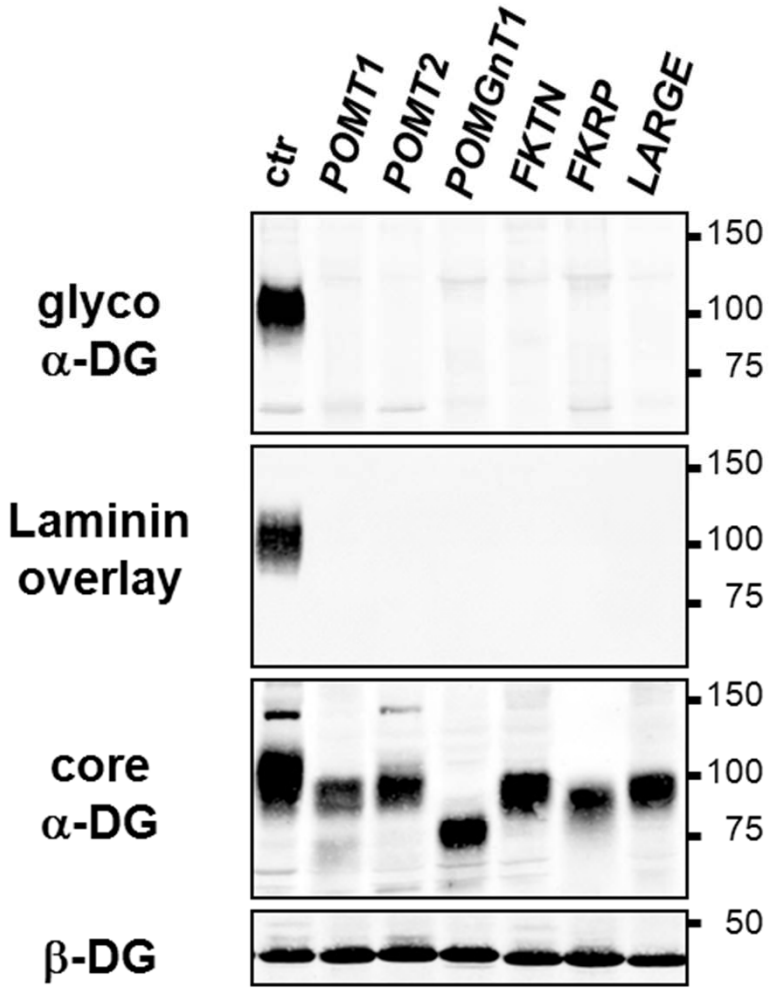
Sequencing/PCR

Western Blot

Sequencing/PCR

Western Blot

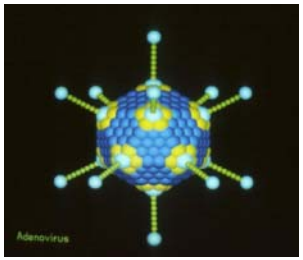
α-DG glycosylation defect in dystroglycanopathy patient skin fibroblasts



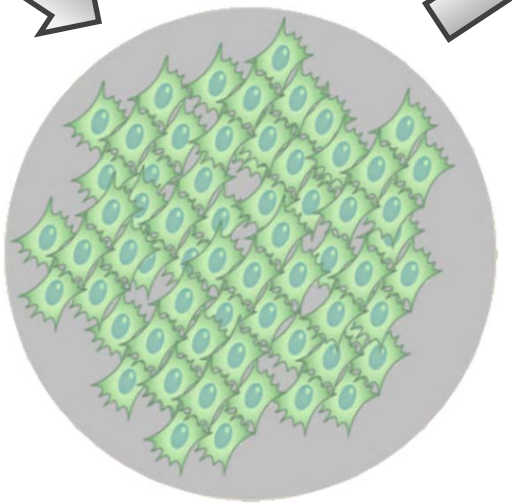
Willer *et al., Nature Genetics* **44**, 575–580 (2012)

Complementation assay

Adenovirus

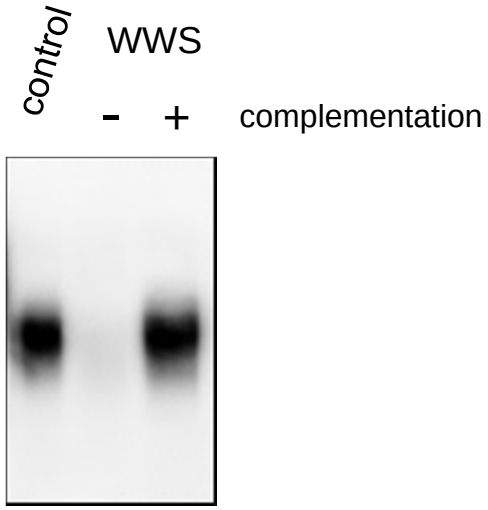


candidate genes



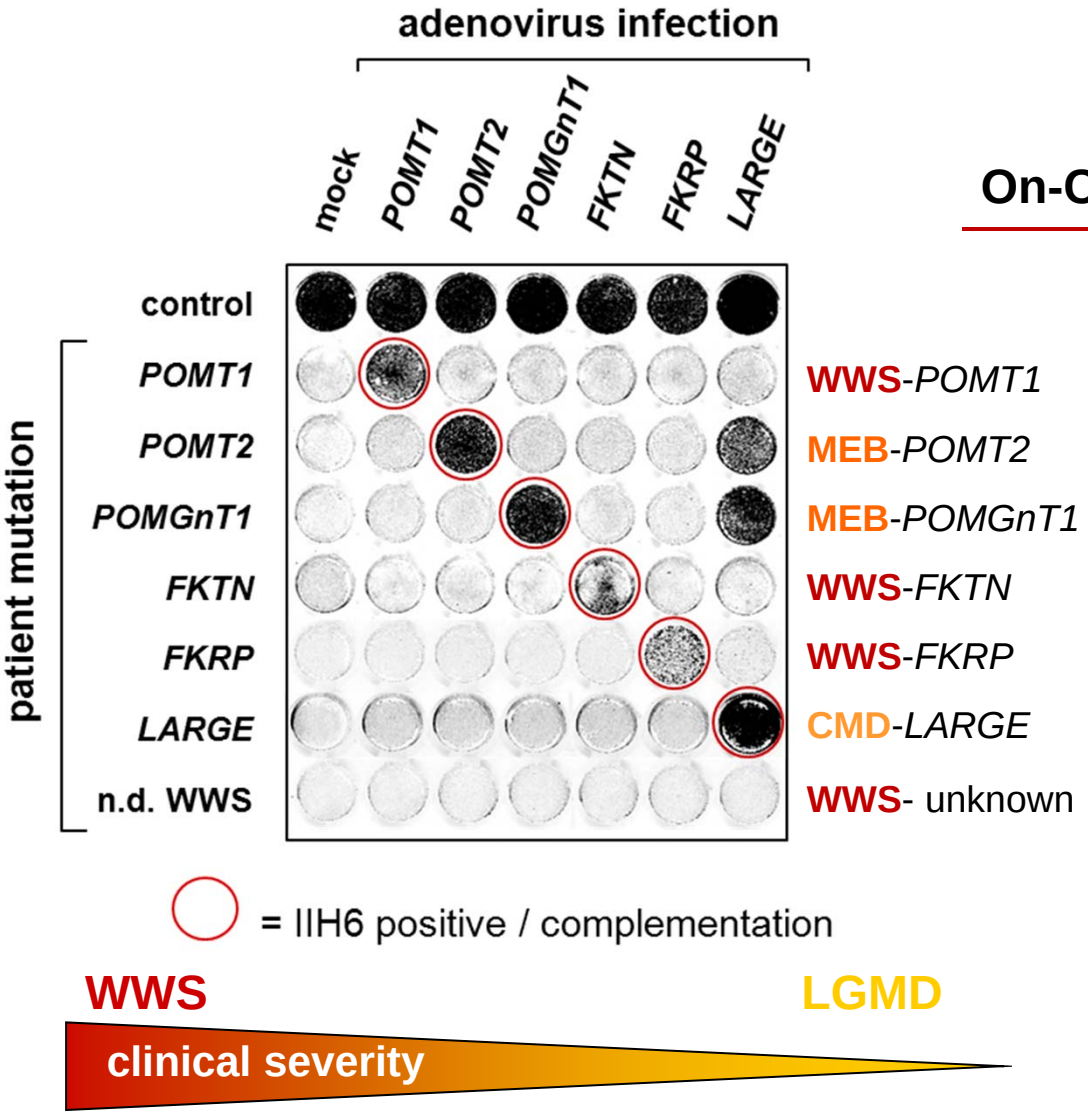
Patient Fibroblasts

Western Blot



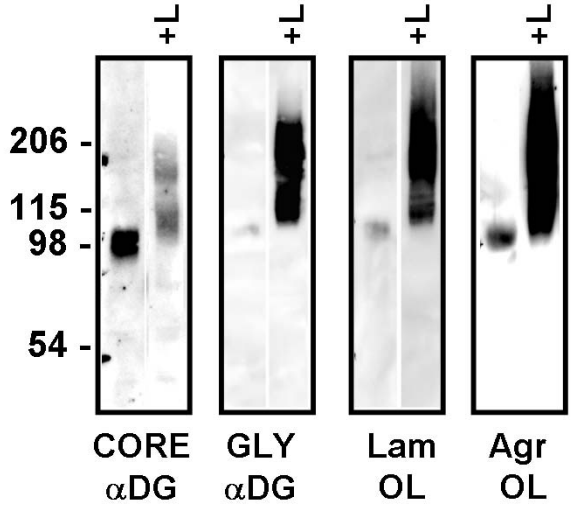
Return of Glycosylation ?

