CONGENITAL DISORDERS OF GLYCOSYLATION (CDG)
BASIC SCIENCE, SELECTED DISORDERS, CLINICAL FEATURES AND THERAPIES

HUDSON FREEZE PhD, Sanford Burnham Prebys Medical Discovery Institute, La Jolla CA

RESOURCES FOR YOUR PATIENTS:

https://cdgcare.org/

Intro to CDG

https://vimeo.com/637115238

Biomarkers

https://www.youtube.com/watch?v=apFBPxfksV0

Resources for you: FCDGC

https://www.rarediseasesnetwork.org/fcdgc

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FCDGC

Disclosures

Rarely consult for

- Glycomine Therapeutics
- BridgeBio

FCDGC

RESEARCH ARTICLE

AGING

scientific and medical professionals confuse Glycosylation and Glycation

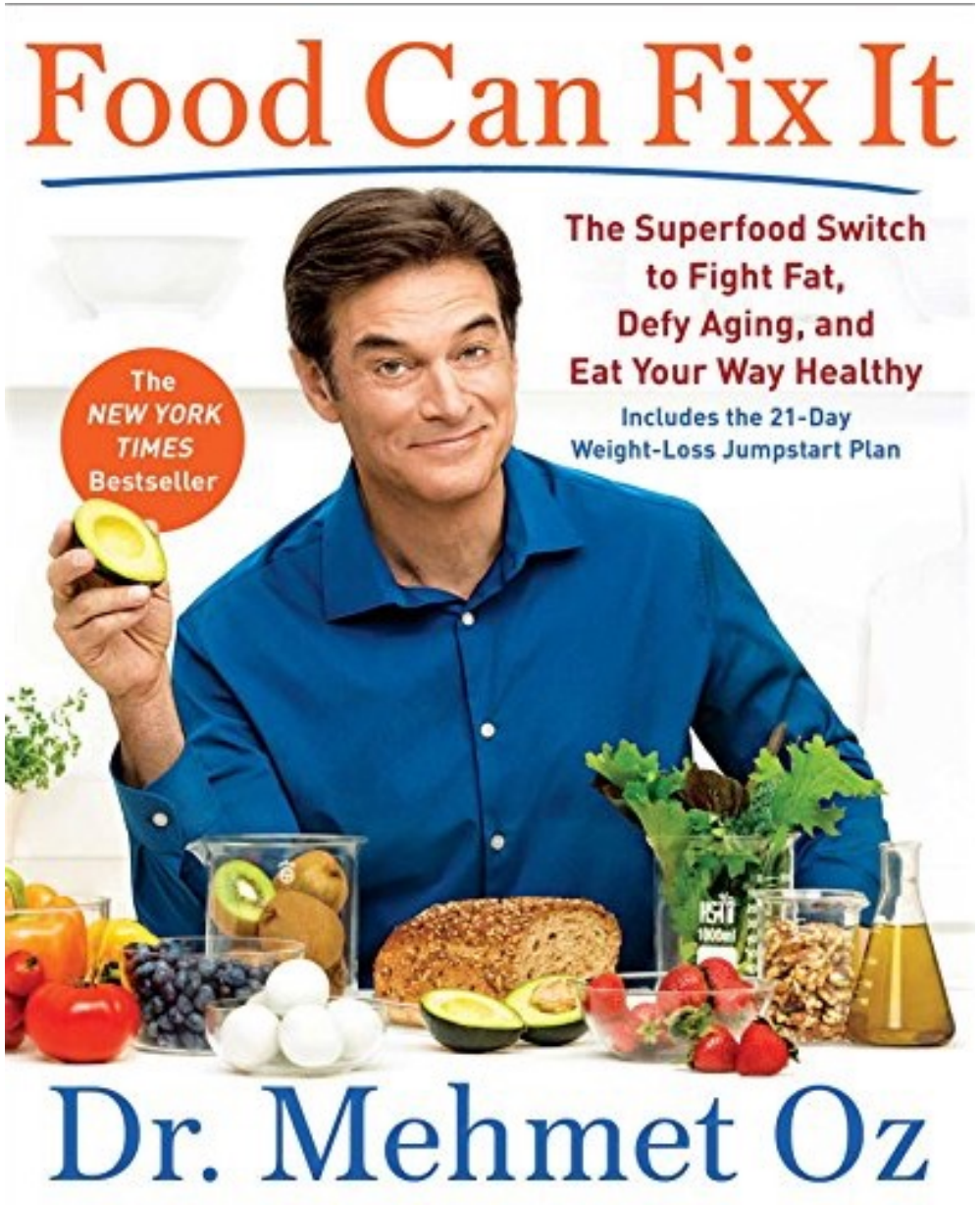
Sex- and age-dependent genetics of longevity in a heterogeneous mouse population

Maroun Bou Sleiman[1]†, Suheeta Roy[2]†, Arwen W. Gao[1], Marie C. Sadler[3,4,5], Giacomo V. G. von Alvensleben[1], Hao Li[1], Saunak Sen[6], David E. Harrison[7], James F. Nelson[8], Randy Strong[8,9], Richard A. Miller[10], Zoltán Kutalik[3,4,5], Robert W. Williams[2]*, Johan Auwerx[1]*

DNA variants that modulate life span provide insight into determinants of health, disease, and aging. Through analyses in the UM-HET3 mice of the Interventions Testing Program (ITP), we detected a sex-independent quantitative trait locus (QTL) on chromosome 12 and identified sex-specific QTLs, some of which we detected only in older mice. Similar relations between life history and longevity were uncovered in mice and humans, underscoring the importance of early access to nutrients and early growth. We identified common age- and sex-specific genetic effects on gene expression that we integrated with model organism and human data to create a hypothesis-building interactive resource of prioritized longevity and body weight genes. Finally, we validated *Hipk1*, *Ddost*, *Hspg2*, *Fgd6*, and *Pdk1* as conserved longevity genes using *Caenorhabditis elegans* life-span experiments.

Bou Sleiman *et al.*, *Science* **377**, eabo3191 (2022) 30 September 2022

worm ortholog, and found that *unc-52* (*Hspg2*) and *ostb-1* (*Ddost*) RNAi both significantly shorten life span ($p < 0.0001$; Fig. 5C and Fig. S6). Although *unc-52* is already known to affect worm life span (*45*, *46*), *ostb-1* or its ortholog (*Ddost*) has not yet been directly linked to longevity. However, Dolichyl-diphosphooligosaccharide–protein glycosyltransferase noncatalytic subunit (DDOST) is implicated in processing advanced glycation end products (AGEs) (*47*), which accumulate with age and exacerbate the aging phenotype (*48*). In the UM-HET3, the



DEFINITIONS AND TERMS:

- Glycosylation is the biosynthetic process of creating and adding sugar chains (glycans) to proteins and lipids. **NOT** glycation—that's HbA1c.
- Glycosylation occurs in every cell of every organism on Earth
- CDG are metabolic disorders that impair the normal initiation, transfer or completion of functional glycans.
- Currently >170 different types of CDG; all are rare, most are ultra rare

CDG NOMENCLATURE: Gene name-CDG

PREVIOUSLY: Type I(a,b,c etc), Type II (a,b,c etc)

BEST APPROACH TO CDG DIAGNOSIS

Exome or Genome Sequencing + Biochemical Analysis

WHEN TO TEST FOR CDG?

"Every time you suspect it…and every time you don't", J. Jaeken

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PI, Eva Morava MD PhD

Frontiers of CDG Consortium Sites

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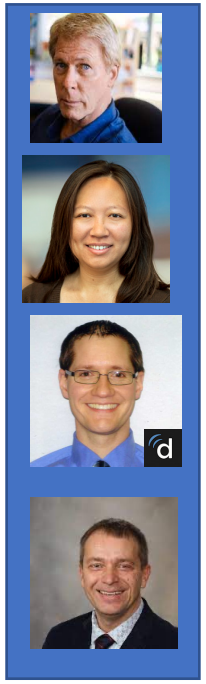
Biomarker discovery/improve diagnostics
Biobank

Natural history studies
Patient reported outcomes

Clinical trials

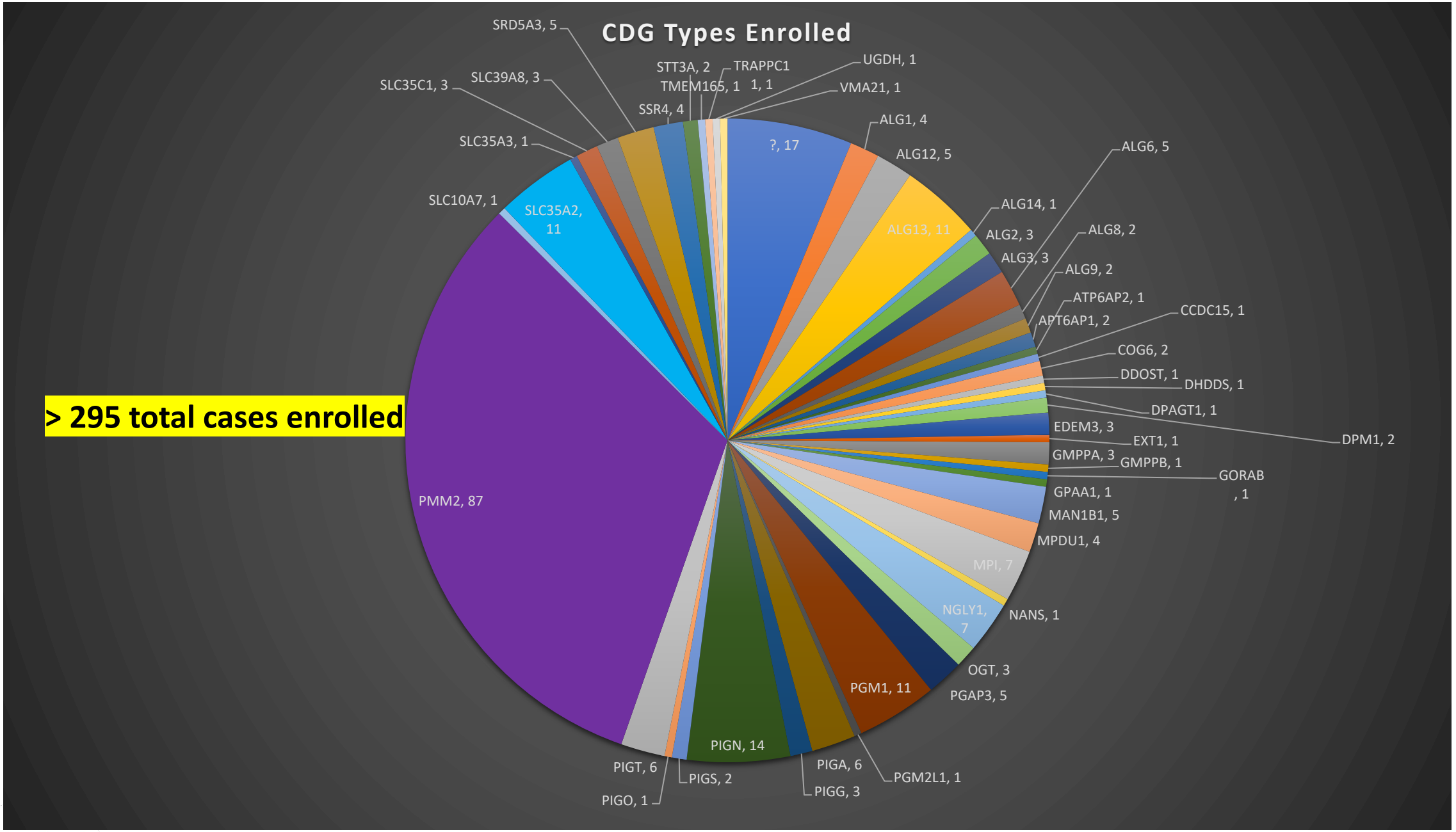
Education/knowledge sharing

RFAs



U54NS115198 from the National Institute of Neurological Diseases and Stroke (NINDS) and the National Center for Advancing Translational Sciences (NCATS) https://www.rarediseasesnetwork.org/fcdgc

Undiagnosed Disorders Network TR002471-03
International Centers are being added in Europe



> 295 total cases enrolled

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YOUR CONTRIBUTIONS ARE WARMLY ACCEPTED

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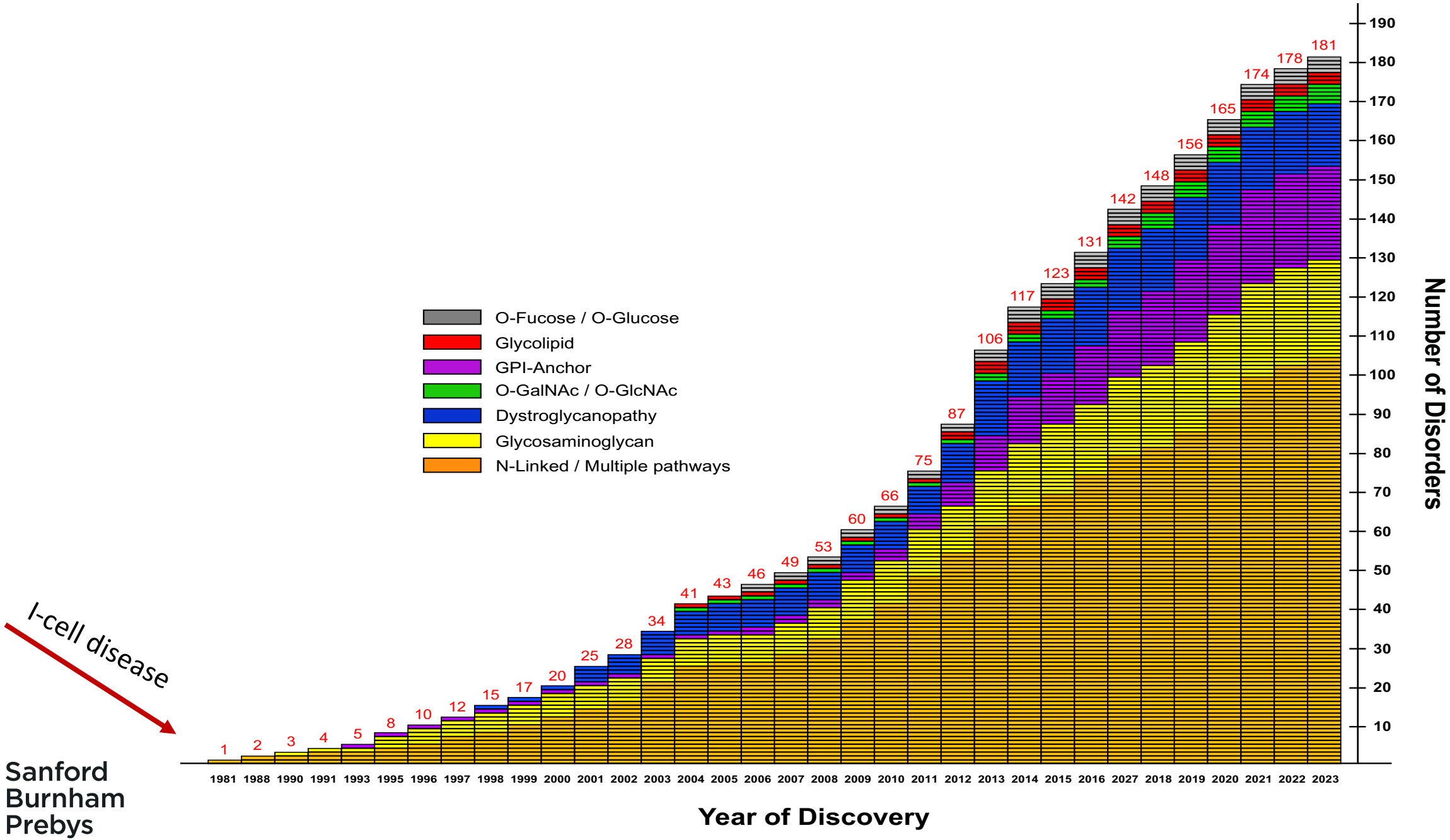
DISCOVERY OF CONGENITAL DISORDERS OF GLYCOSYLATION