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



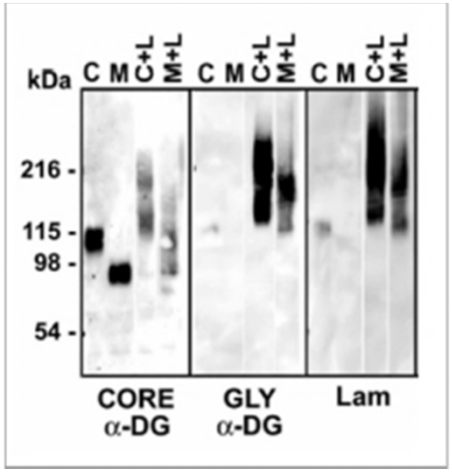
Willer *et al., Nature Genetics* **44**, 575–580 (2012)

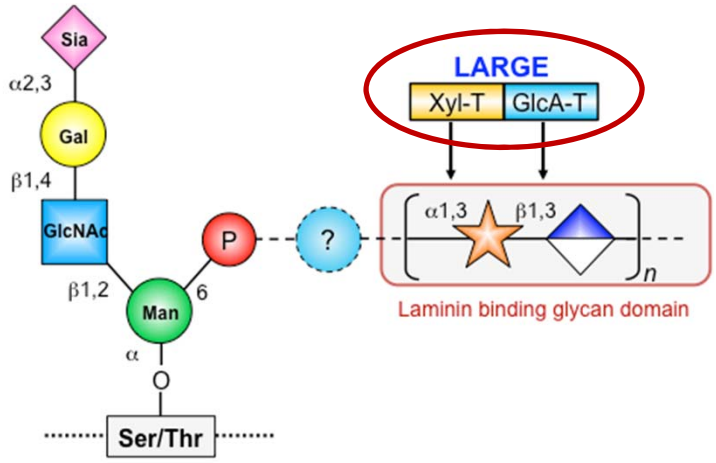
LARGE can bypass α-DG glycosylation defects in CMD patients



The "LARGE" effect

Barresi *et al*., NatMed (2004)

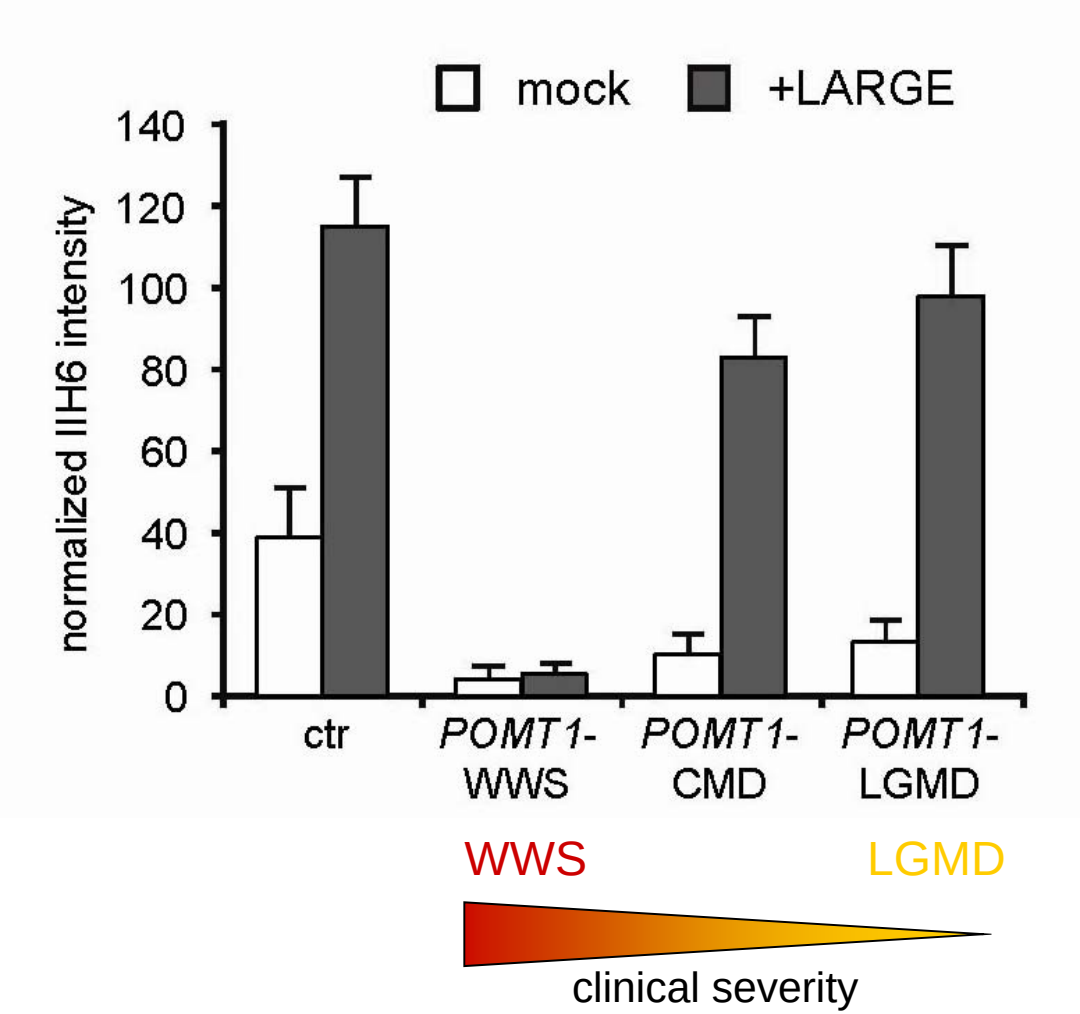


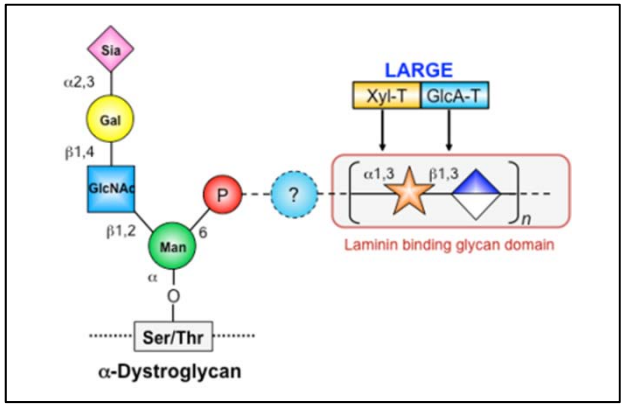


α-Dystroglycan

Inamori *et al*., Science (2012)

Bypass of α-DG glycosylation defects by LARGE correlates with residual activity of impaired CMD gene





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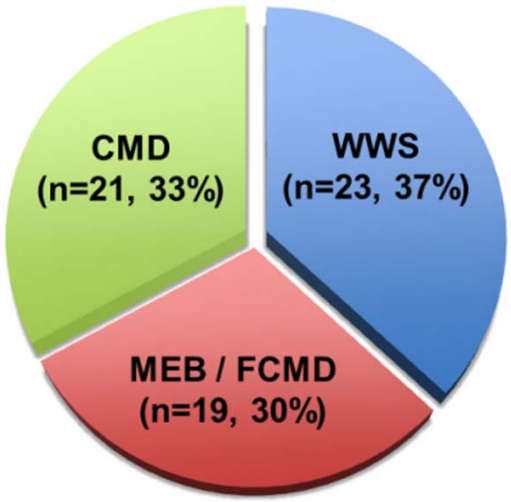
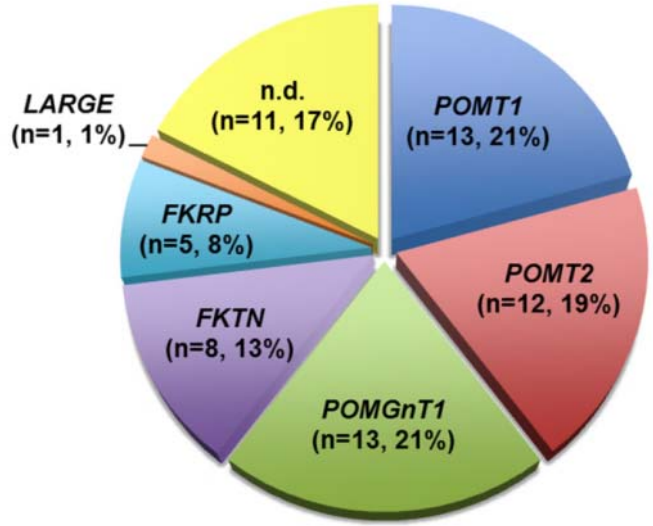
Genetic and Phenotypic distribution of cells analyzed by On-Cell Western Blot complementation (n=63)

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





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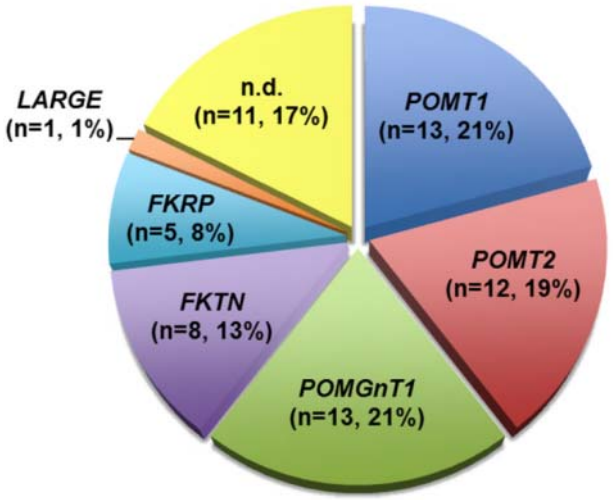
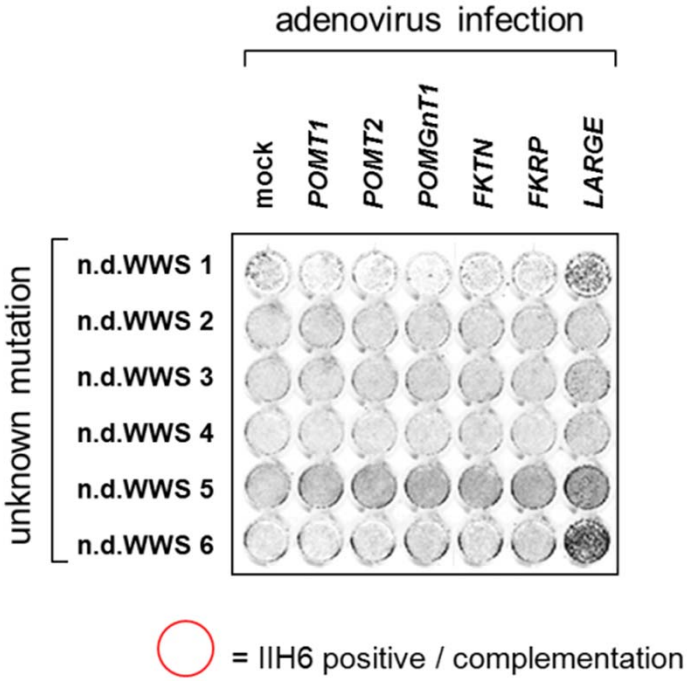
Patient fibroblast complementation asssay: unknowns

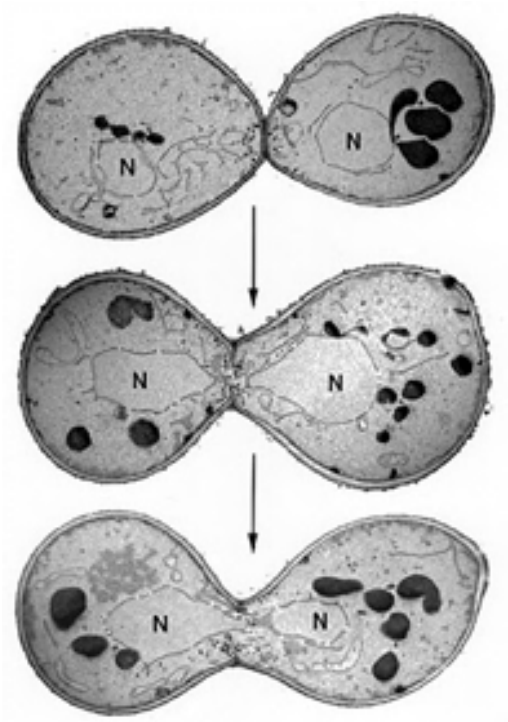




Yeast mating complementation

cell fusion

Yeast cell mating



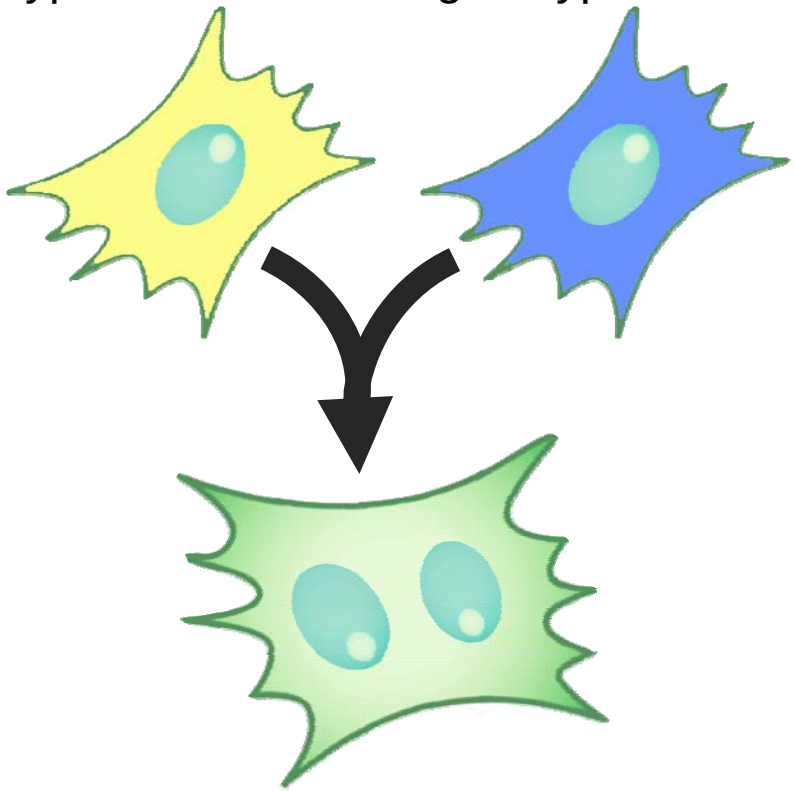
Hypothesis:

Fusion between co-cultured cells harboring recessive mutations with independent genetic defects would result in successful rescue.