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Activated sugars from *de novo*, diet or salvage pathways

Sugars

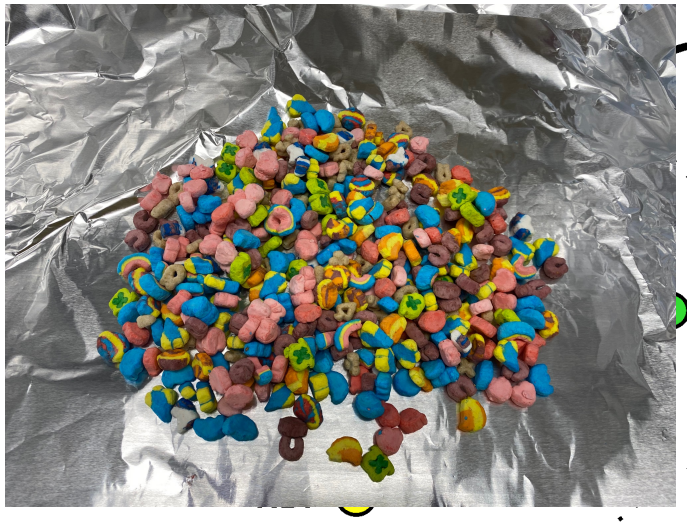
Diet

Sugar 1

Sugar 2

De novo

Lysosome



Salvage

Activated sugars

Glycoproteins

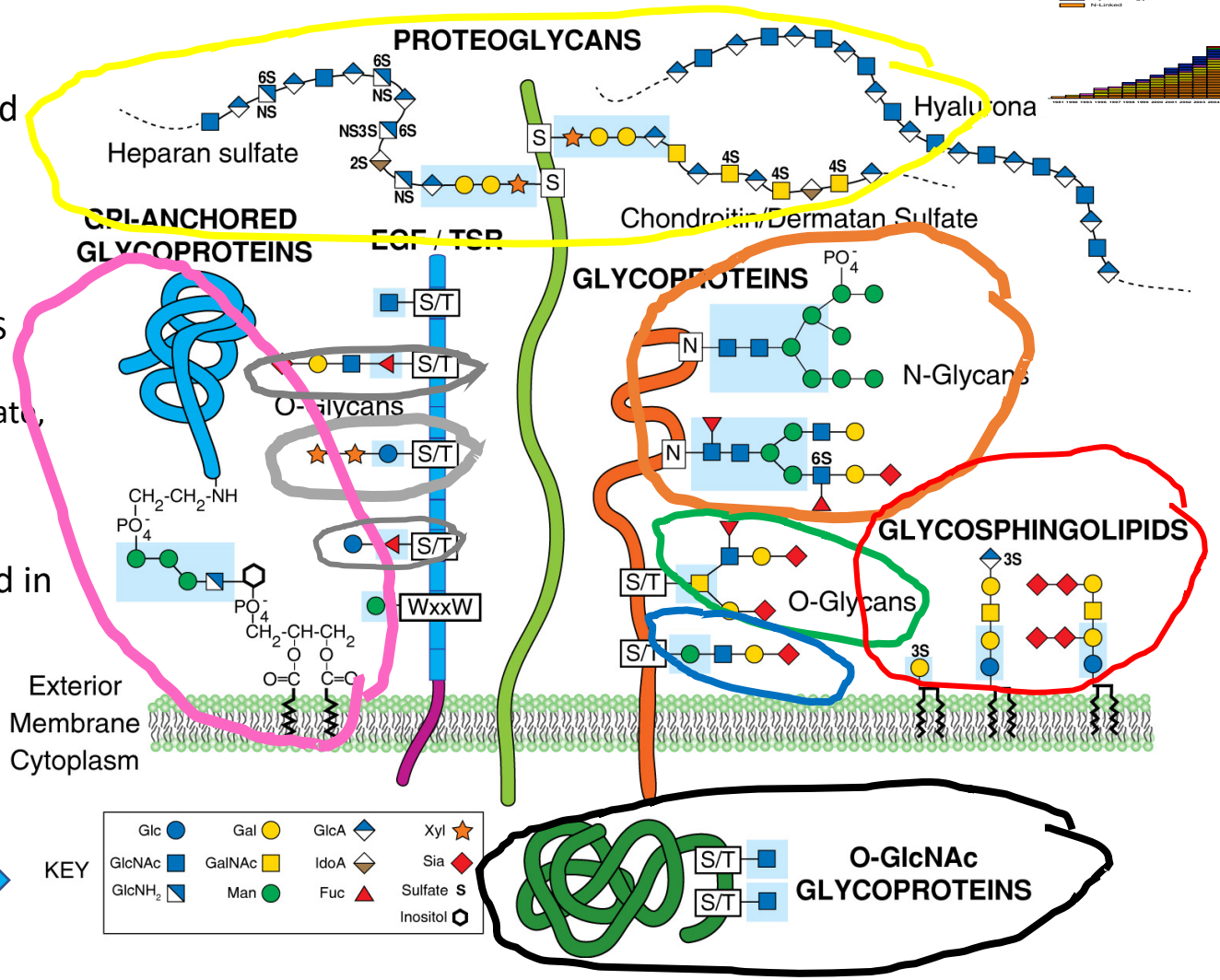
GLYCOSYLATION PATHWAYS: COMMON FEATURES

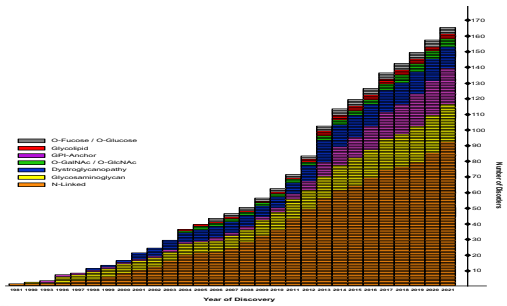
- Activated sugars from *de novo,* diet or salvage pathways
- Requires:
  - Glycosyltransferases—enzymes that add activated sugars
  - Nucleotide sugar donors
  - Transport into Golgi
  - Highly organized dynamic Golgi—proper trafficking
  - Co-localized transferases, donors, acceptors
  - Metal ions, Mg^{+2}, Mn^{+2}
  - Proper intra-vesicular pH

Common Classes of Animal Glycans

PATHWAYS AND CLIENT PROTEINS/LIPIDS

- N-linked—GlcNAc-Asn— nearly all membrane and secreted proteins
- O-linked—Ser/Thr
  - O-GalNAc—mucins &>600 proteins, 1-2 glycans
  - O-Mannose-α-dystroglycan
  - O-Fucose—EGF or TSR1 modules, Notch, ADAMTS
  - O-Glc—Notch, Delta, Jagged signaling
  - O-Xylose—GAG chains, heparan, chondroitin sulfate, heparin
  - O-GlcNAc—regulates activity of 1000's of cytoplasmic/nuclear proteins
- GPI-Anchor~ 150 Transmembrane proteins, found in lipid rafts
- GSL— Glycolipids also in membrane lipid rafts





Some proteins contain multiple types of glycans

Chapter 1, Figure 6. Essentials of Glycobiology, Third Edition

Symbol Nomenclature for Glycans (SNFG)

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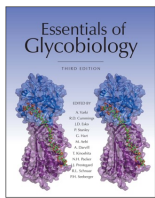
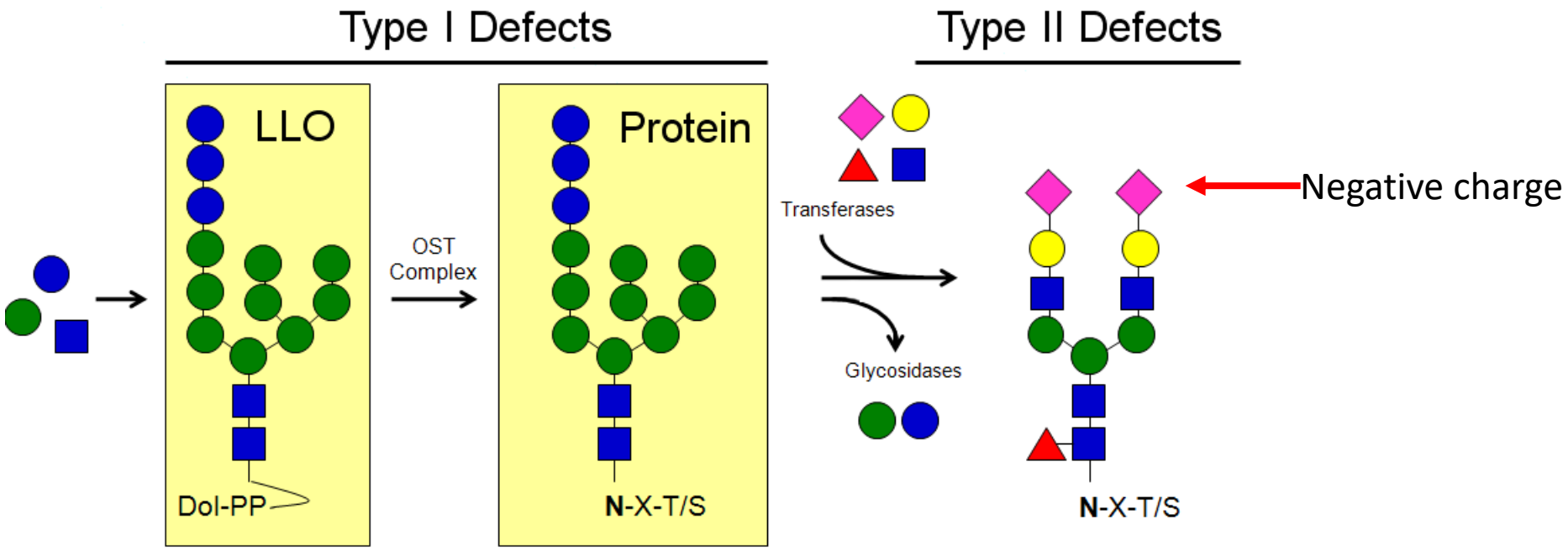
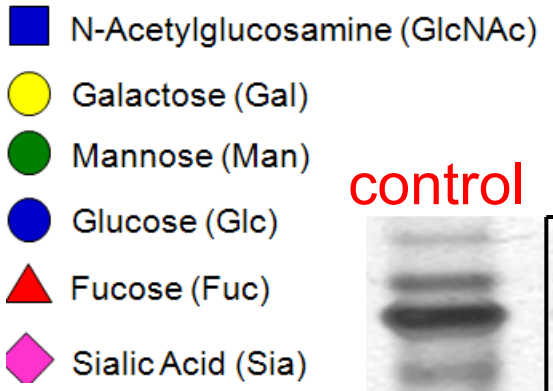
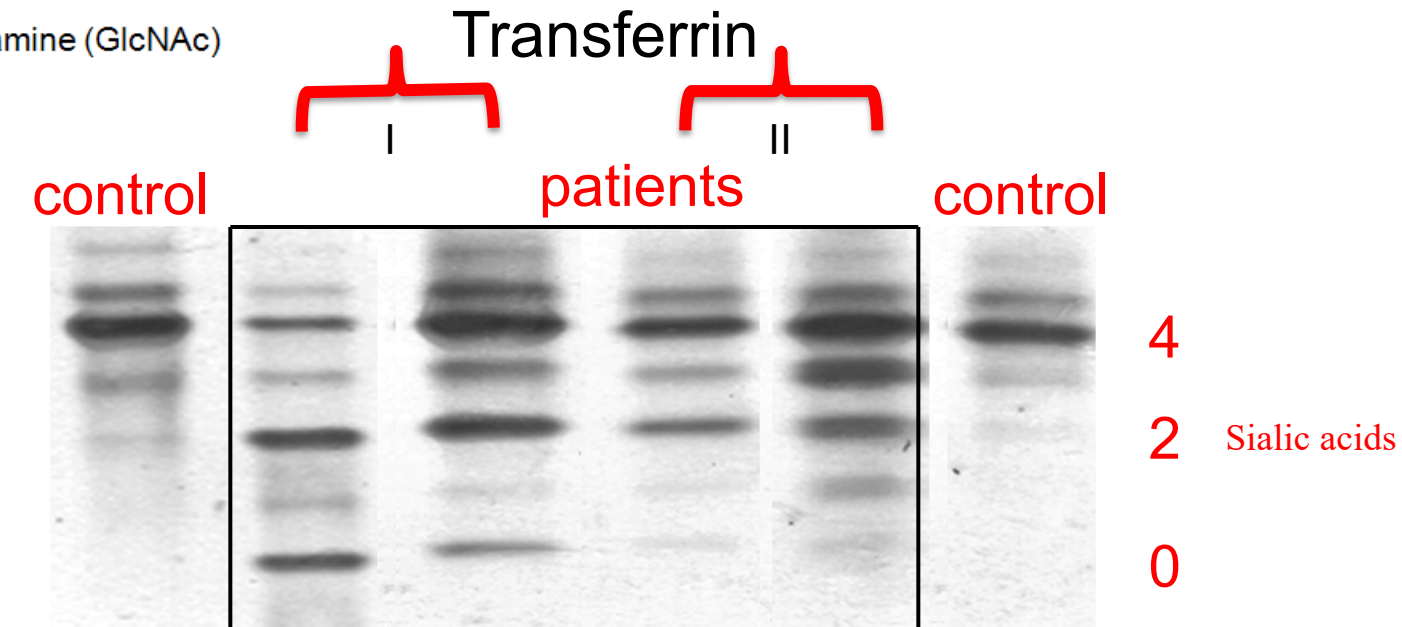


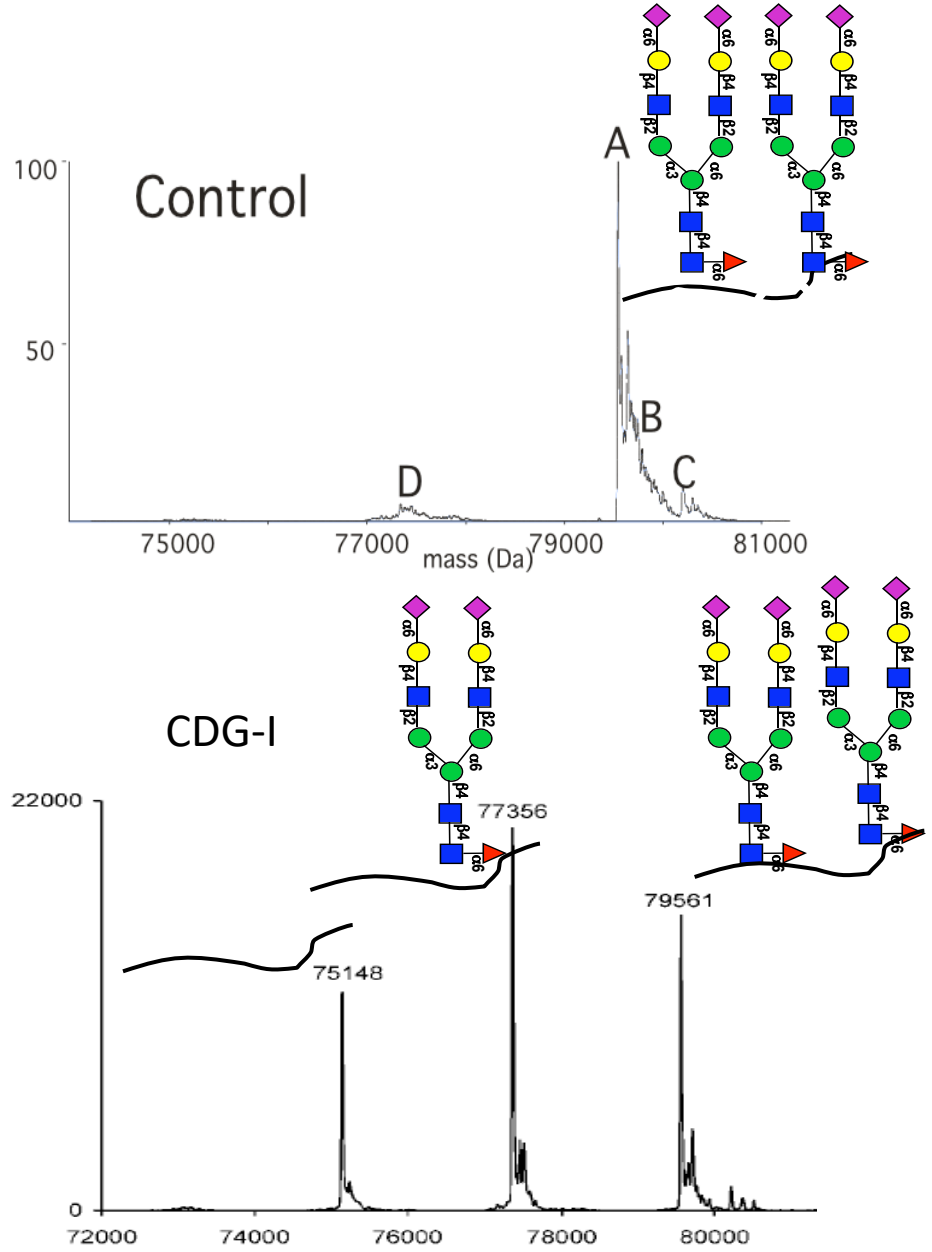
Glycosylation Disorder--Biochemical Diagnosis