PEG induced cell fusion of mammalian cells

IIH6 negative
genotype: aaBB

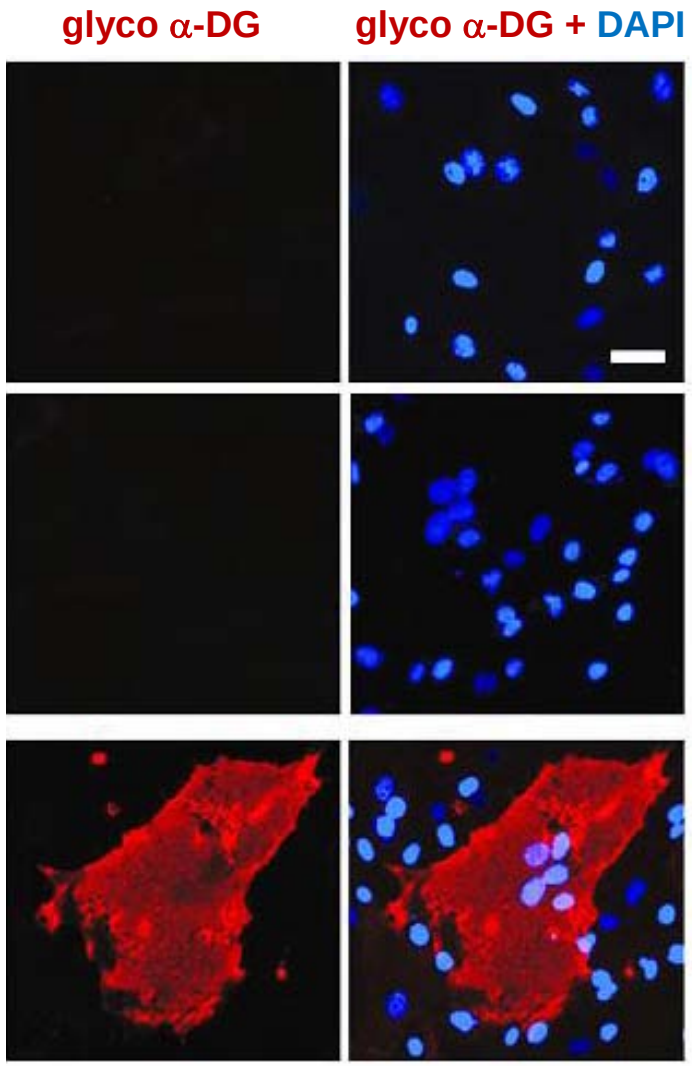
IIH6 negative
genotype: AAbb



IIH6 positive
genotype: aaAABBbb

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

PEG induced cell fusion assay



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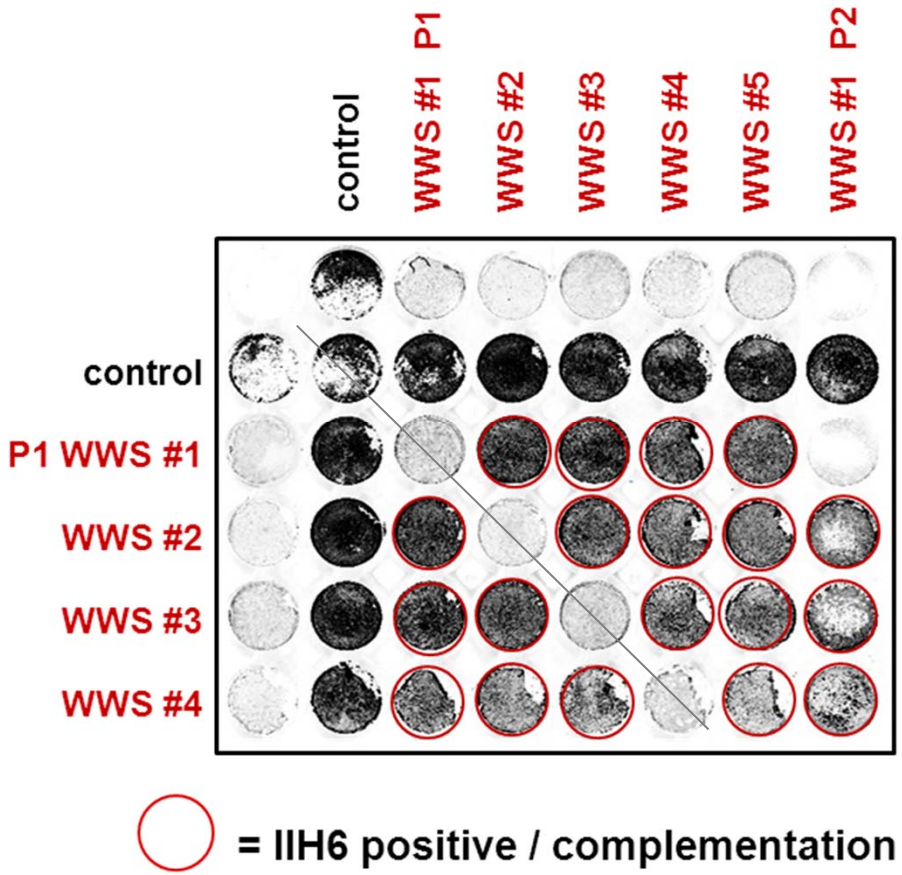
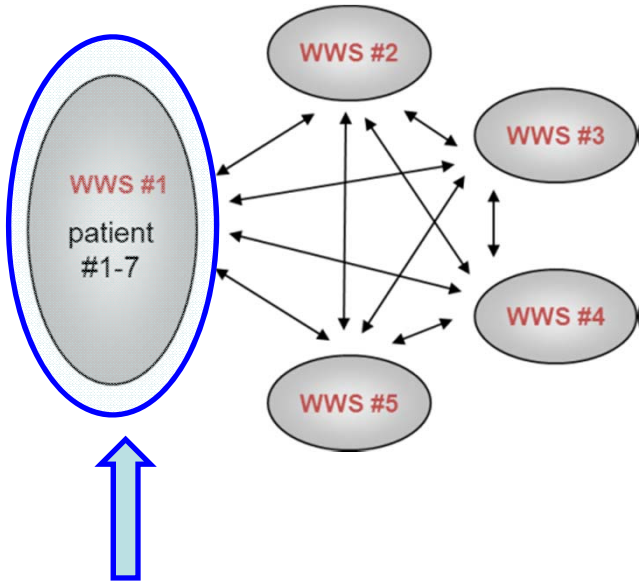
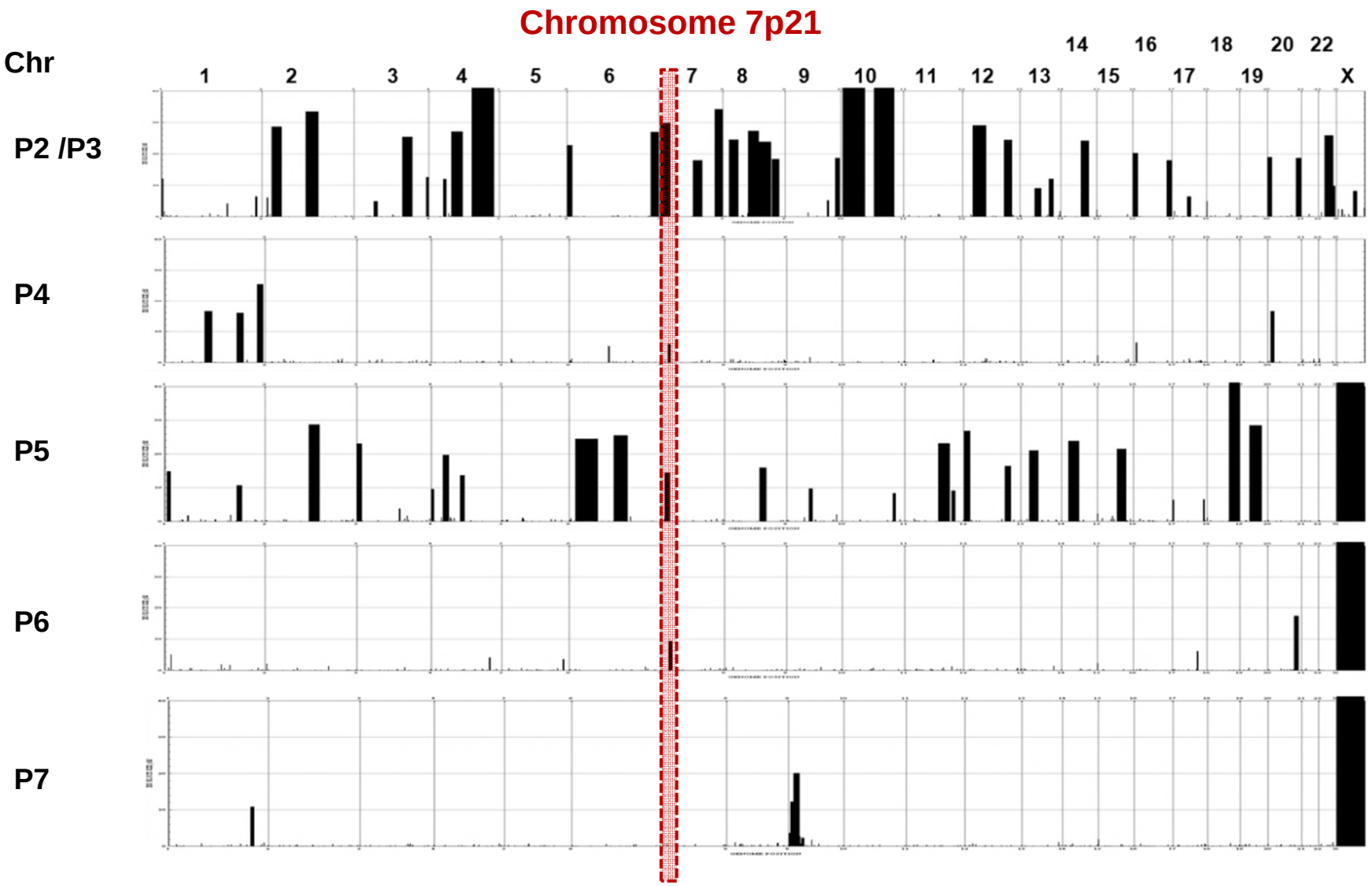