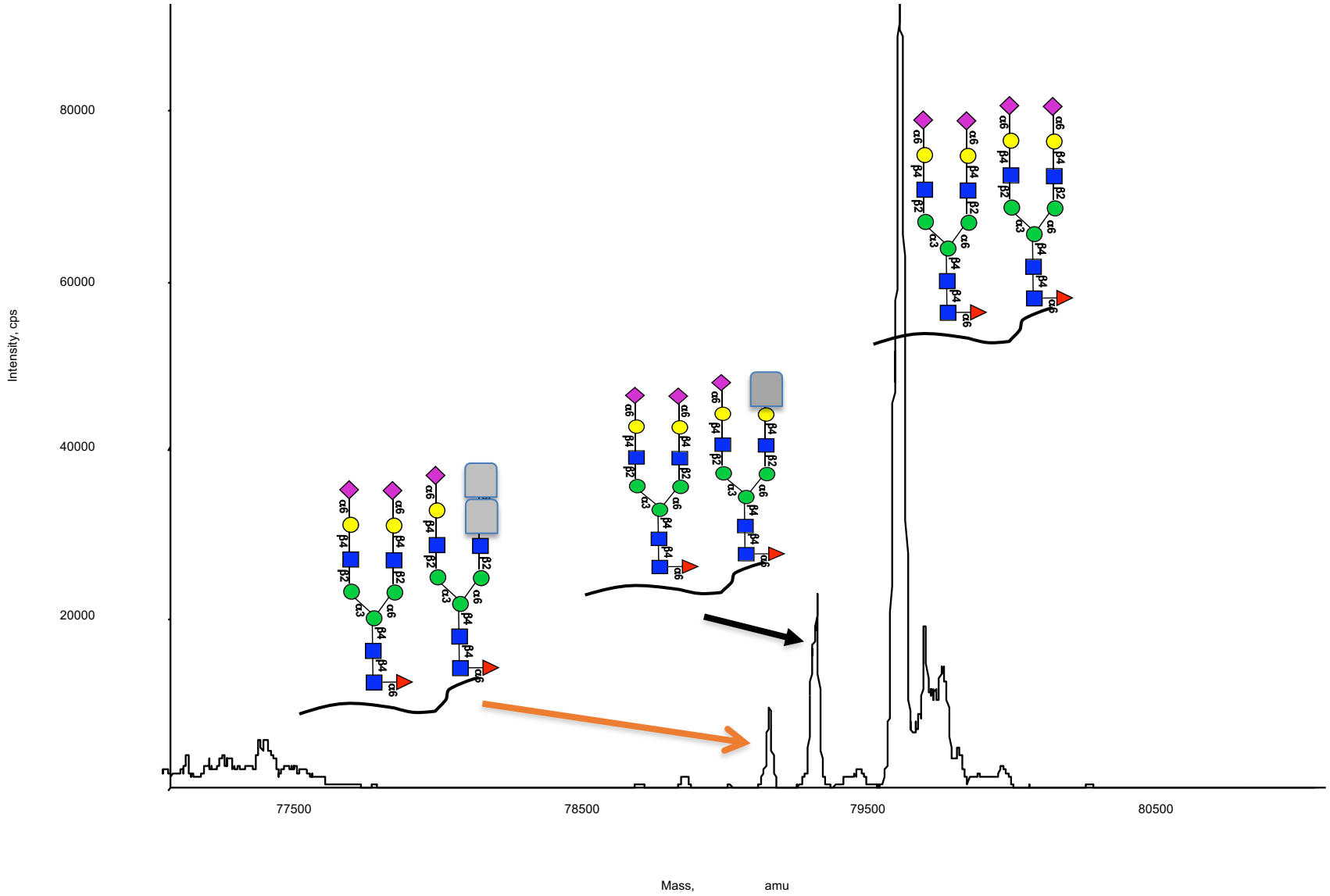




ESI-MS OF TRANSFERRIN: A Key to Identifying Many CDG Patients

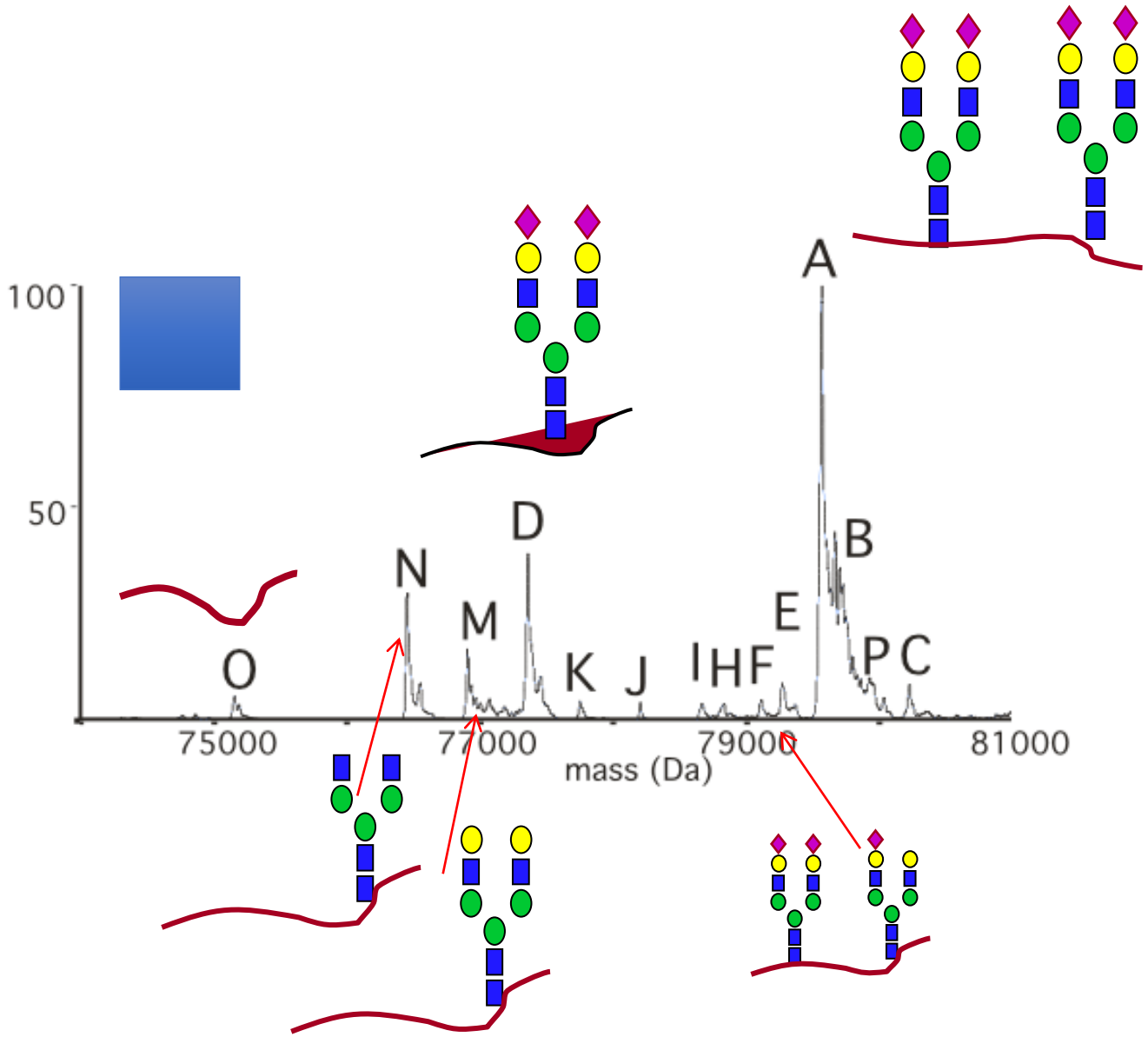


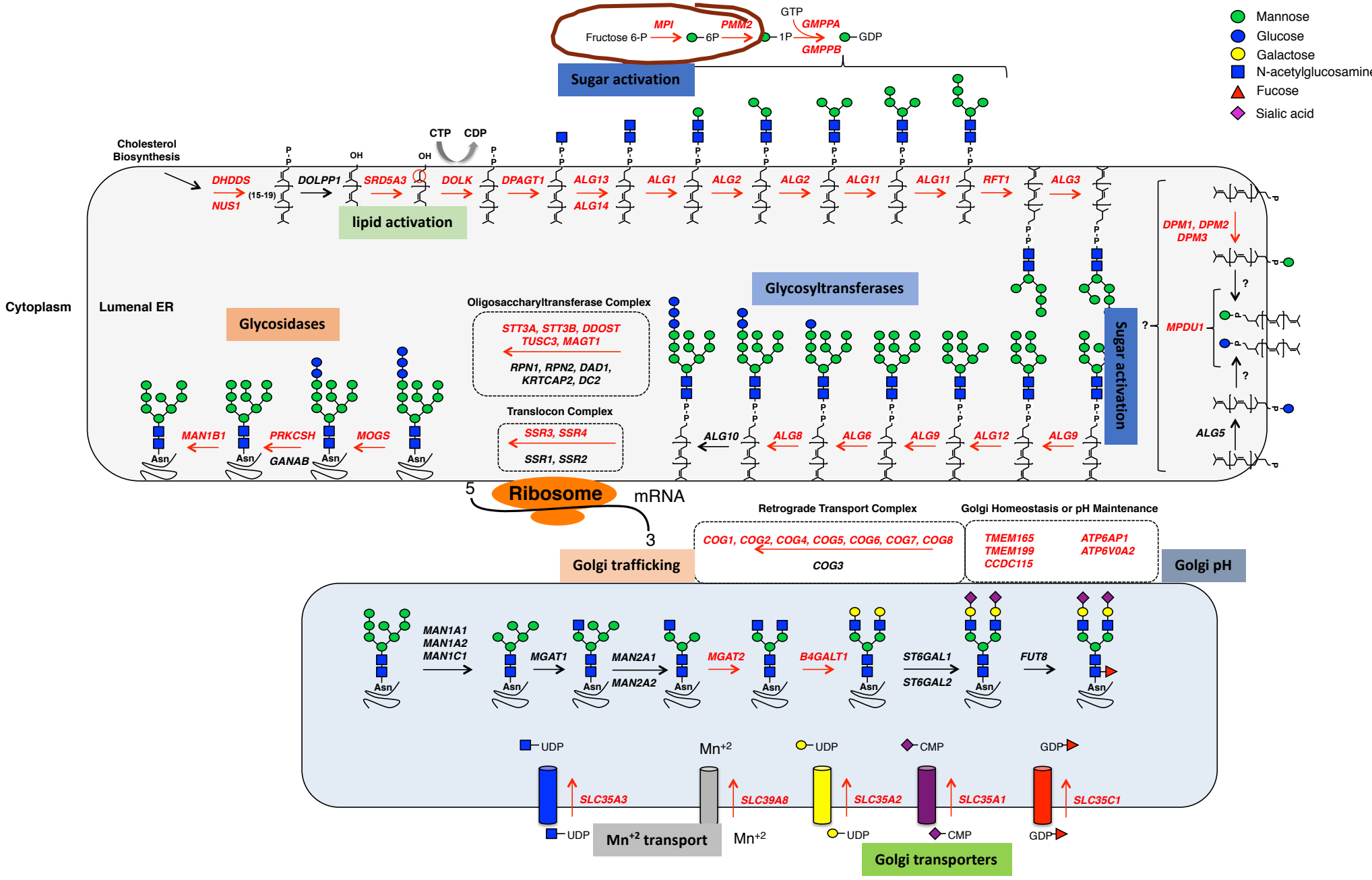
EXAMPLE OF LOSS OF SPECIFIC SUGARS



CDG patient missing both entire glycans AND individual sugars

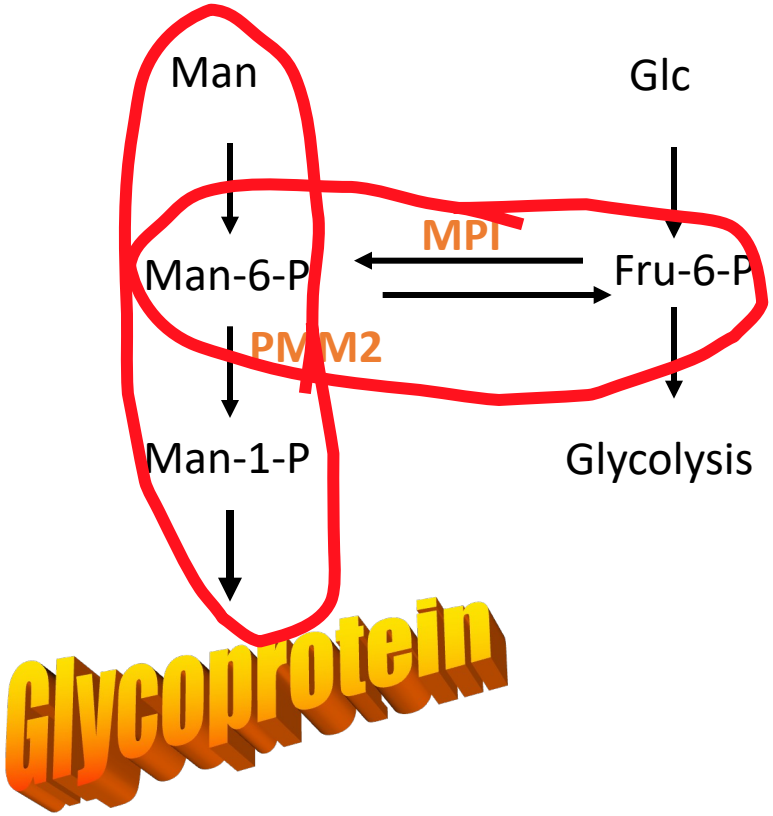
NOW MANY PATIENTS HAVE BEEN IDENTIFIED WITH THIS PATTERN




Mannose
Glucose
Galactose
N-acetylglucosamine
Fucose
Sialic acid
Fructose 6-P
MPI
PMM2
6P
1P
GTP
GMPPA
GMPPB
GDP
Sugar activation
Cholesterol Biosynthesis
CTP
CDP
DHDDS
NUS1
(15-19)
DOLPP1
SRD5A3
DOLK
DPAGT1
ALG13
ALG14
ALG1
ALG2
ALG2
ALG11
ALG11
RFT1
ALG3
lipid activation
Cytoplasm
Lumenal ER
Glycosidases
Oligosaccharyltransferase Complex
STT3A, STT3B, DDOST
TUSC3, MAGT1
RPN1, RPN2, DAD1,
KRTCAP2, DC2
Translocon Complex
SSR3, SSR4
SSR1, SSR2
Glycosyltransferases
Sugar activation
DPM1, DPM2
DPM3
MPDU1
ALG5
MAN1B1
PRKCSH
GANAB
MOGS
ALG10
ALG8
ALG6
ALG9
ALG12
ALG9
Asn
5
Ribosome
mRNA
3
Retrograde Transport Complex
COG1, COG2, COG4, COG5, COG6, COG7, COG8
COG3
Golgi Homeostasis or pH Maintenance
TMEM165
TMEM199
CCDC115
ATP6AP1
ATP6V0A2
Golgi trafficking
Golgi pH
MAN1A1
MAN1A2
MAN1C1
MGAT1
MAN2A1
MAN2A2
MGAT2
B4GALT1
ST6GAL1
ST6GAL2
FUT8
UDP
Mn+2
UDP
CMP
GDP
SLC35A3
SLC39A8
SLC35A2
SLC35A1
SLC35C1
Mn+2 transport
Golgi transporters

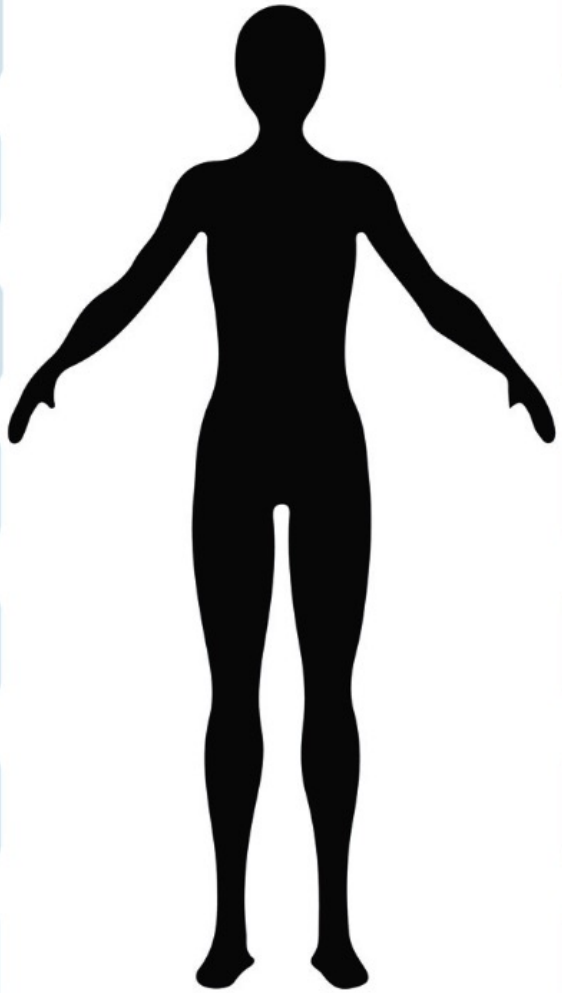
BASIC BIOCHEMISTRY OF COMMON TYPES OF CDG

MOST COMMON CDG TYPES
Number of known patients
ALG1 –59 *
ALG3—26
ALG6—89
ALG13—60 *
DPAGT1—39
PGM1—60 *
PMM2-~1000*
PIGA—80 *
SLC37A2—65 *
MPI—37 *
NGLY1—60*



Organ Systems Affected by Congenital Disorders of Glycosylation

All cell types and organs are affected by the glycosylation defect



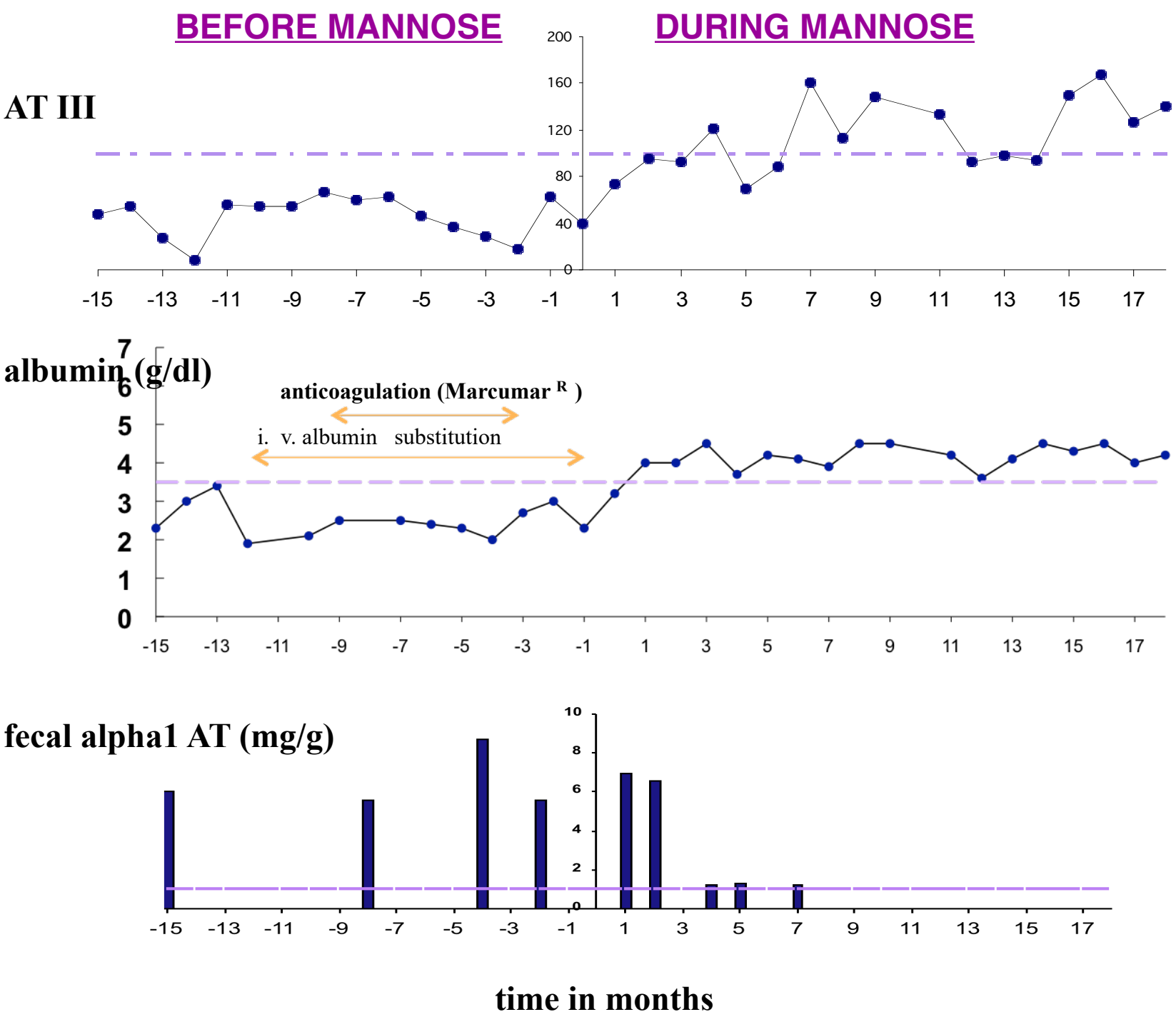
- **Congenital malformations**
- **Ophthalmologic involvement**
- **Endocrine involvement**
  - **Growth failure**
  - **Hypothyroidism**
  - Hypoglycemia
  - Hypogonadism
- **Muscle involvement**
- **Hepatic involvement**
  - Hepatopathy
  - Hepatomegaly
  - Cholestasis
  - Liver failure
- **Lipid abnormalities**
  - Hypocholesterolemia
  - Hypercholesterolemia
- **Infections and immune involvement**
  - Recurrent infections
- **Neurologic involvement**
  - Developmental delay
  - Seizures
  - Stroke-like episodes
  - Peripheral neuropathy
  - Cerebellar ataxia
  - Hypotonia
- **Cardiac involvement**
  - Pericarditis
  - Cardiomyopathy
  - Structural heart defects
- **Gastrointestinal involvement**
  - Failure to thrive
  - Vomiting
  - Diarrhea
  - Protein-losing enteropathy
- **Renal involvement**
  - Kidney cysts
  - Nephrotic syndrome
  - Dysplastic kidneys
- **Hematologic involvement**
  - Thrombosis
  - Bleeding
  - Anemia
- **Skeletal involvement**
  - Kyphoscoliosis
  - Skeletal dysplasia
  - Osteopenia

Figure from Genetics in Medicine(2020) 22:268–279; https://doi.org/10.1038/s41436-019-0647-2

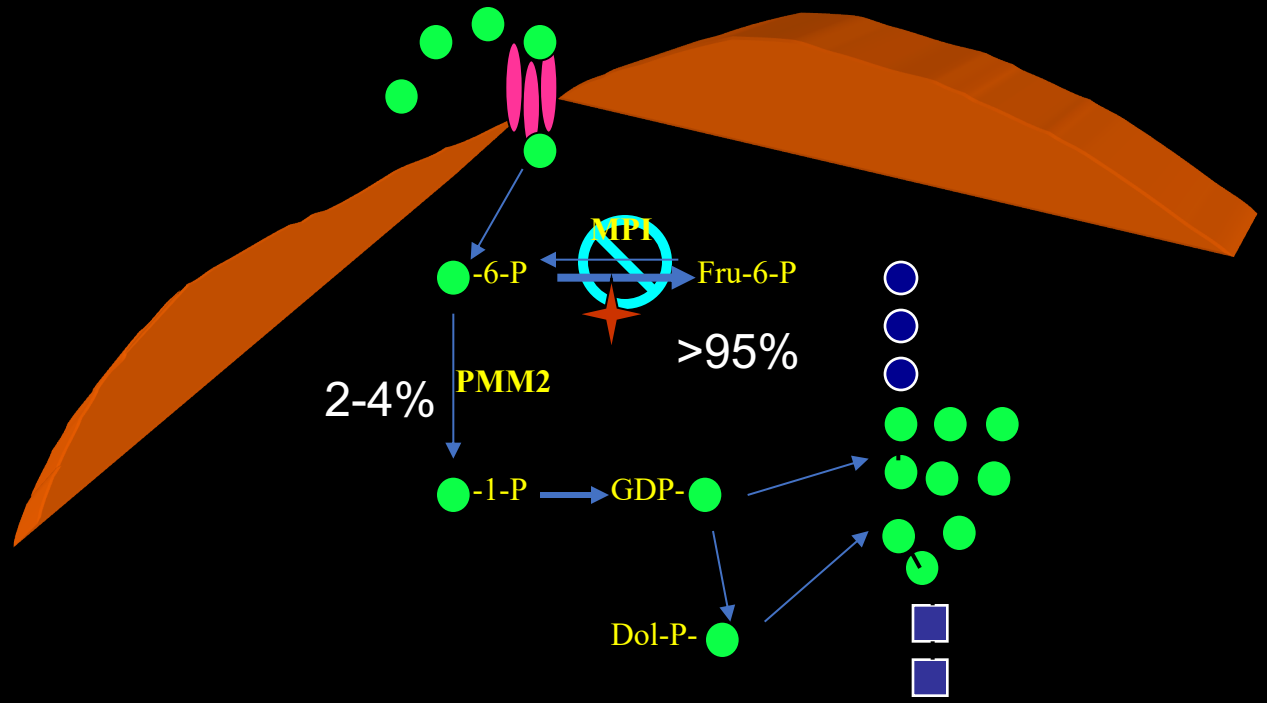
FCDGC

SOME PATIENTS HAVE COAGULOPATHY, HEPATIC FIBROSIS AND PROTEIN-LOSING ENTEROPATHY

Collaboration with Thorsten Marquardt, University of Munster


BEFORE MANNOSE
DURING MANNOSE
AT III
albumin (g/dl)
anticoagulation (Marcumar R)
i. v. albumin substitution
fecal alpha1 AT (mg/g)
time in months

Therapy for MPI-CDG (-Ib)



Mannose improves most symptoms, but some patients may require liver transplant

CDG defect sugar transporter Man GlcNAc

Classic clinical presentation in PMM2-CDG

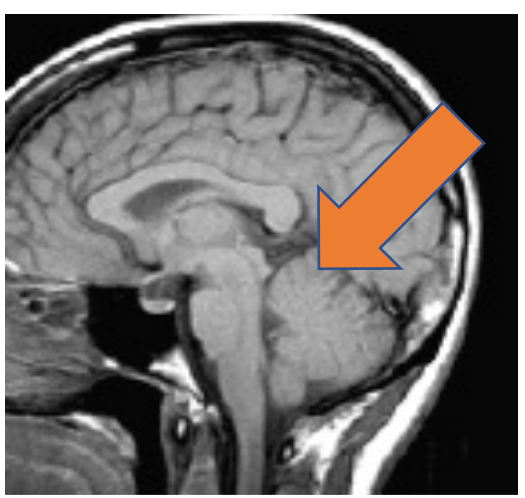
Muscle hypotonia

Developmental delay/Speech delay

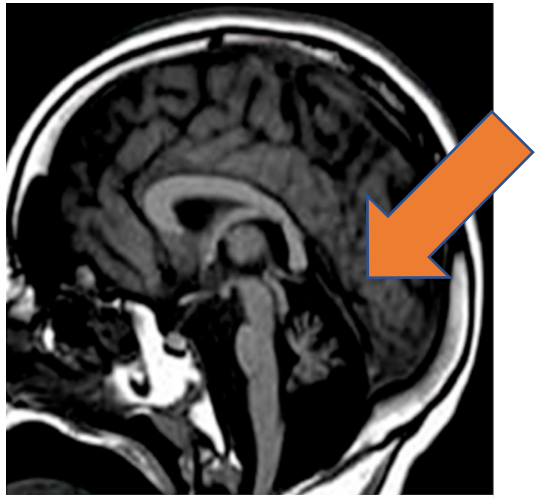
Epilepsy/encephalopathy/SLE

Ataxia/vermis atrophy

Neuropathy



Normal vermis



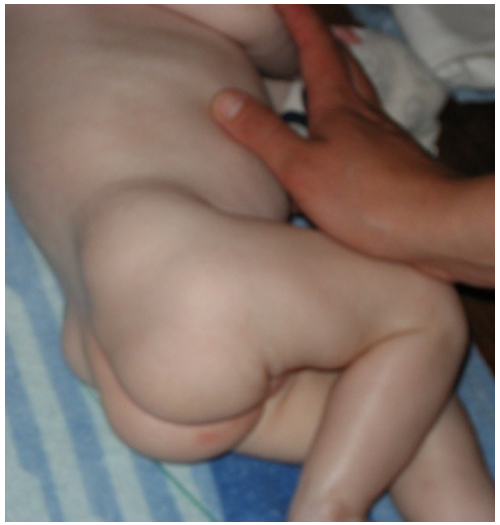
Vermis atrophy

Characteristic facial features

Strabismus

Abnormal fat distribution/Inverted nipples

Long fingers



Failure to thrive

Endocrine failure

Coagulation abnormalities

MAYO CLINIC

PMM2-CDG

Prevalence: 1/20,000-1/50,000

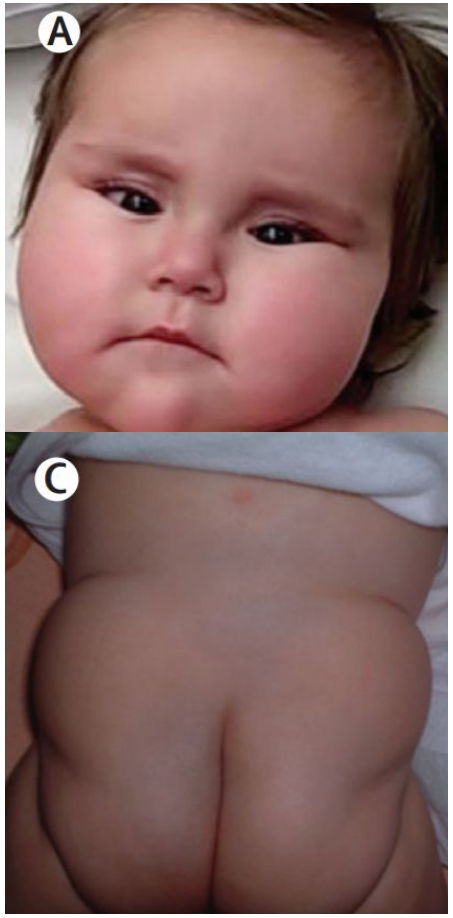
Discovery: 1980

Biochemical defect: Mannose-6-P→Mannose-1-P

Pathogenesis: deficient GDP-Man and Dol-P-Man effecting 100's of proteins

PMM2-CDG (CDG-Ia)