Target group for new gene discovery

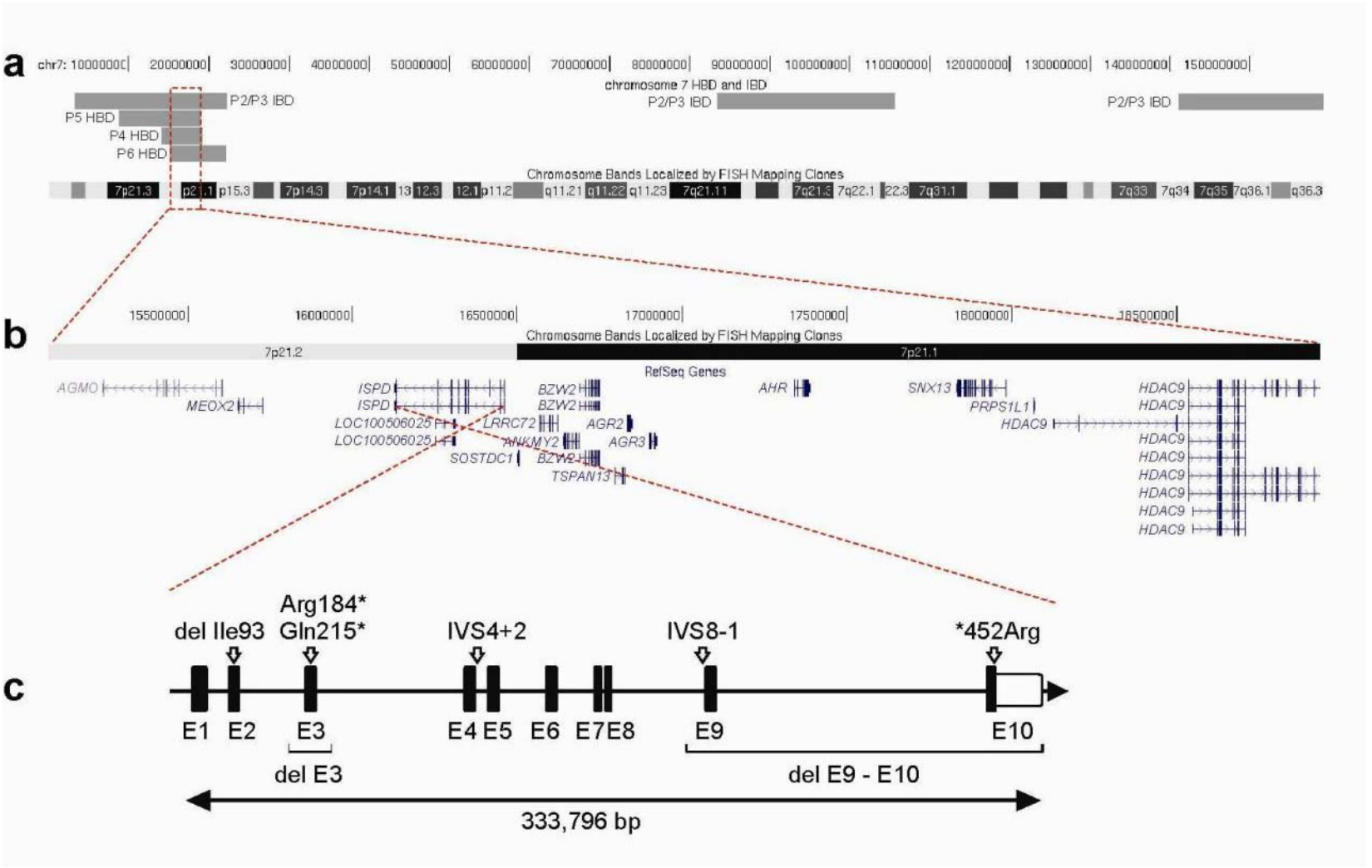
Willer *et al., Nature Genetics* **44**, 575–580 (2012)

Linkage analysis of inbred samples in WWS #1 group



Willer *et al.*, *Nature Genetics* **44**, 575–580 (2012)

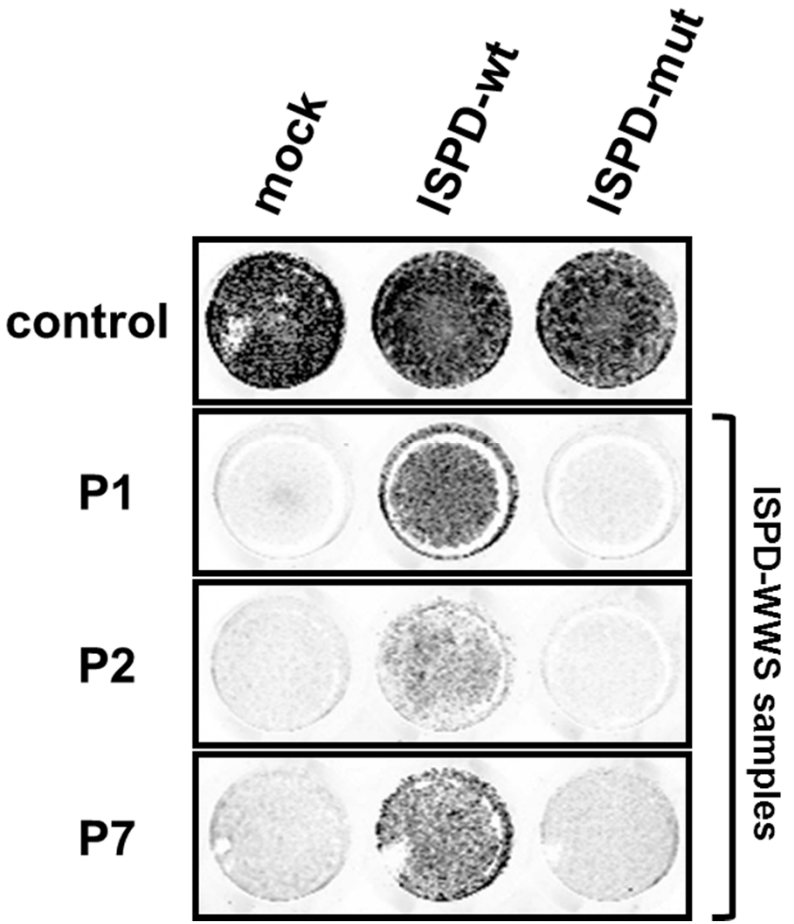
Inbred samples in WWS #1 complementation group have overlapping linkage at Chr. 7p21 and share mutations in *ISPD*



ISPD : Isoprenoid synthase domain containing

Willer *et al., Nature Genetics* **44**, 575–580 (2012)

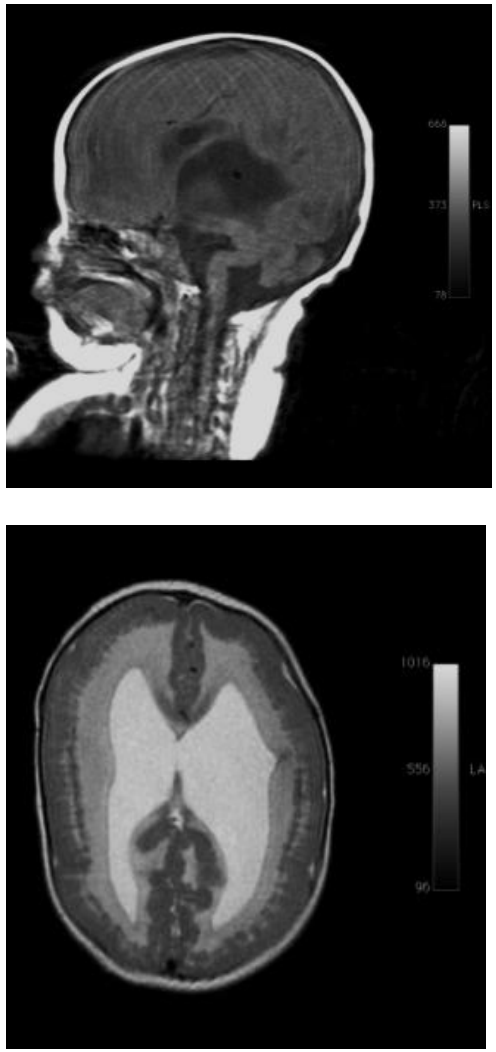
Validation of pathogenic *ISPD* mutations with fibroblast complementation



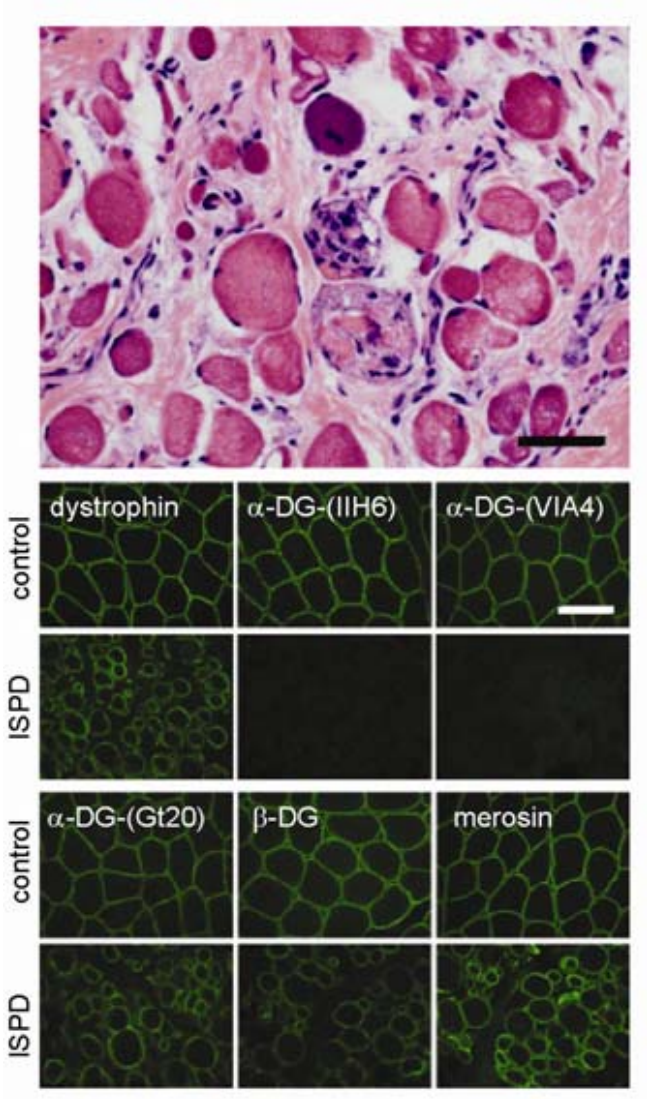
Willer *et al., Nature Genetics* **44**, 575–580 (2012)

ISPD-WWS patient P1 : clinical presentation

Brain MRI



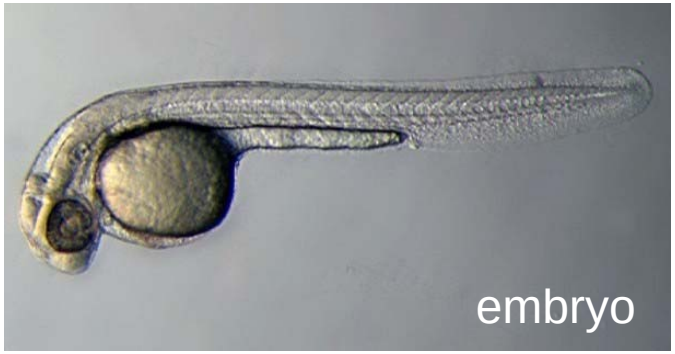
Muscle biopsy

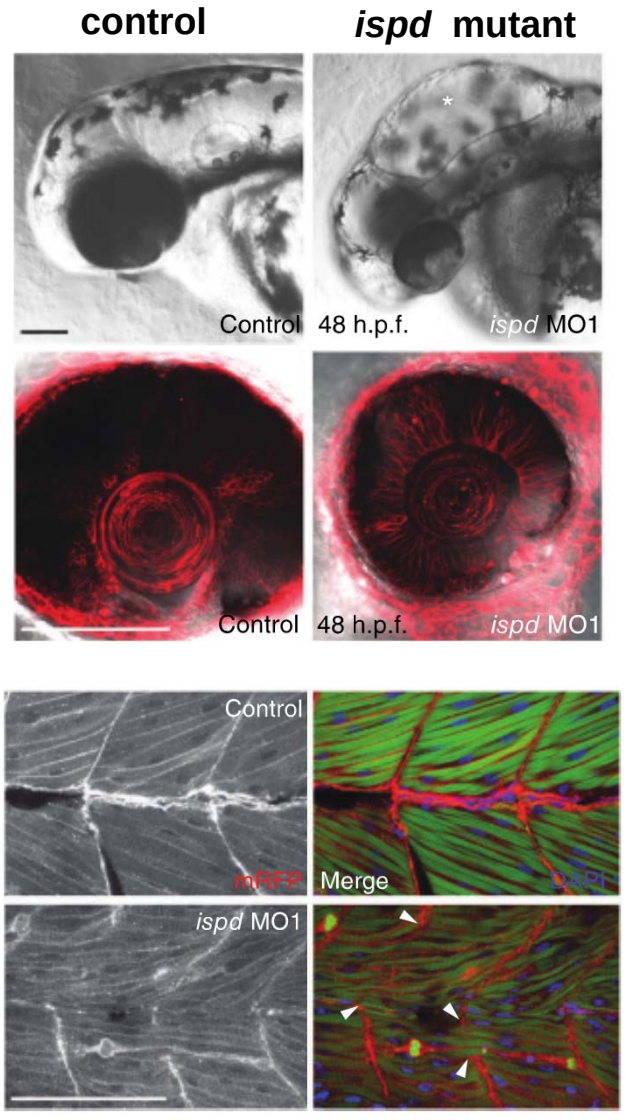


Willer *et al., Nature Genetics* **44**, 575–580 (2012)

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

Zebrafish (*Danio rerio*)

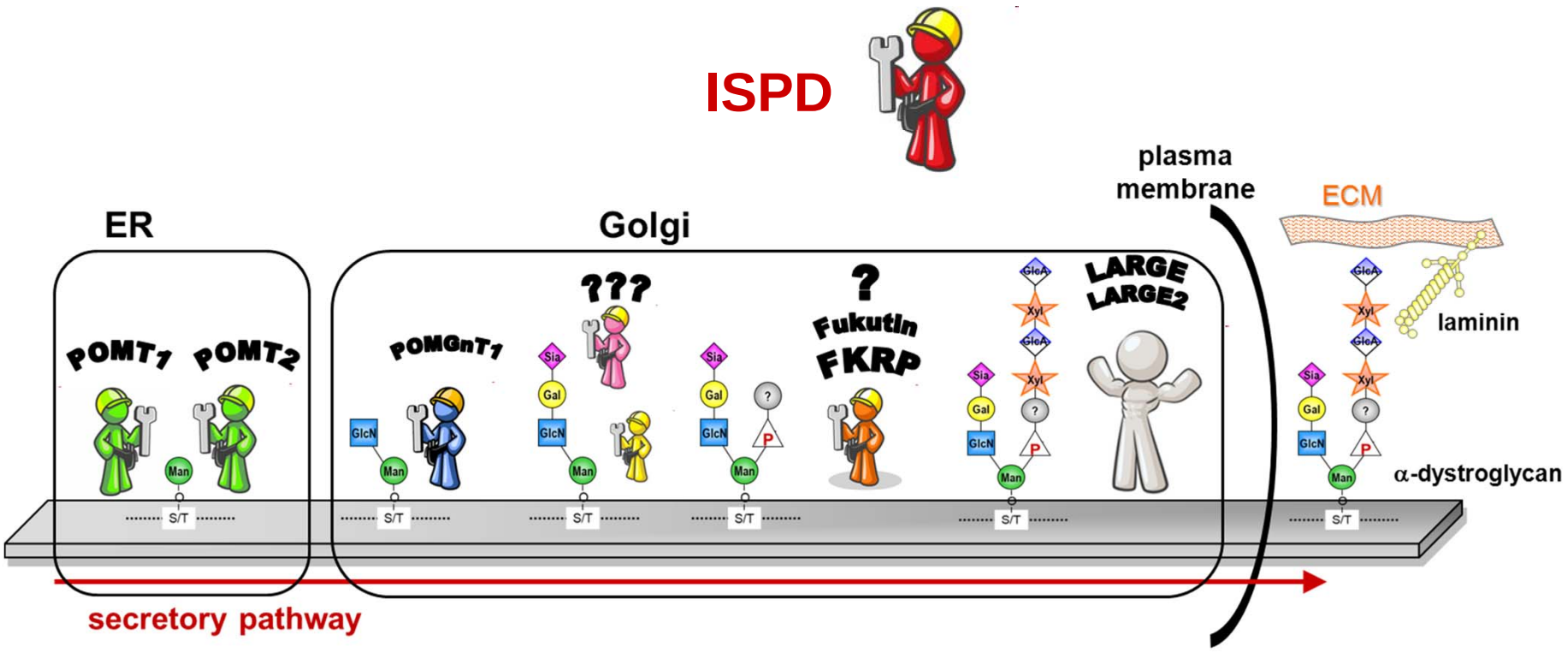




Roscioli *et al., Nature Genetics* **44**, 581–585 (2012)

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 ?