- By far the most common CDG
- *PMM2* = phosphomannomutase; enzyme that converts mannose-6P to mannose-1P
- Wide spectrum of severity from neonatal lethal to late onset
- Symptoms in almost every body system
- Not treatable
- Type-I N-linked CDG pattern on mass spec or transferrin isoelectric focusing
- Characteristic facial dysmorphisms and subcutaneous fat distribution
- Inverted nipples, supragluteal fat pads



Courtesy, Austin Larson

PMM2 - Infantile multisystem disease

- 20% of patients die in infancy
- The most common mutation (p.Arg141His) is only ever seen in a compound heterozygous state
- Severe diarrhea, malabsorption, failure to thrive
- Severe proteinuria and hypoalbuminemia
- Liver disease
- Severe edema (anasarca)
- Coagulation abnormalities with risk for both excessive bleeding and clotting
- Hypotonia, developmental delay, seizures

Martinez-Monseny A, Cuadras D, Bolasell M, et al. From gestalt to gene: early predictive dysmorphic features of PMM2-CDG. *J Med Genet*. 2019;56(4):236-245.

Courtesy, Austin Larson

PMM2 - Childhood disease

- Hypotonia and ataxia
- Moderate developmental delay with IQ of 40-70
- Typically a cheerful, social personality
- May have reversible stroke-like episodes
- Liver function tests slowly normalize with age
- Coagulation abnormalities persist
- Abnormal endocrine labs (thyroid)
- Development of retinitis pigmentosa
- Esotropia is almost universal, sometimes requires surgery

PMM2 - Adolescent/adult disease

- Stable intellectual disability
- Progressive ataxia
- Progressive peripheral neuropathy
- Lack of spontaneous puberty, especially girls
- Osteoporosis and osteopenia
- Persistently abnormal coagulation studies

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Courtesy, Austin Larson

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Therapy in PMM2-CDG

Symptom specific

Hormone supplements/Diazoxide

Fresh frozen plasma/Factor supplements

Seizure treatment

OT/PT/ST

Hydration, tube feeding

Organ specific

Surgery (skeletal, cardiac, etc.)

Transplantation

Experimental

- Off label use (Acetazolamide)[1]
- Chaperones[2]
- Small molecules
- Drug repurposing
- Preclinical “activated sugar therapy”
- Preclinical gene therapy

1. Martinez-Monseny, 2019 doi: 10.1002/ana.25457
2. Vilas A, 2020, doi: 10.1016/j.bbadis.2020.165777

TABLE 1 Suggested surveillance for PMM2-CDG patients

Systems	At diagnosis, if not previously obtained	At follow up 1-2 years interval and as needed	As needed depends on the symptoms
Neurology			
Developmental and cognitive assessment	✓	✓	✓
Electroencephalogram			✓
Brain MRI	✓		✓
Audiology	✓		✓
Endocrine			
Height	✓	✓	
Calcium, magnesium and phosphate	✓	✓	
Gonadotropins	✓	✓	✓
Glucose	✓		
Insulin and other labs in case of hypoglycemia[a]	✓		✓
Thyroid function	✓	✓	✓
Cardiology			
Echocardiogram	✓		✓
Electrocardiogram	✓		✓
Holter			✓
Cardiac MRI			✓
Gastroenterology			
Growth and anthropometric parameters	✓	✓	
Swallowing evaluation			✓
Transaminases	✓	✓	✓
Hematology			
Complete blood counts and differential	✓	✓	✓
Coagulation factors	✓	✓	✓
Renal			
Creatinine	✓	✓	✓
Protein	✓		✓
Immunology			✓
Ophthalmology			
Exam	✓	✓	✓
Electroretinogram			✓
Skeletal	✓		✓
Psychiatric evaluation			✓

International clinical guidelines for the management of phosphomannomutase 2-congenital disorders of glycosylation: Diagnosis, treatment and follow up *J Inherit Metab Dis.* 2019;42:5–28.

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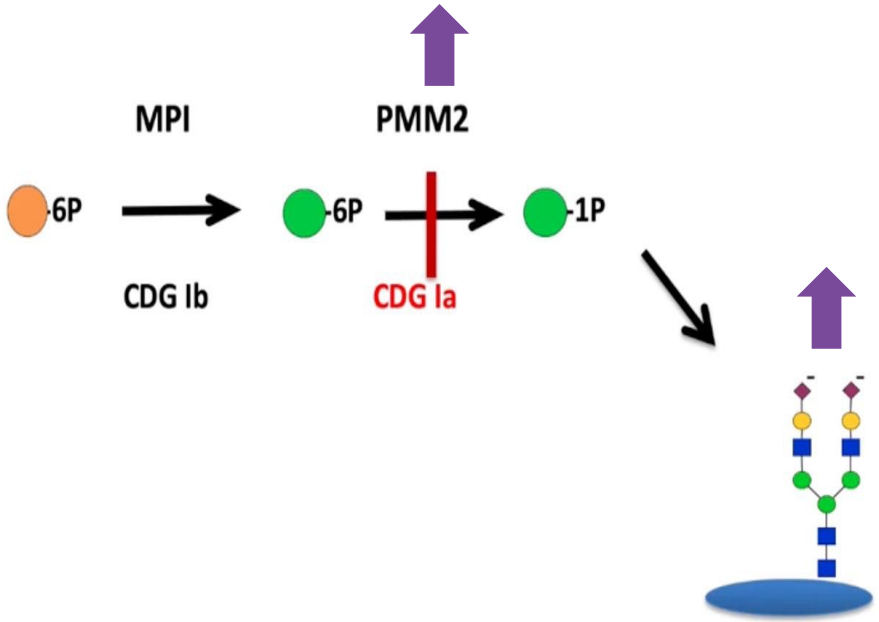
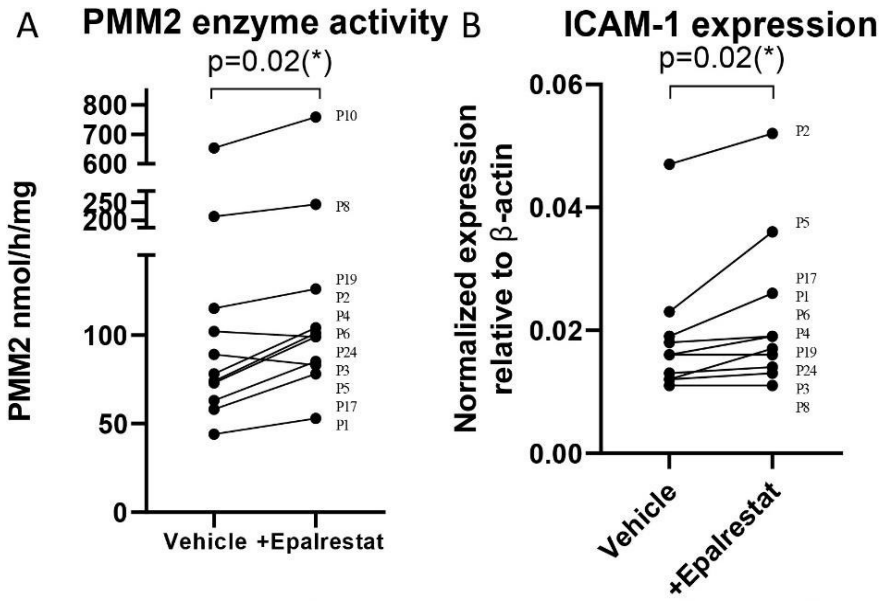
The Role of Aldose Reductase (AR) inhibitors in PMM2-CDG

- PMM2 worm model was used to screen small molecule libraries
- AR inhibitors, including epalrestat increased PMM2 activity in worms
- The AR inhibitor epalrestat increased PMM2 activity patient fibroblasts





. Iyer et al Disease Models & Mechanisms 2019, Ligezka et al, ANA, 2021

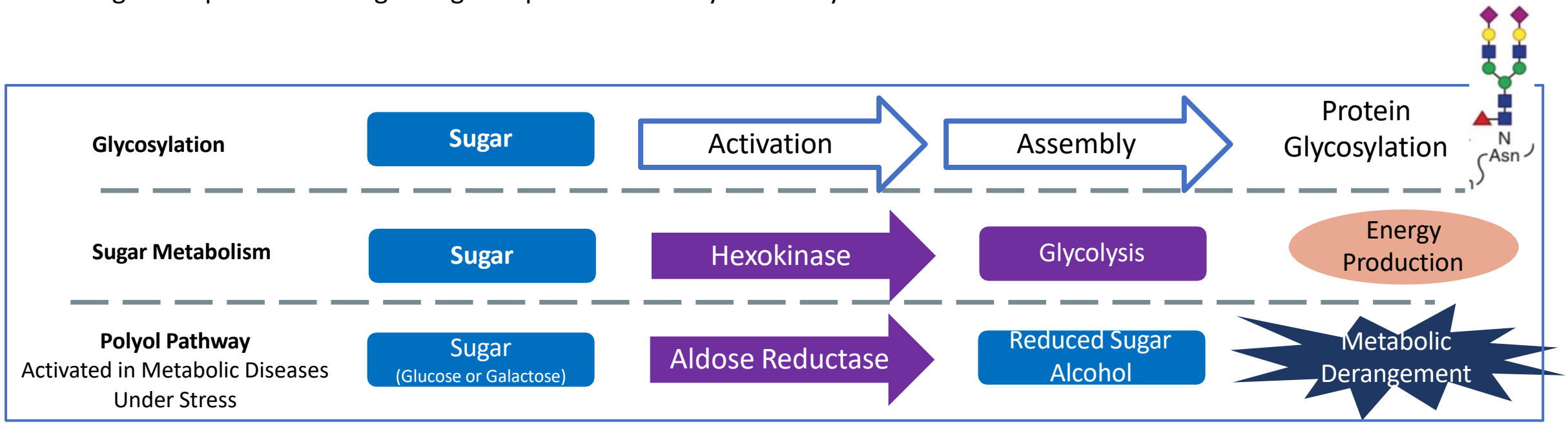
Courtesy of Eva Morava

AR inhibitor epalrestat increased PMM2 activity in worms and patient fibroblasts

Aldose Reductase inhibition might increase G1,6BP stabilizing PMM2

Altered sugar flux can increase GDP mannose availability

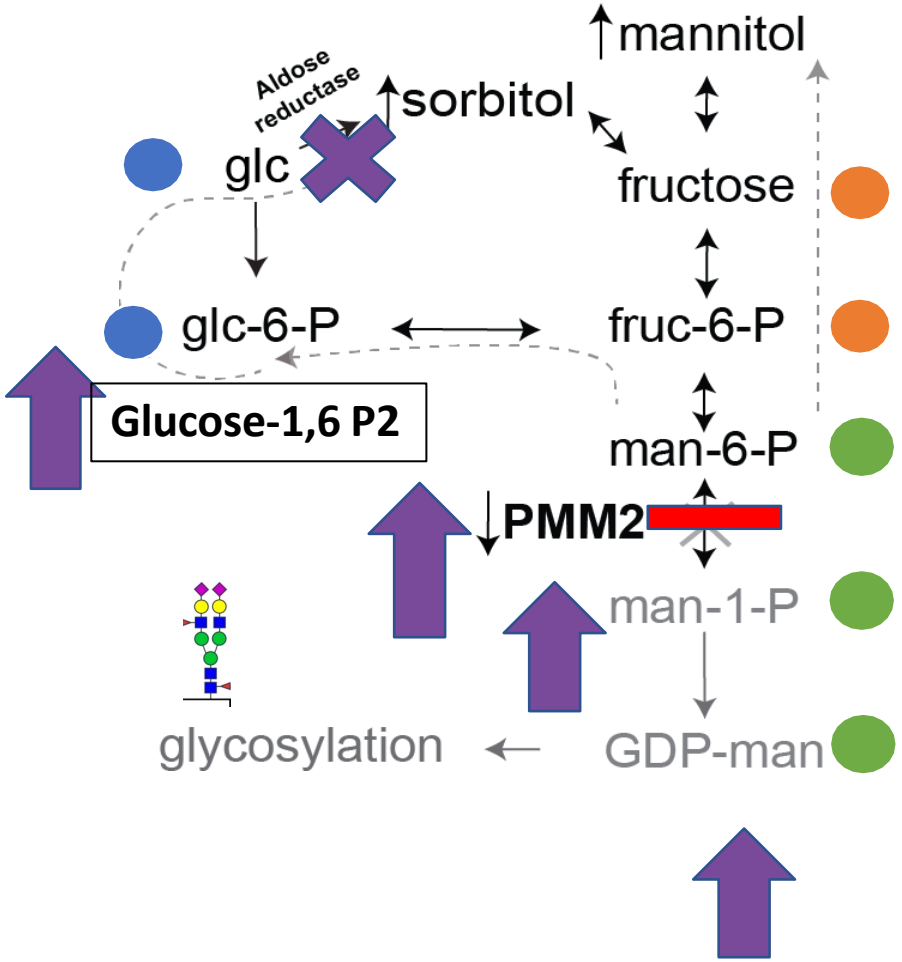
Reversing redox potential changes might improve secondary PMM2 dysfunction

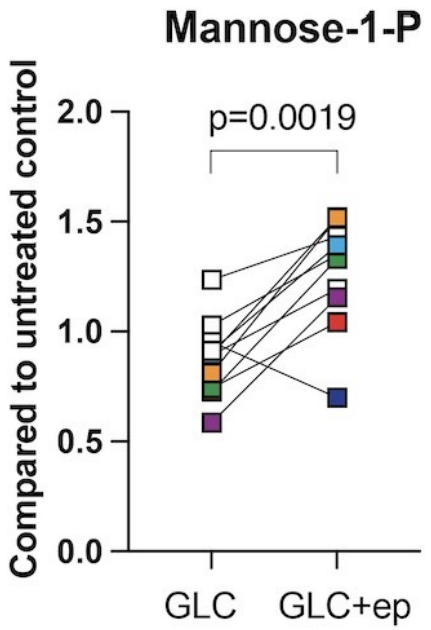


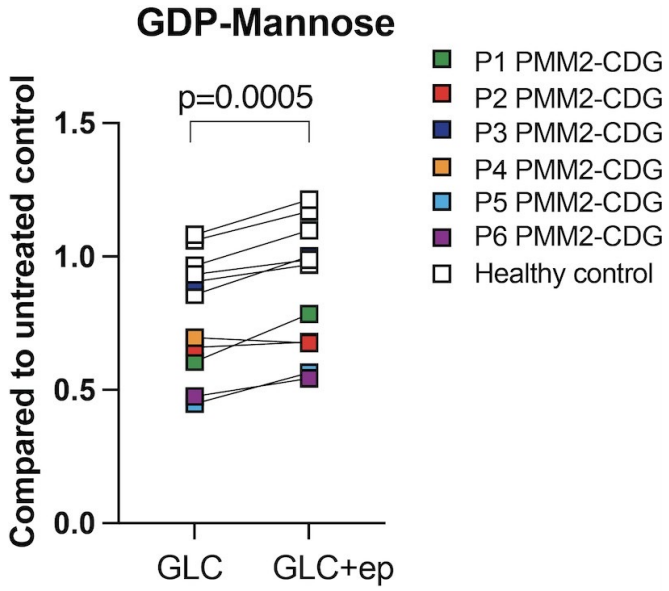
2. Morava editorial Nature Genetics 2020; . 1. Iyer et al Disease Models & Mechanisms 2019

Courtesy of Eva Morava

measuring mannose metabolites



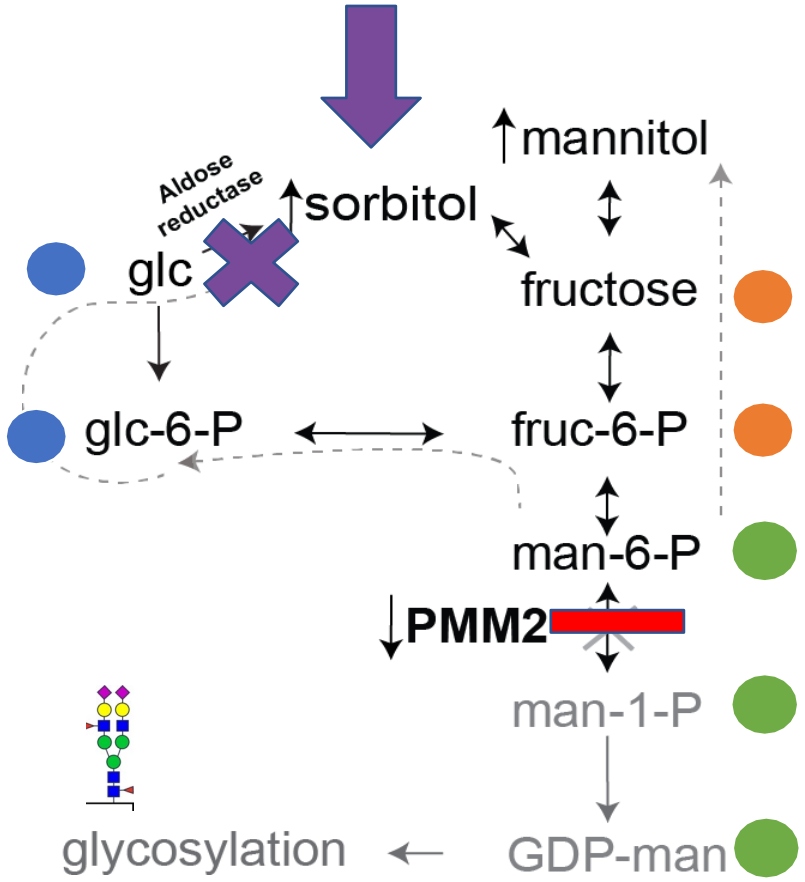


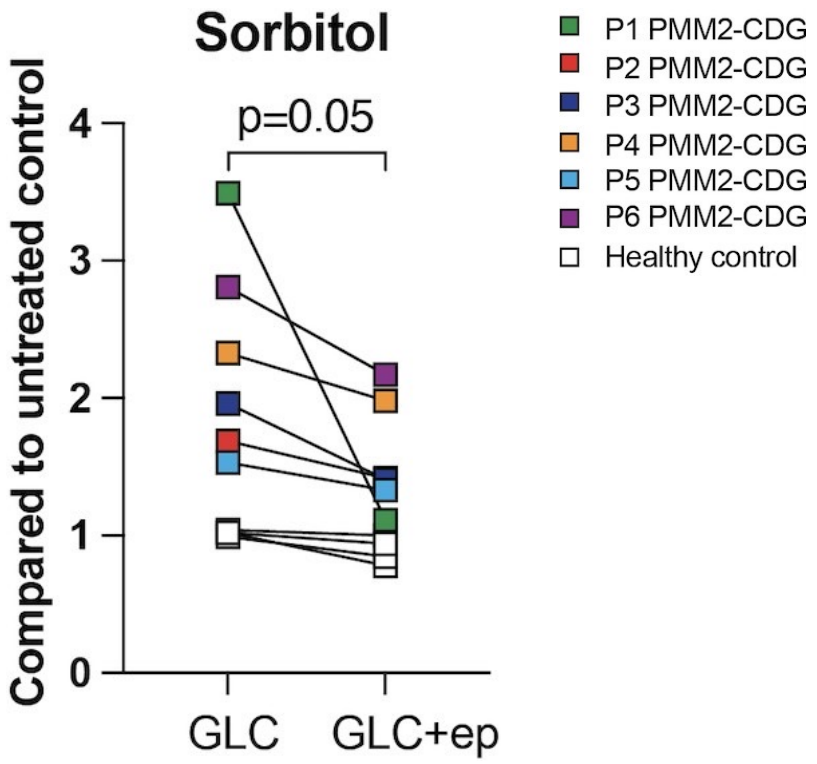


3. Ligezka and Radenkovic et al, Annals of Neurology, 2021, 90: 887-900; Radenkovic Cell Report Med 2023 PMID: 37257447,

Courtesy of Eva Morava

measuring polyols

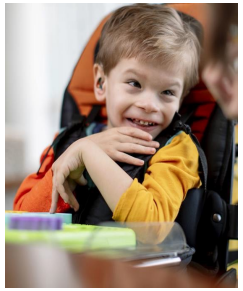


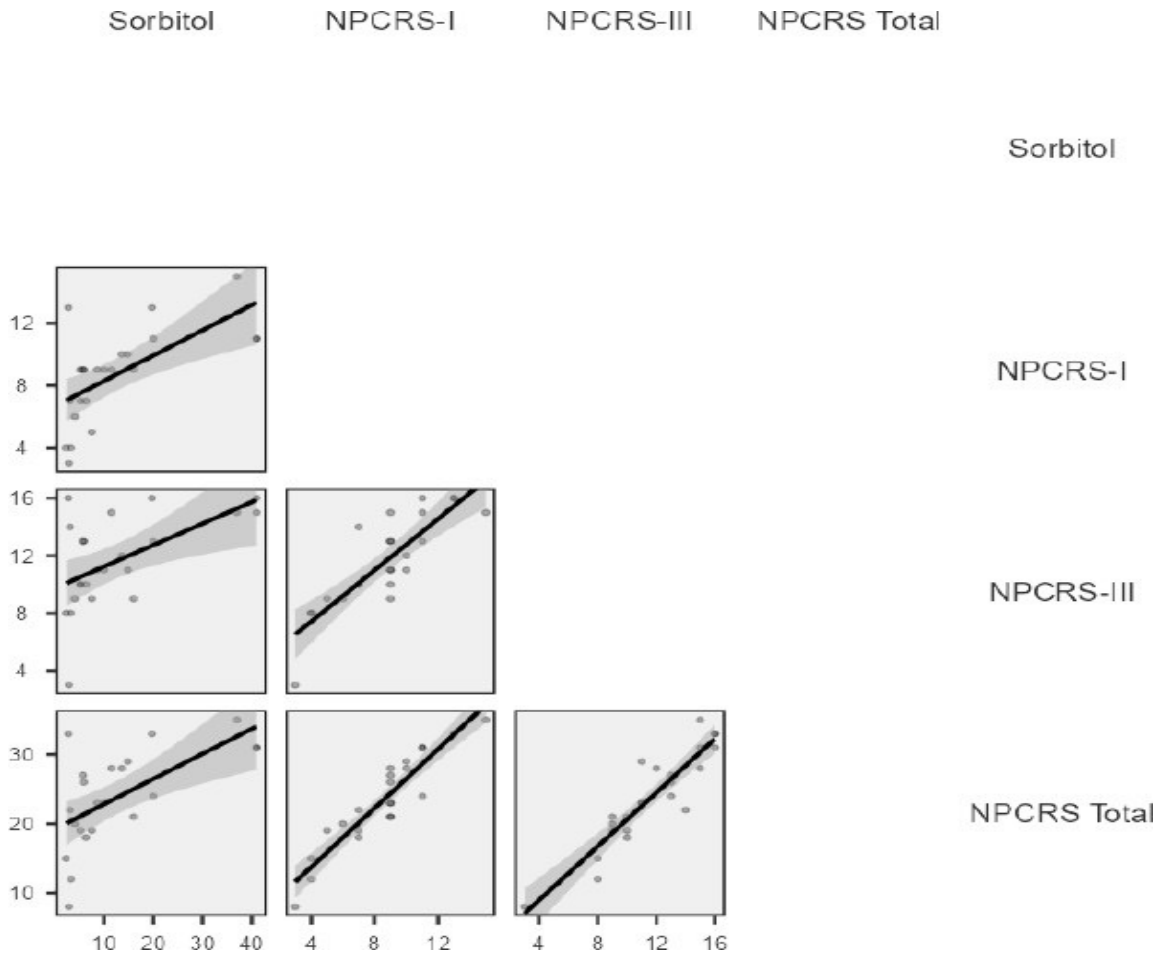


3. Ligezka and Radenkovic et al, Annals of Neurology, 2021: Radenkovic et al 2023

Courtesy of Eva Morava

Sorbitol elevated in most PMM2-CDG patients' urine and correlates with disease severity





Dr Kimiyo Raymond

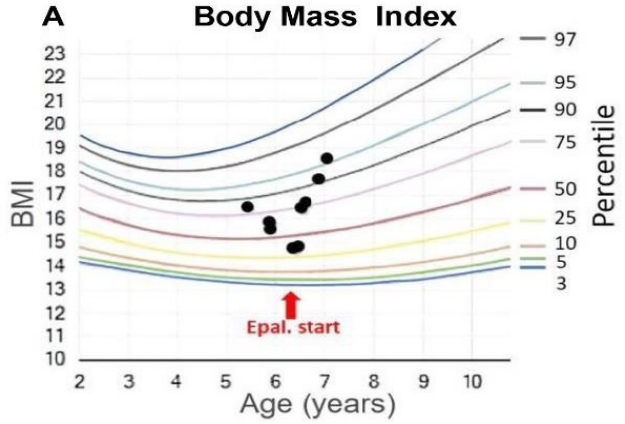
MAYO CLINIC

3. Ligezka and Radenkovic et al, Annals of Neurology, 2021, in press

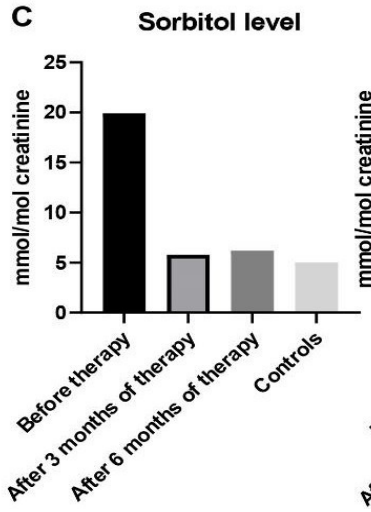
Courtesy of Eva Morava

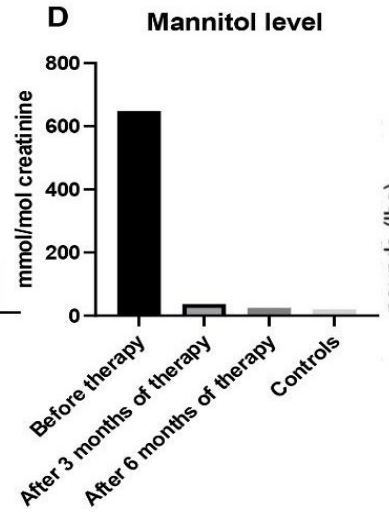
Recruited 38 patients for Phase IIb clinical trial (NCT04925960)

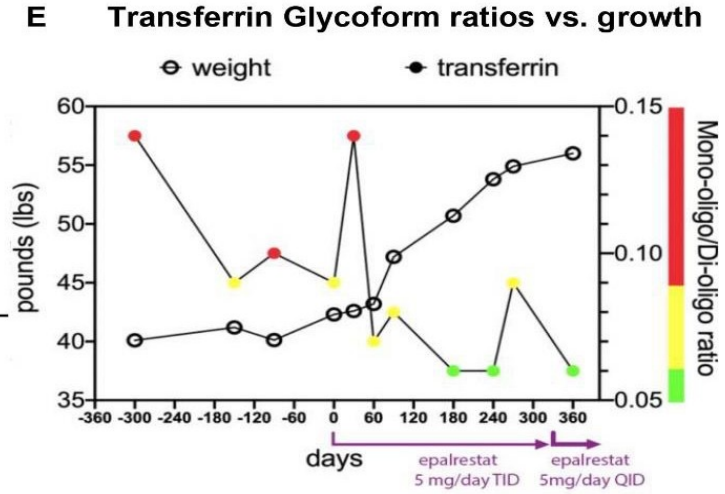
Single patient trial



ICARS score improved 13%







Courtesy of Eva Morava

ADDITIONAL THERAPY FOR PMM2-CDG

Acetazolamide to the Rescue

Martinez-Monseny, et al, Ann Neurol 2019;85:740-751

- Ataxia is large burden for PMM2 patients
- Stroke-like episodes causing hemiparesis
- Acetazolamide (carbonic anhydrase inhibitor) improves PMM2 patients:
  - International Cooperative Ataxia Rating Scale (ICARS)
  - Nijmegen Pediatric CDG Rating Scale (NPCRS)
  - Cognition scores and syllable repetition test PPATA)
- Recruiting for US Clinical Trials (NCT04679389)
- Natural History trials (NCT03173300)