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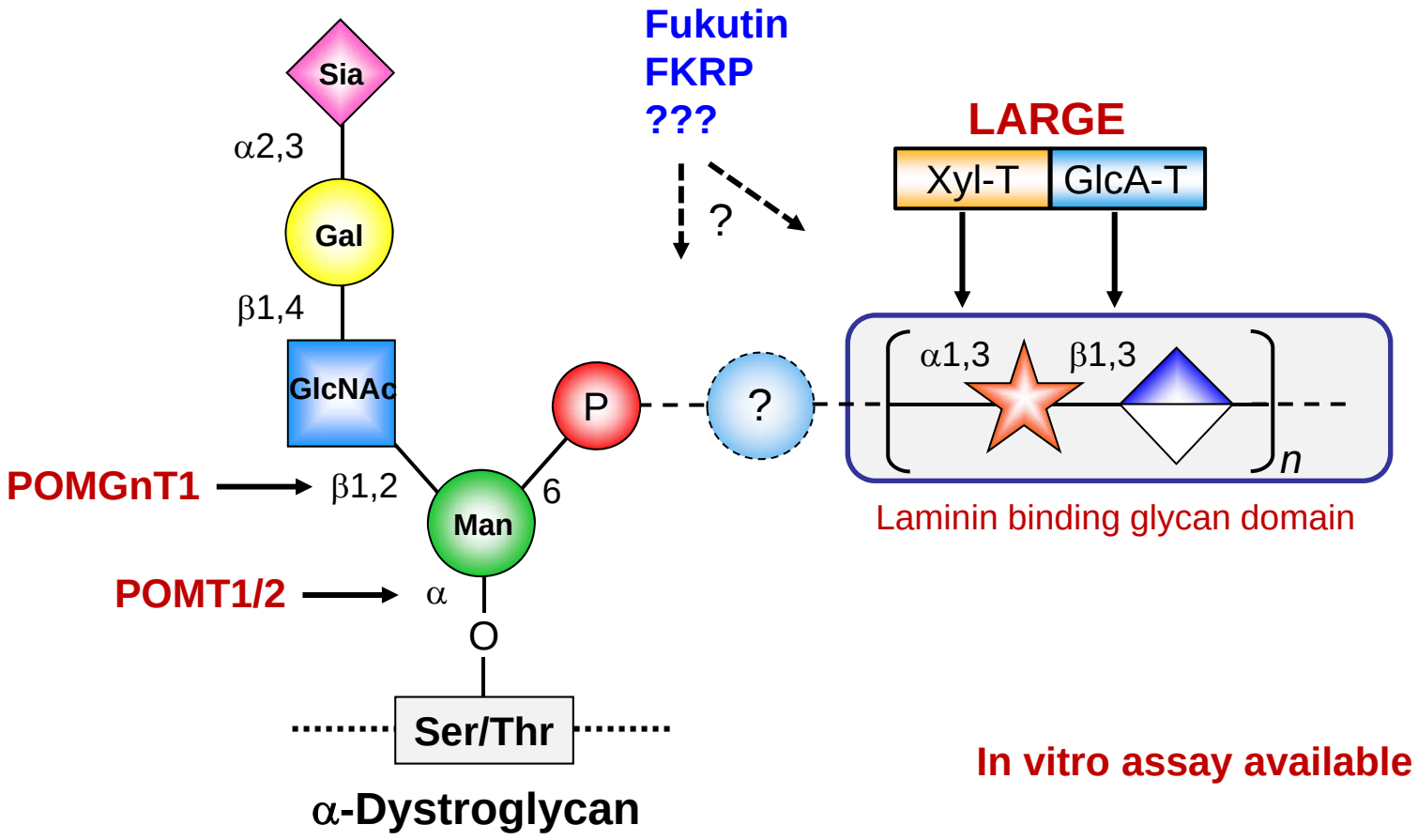
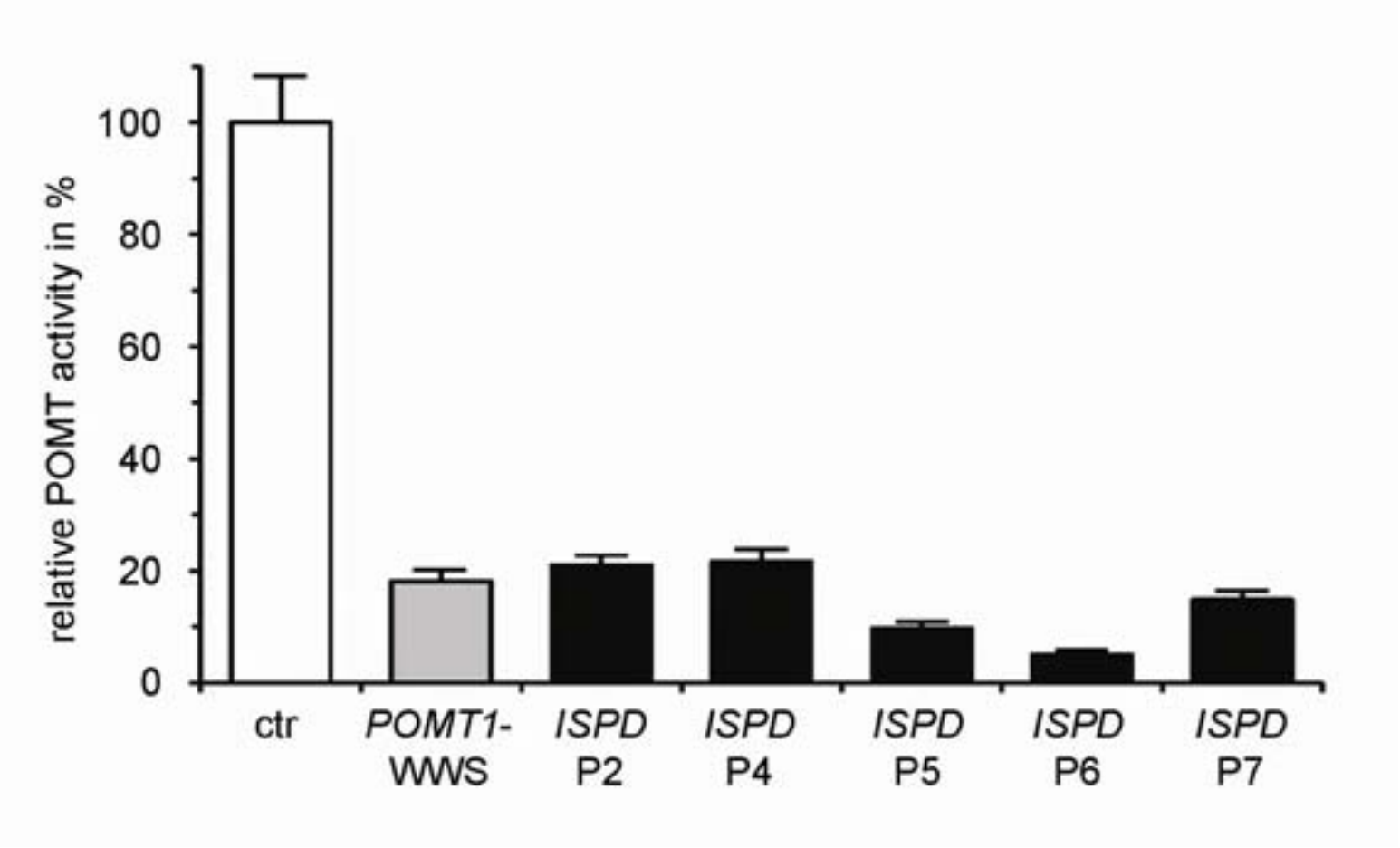