Other planned clinical trials for PMM2-CDG

- Liposome encapsulated Mannose-1-P for IV injection—Phase 1, 2023

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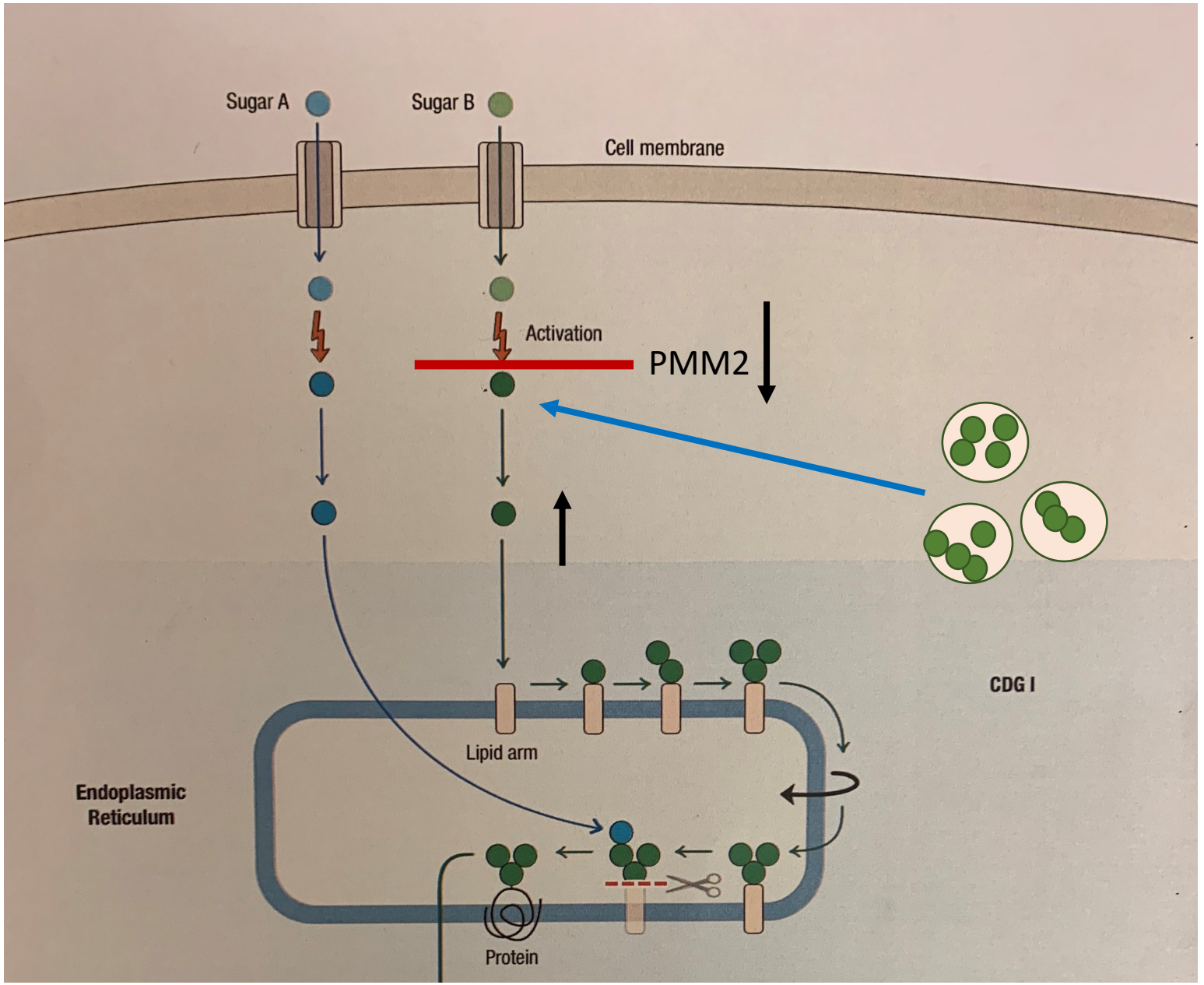
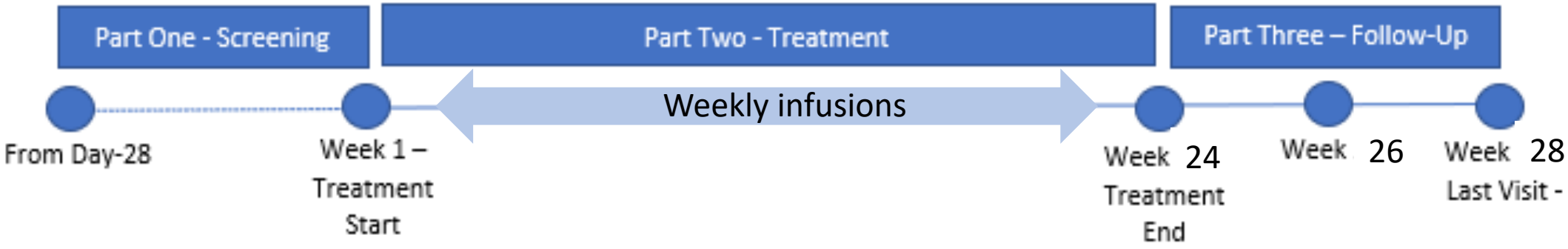
Liposomal M-1-P targeting

-In vitro studies: GDP mannose ↑
-Increase in glycosylated proteins by glycoproteomics



GLM101-002 Study Design & Primary Objective, Endpoints

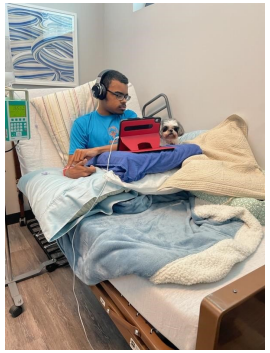


Primary Objective: Gain adult patient exposure and select dose for future pediatric studies

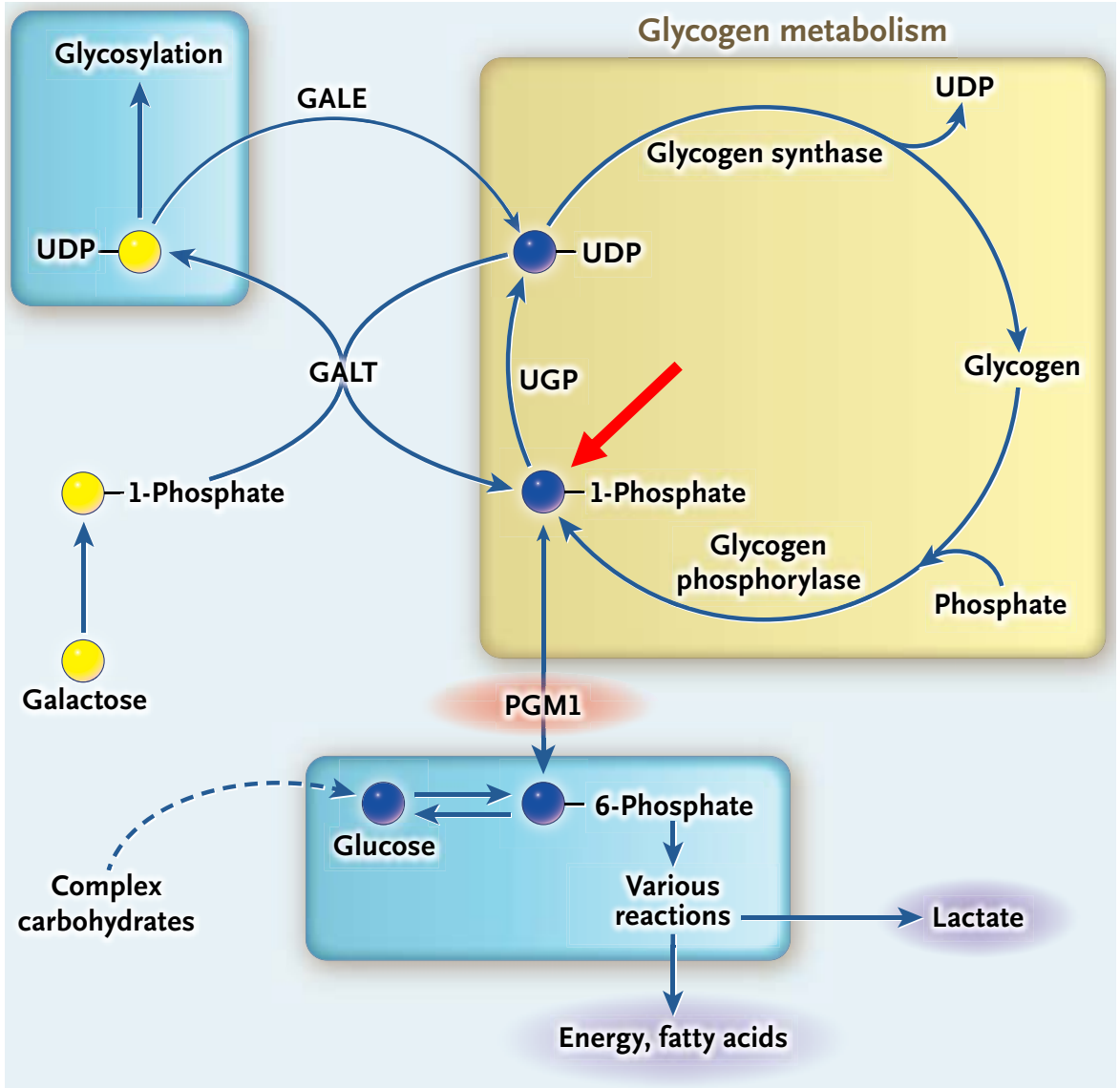
Primary Endpoints: Evaluate changes in **ATIII and FXI** in adult participants with PMM2-CDG

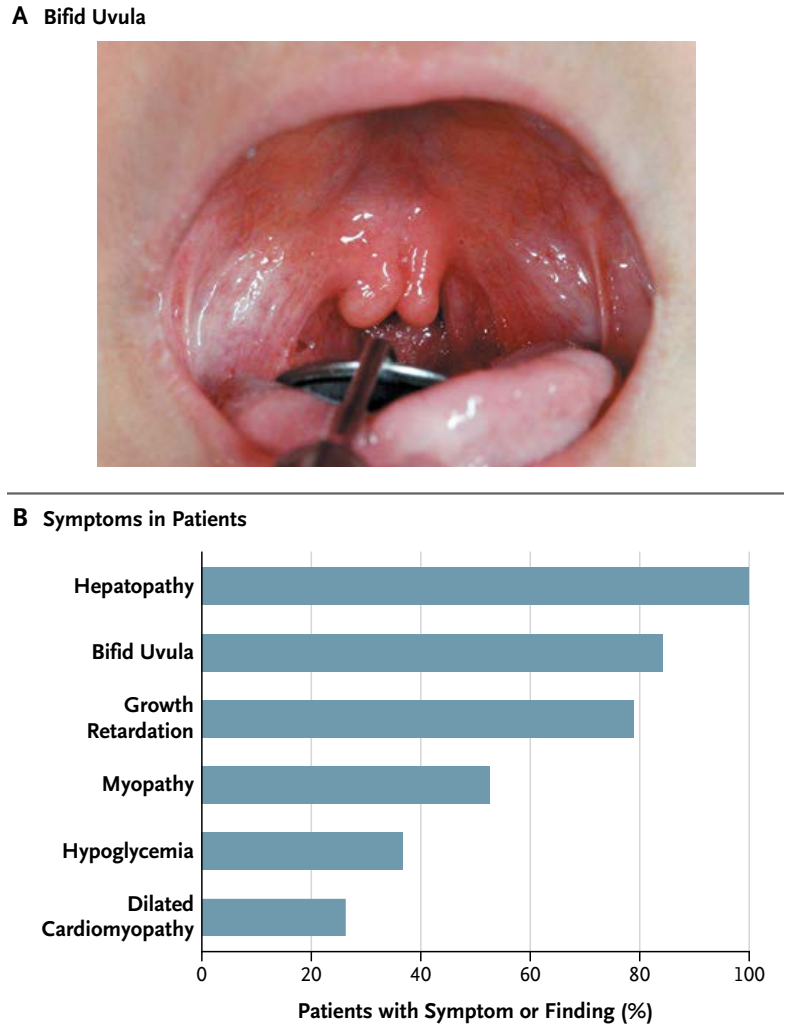
Secondary Endpoints: Safety, PK, clinical labs

Exploratory Endpoints: Transferrin ratios, Glycomics, Mannose, GDP-mannose



PGM1-CDG





Initial studies suggested Galactose improved clinical, biochemical outcome

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PGM1-CDG

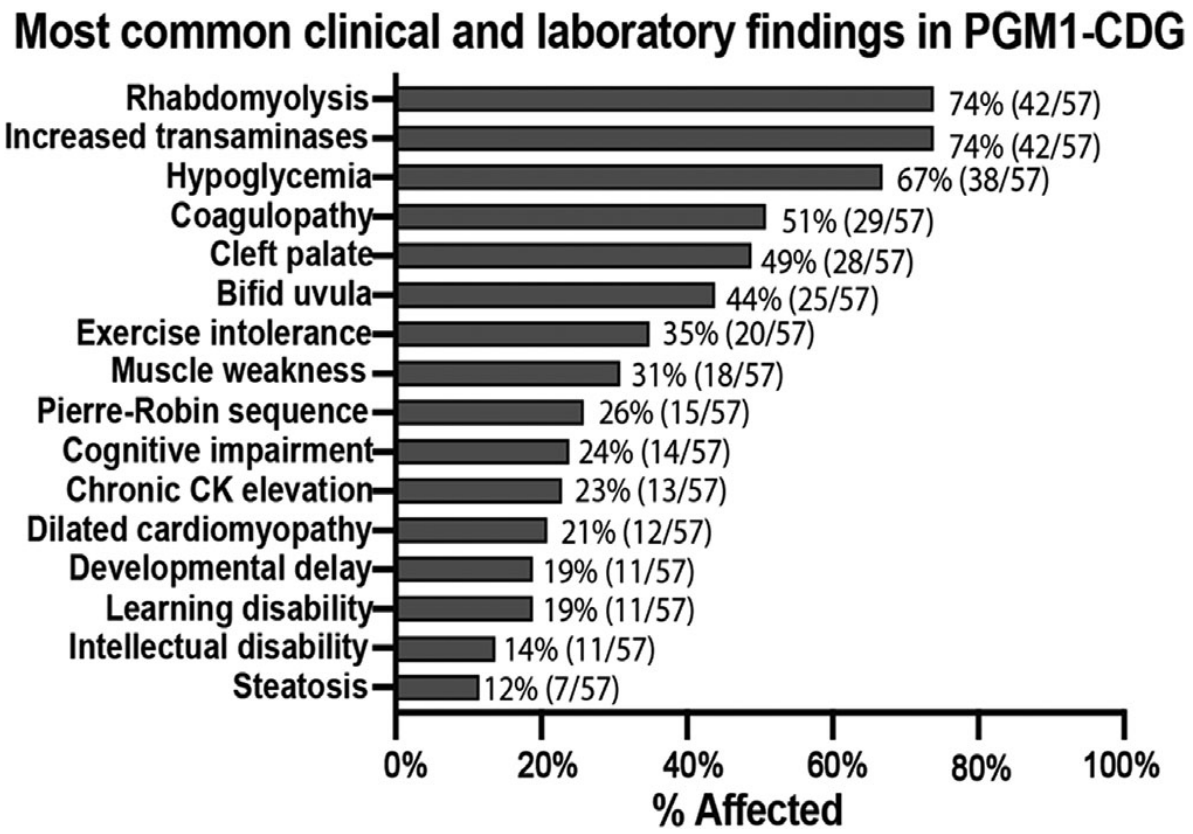

TABLE 1 Clinical presentation in patients with PGM1 deficiency and suggested surveillance

Phenotype		Suggested surveillance frequency
Congenital malformations	Cleft palate, micrognathia, bifid uvula, Pierre Robin sequence, vertebral malformations, anal atresia	Complete physical examination at the time of diagnosis and referral to necessary services
Neurological	Cognitive delay, seizure	Complete physical examination at the time of diagnosis and yearly developmental assessment, especially in patients who had suffered hypoglycemia attacks. EEG and brain MRI if clinically indicated
Ophthalmological	Strabismus, abnormal eye movements, nasolacrimal duct obstruction, and/or epiphoria	Eye exam at the time of diagnosis and monitoring if clinically indicated
Endocrine	Hypothyroidism, hypogonadotropic hypogonadism, delayed puberty, hyperinsulinemia	Assessment of growth at the time of diagnosis and on follow-up. Serum levels of IGF-1, IGFBP3, TGB, and TSH at the time of diagnosis and regularly monitored. Serum cortisol and ACTH levels at the time of diagnosis; further on if clinically indicated
Cardiac	Cardiomyopathy (dilated cardiomyopathy), structural, and conductive heart abnormalities	Electrophysiology (ECG) and echocardiography at the time of diagnosis and monitored if clinically indicated. Annual cardiac screening in childhood and adolescence.
Muscle	Exercise intolerance, myopathy, rhabdomyolysis	CK at the time of diagnosis, then if clinically indicated (during acute illnesses); neurophysiological study if clinically indicated
Liver	Elevated transaminases, steatosis, cholestasis, fibrosis, acute hepatic failure	Transaminases and hepatic function at time of diagnosis and monitored regularly
Hematological	Antithrombin III, factors XI, VII, IX, X, and XI deficiencies low proteins C and S, increased PT and prolonged aPPT	Coagulation profiles at the time of diagnosis and monitored regularly
Metabolic	Hypoketotic and ketotic hypoglycemia	Glucose level at the time of diagnosis and during illnesses with urine ketones and insulin levels
Other	Malignant hyperthermia	Caution is advised with anesthesia prior to surgeries