ISPD mutations impair protein *O*-mannosylation



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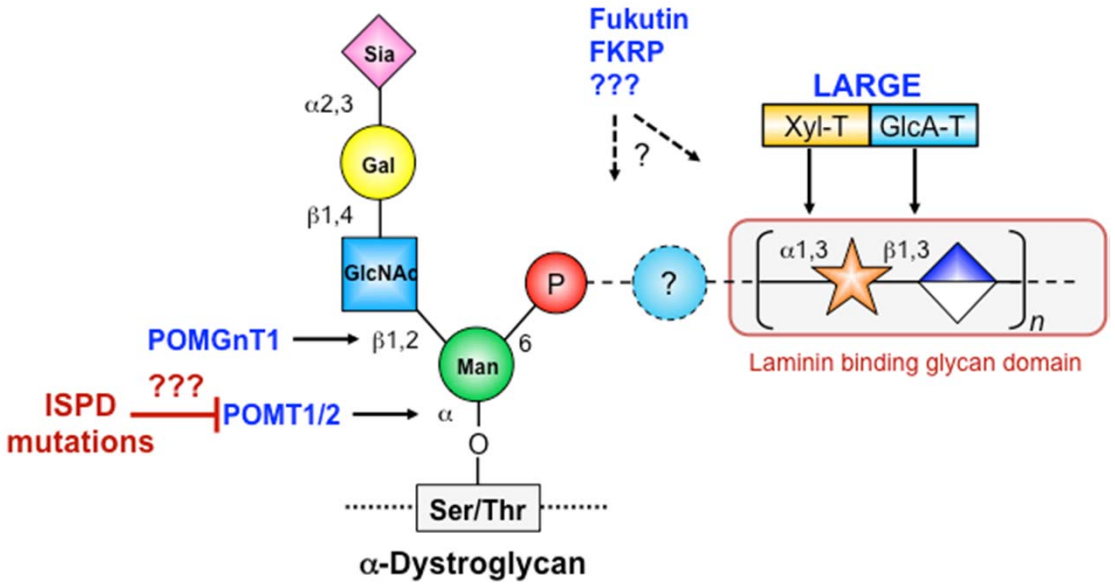
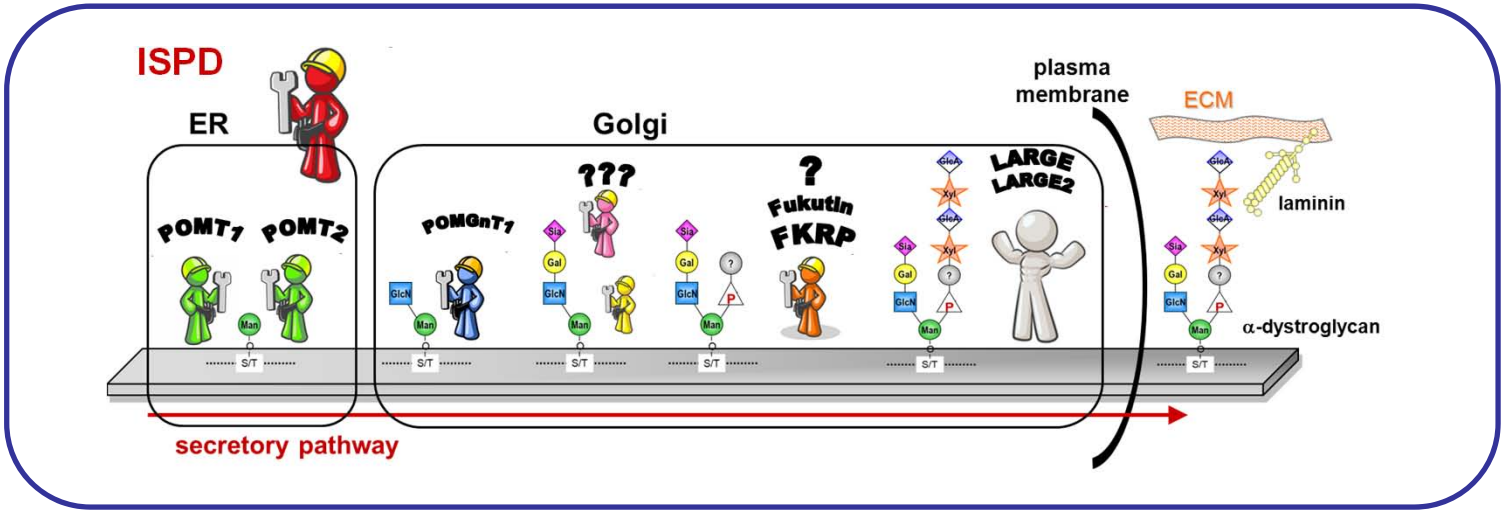




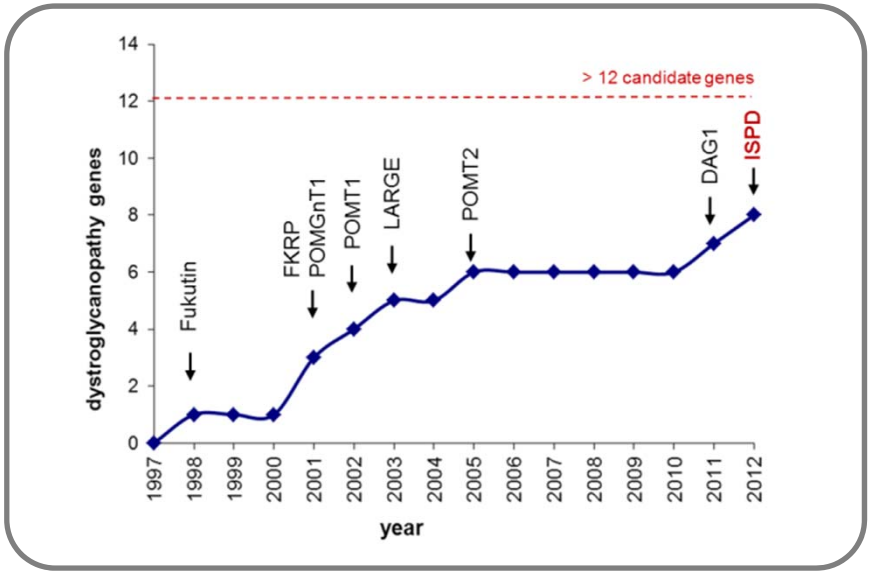
Summary

- Patient fibroblasts can be used to study α-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 α-dystroglycan glycosylation defect
- *ISPD* mutations lead to impaired α-dystroglycan *O*-mannosylation, establishing a new pathway and mechanism for disease in WWS.

Outlook

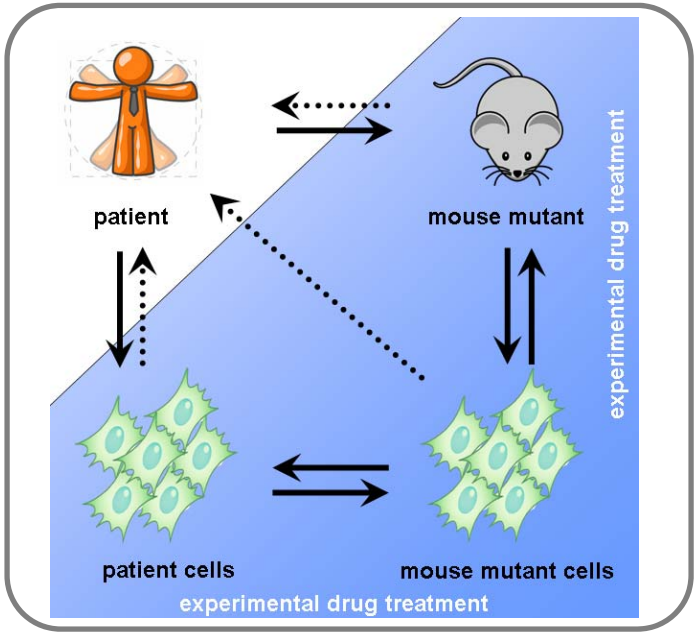
New dystroglycanopathy gene discovery:

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

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

Mark Lommel / Sabine Strahl
University of Heidelberg, Germany

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

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)