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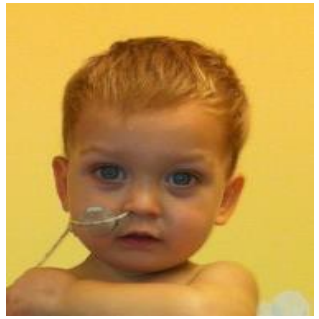
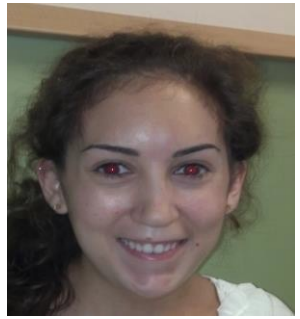
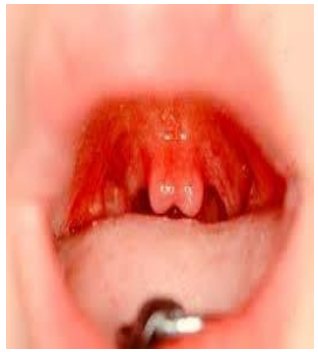
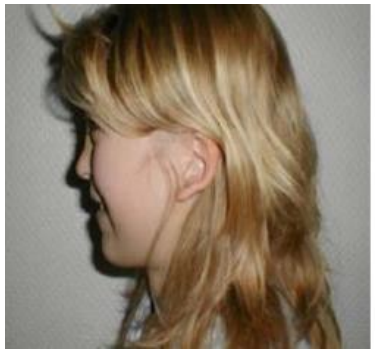
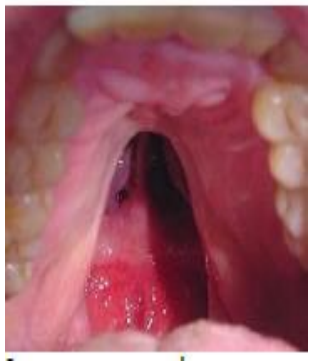
International consensus guidelines for phosphoglucomutase 1 deficiency (PGM1-CDG): Diagnosis, follow-up, and management. Altassan, R et al, J Inherit Metab Dis. 2020;1–16.

FCDGC

Phosphoglucomutase 1 deficiency (PGM1-CDG)



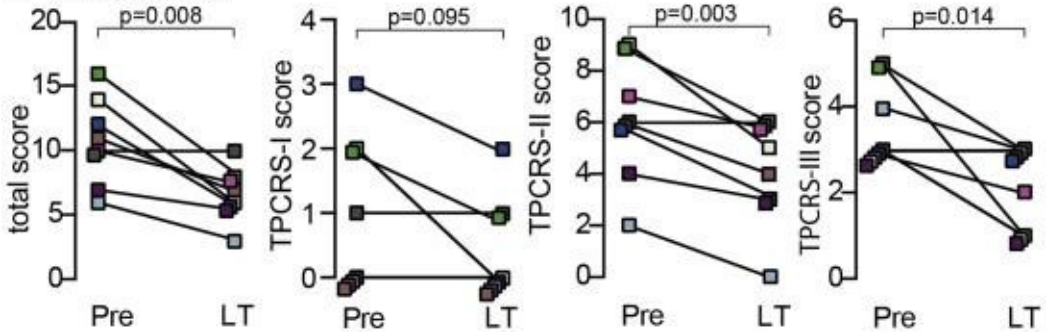
Hypoglycemia, hyperinsulinism, bleeding disorder

cardiomyopathy

A clinical progression scores

Person #	Total	TPCRS I: Vision	Hearing	Communication	Feeding	Mobility	TPCRS II: Seizures	Enceph	Hemostatic	GI	Endocrine	Respiratory	Cardiac	Renal	Liver	Blood	Congenital malformation	TPCRS III: Growth	Vision with glasses	Strabismus, abnormal eye movement	Myopathy	Ataxia	Pyramidal	Extrapyramidal	Neuropathy
Pre-galactose treatment																									
P2	12	0	0	0	3	0	0	0	1	1	1	0	0	0	0	0	3	3	0	0	0	0	0	0	0
P7	10	0	0	1	0	0	0	0	1	0	2	0	0	0	1	0	2	3	0	0	0	0	0	0	0
P8	11	0	0	0	0	0	1	0	0	1	0	0	0	0	1	0	3	3	0	0	2	0	0	0	0
P9	7	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	3	3	0	0	0	0	0	0	0
P10	6	0	0	0	0	0	1	0	0	0	0	0	0	0	1	0	0	3	0	0	1	0	0	0	0
P11	16	0	0	1	0	1	0	0	1	0	2	0	2	0	2	0	2	3	0	0	2	0	0	0	0
P12	10	0	0	0	0	0	0	0	3	0	0	0	0	0	1	0	3	1	0	0	2	0	0	0	0
P13	14	0	0	0	2	0	0	0	1	1	2	0	2	0	0	0	3	2	0	0	1	0	0	0	0
Long-Term galactose treatment																									
P2	6	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	3	1	0	0	0	0	0	0	0
P7	10	0	0	1	0	0	0	0	1	0	2	0	0	0	1	0	2	3	0	0	0	0	0	0	0
P8	7	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	3	3	0	0	0	0	0	0	0
P9	6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	3	0	0	0	0	0	0	0
P10	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	0	0	0	0	0	0	0
P11	8	0	0	1	0	0	0	0	1	0	1	0	2	0	0	0	2	1	0	0	0	0	0	0	0
P12	8	0	0	0	0	0	0	0	3	0	0	0	0	0	0	0	3	1	0	0	1	0	0	0	0
P13	6	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	3	1	0	0	0	0	0	0	0

B TPCRS scores

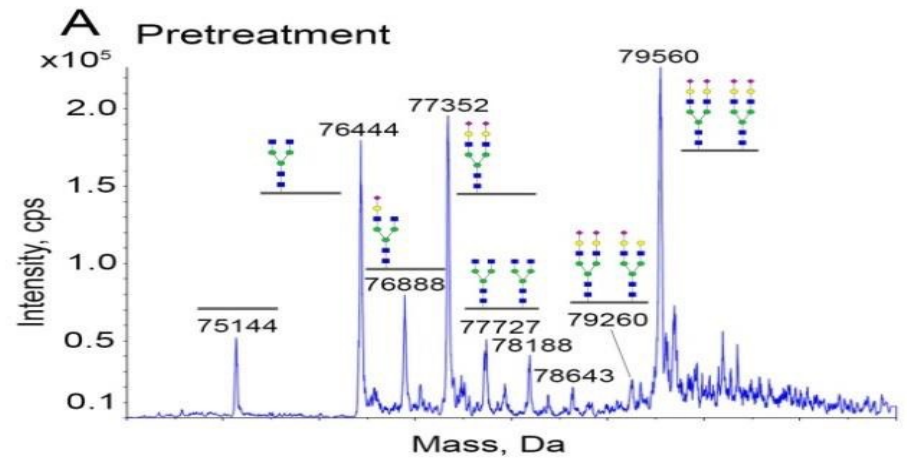


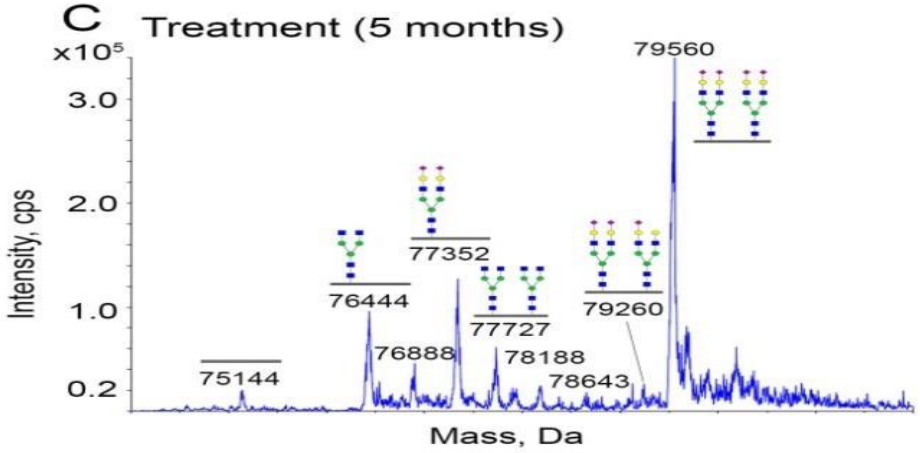
Radenkovic et al, AJHG, 2019

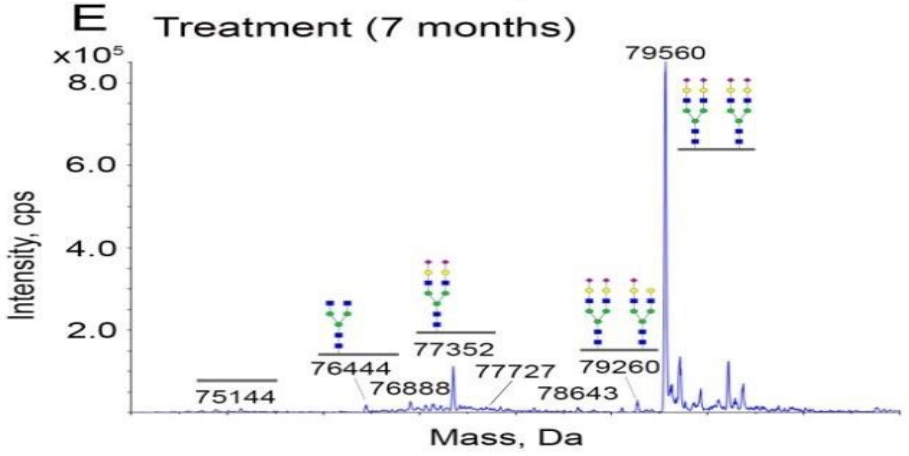
Courtesy of Eva Morava

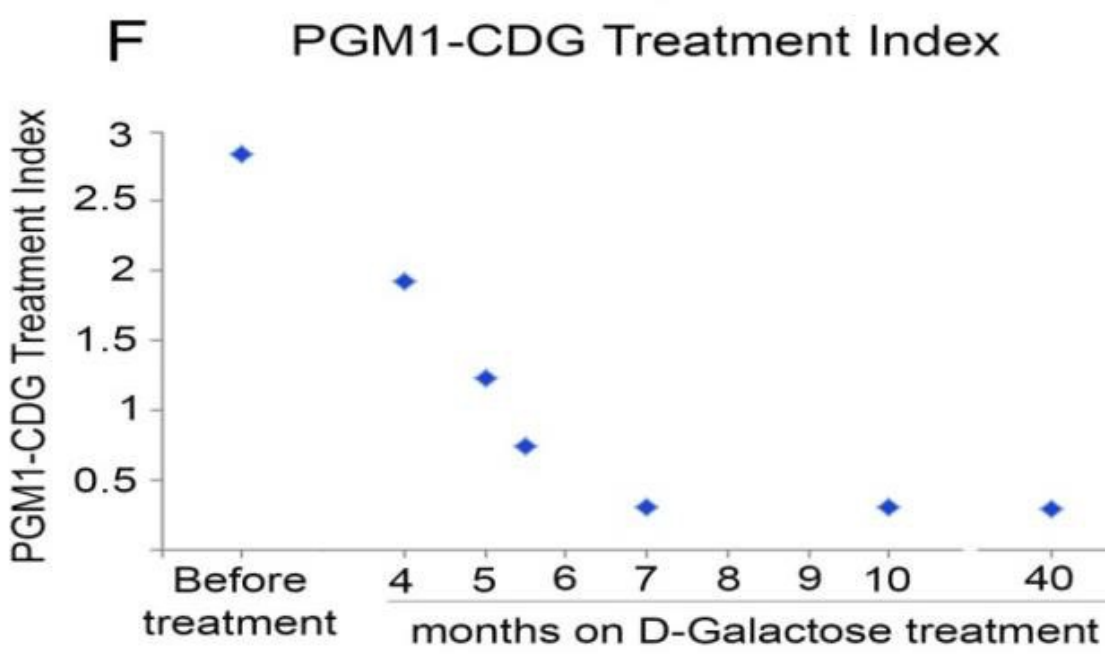
PGM1-CDG: Galactose Therapy

- 16 PGM1-CDG patients (six females and ten males; 4 mo- 26 yr at dx
- 18 weeks (weeks 0-6:0.5 g/kg/day; weeks 6-12:1.0 g/kg/day; weeks 12-18:1.5 g/kg/day)
- safe and well tolerated
- Transferrin pattern clearly improves









Preparing Phase 2 clinical trials design in progress

Courtesy of Eva Morava

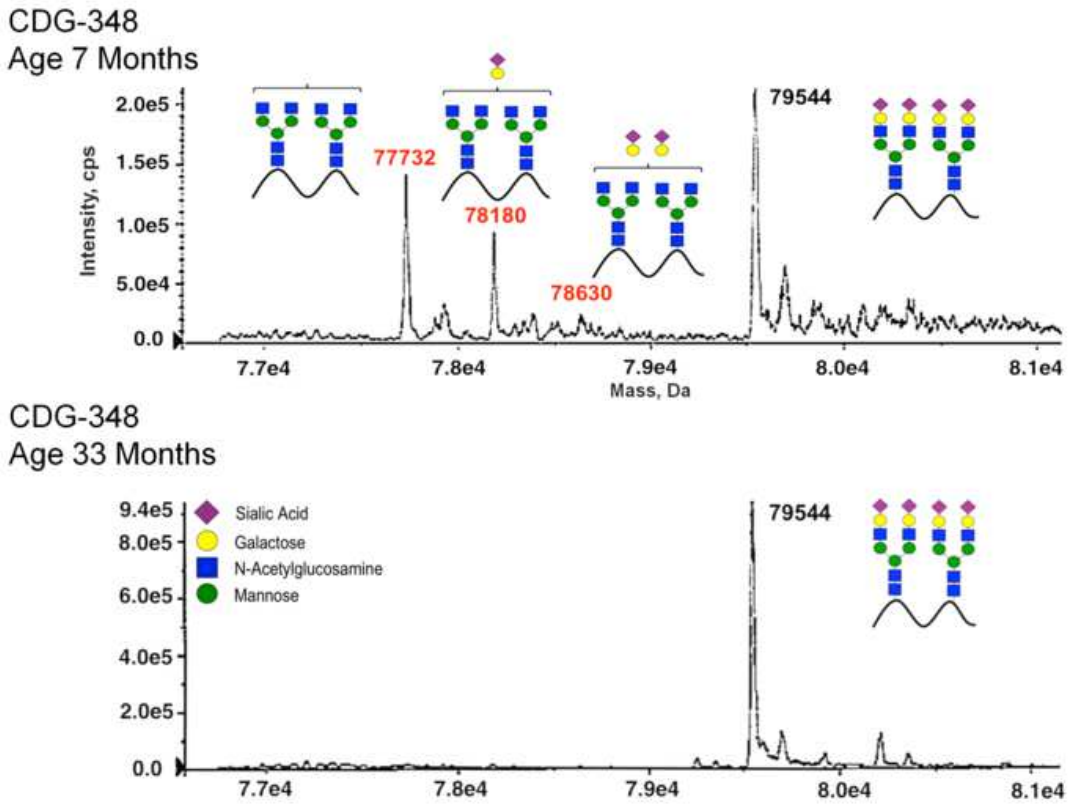
Perales-Clemente, et al J Inherit Metab Dis. 2021 Sep;44(5):1263-1271. A new D-galactose treatment monitoring index for PGM1-CDG

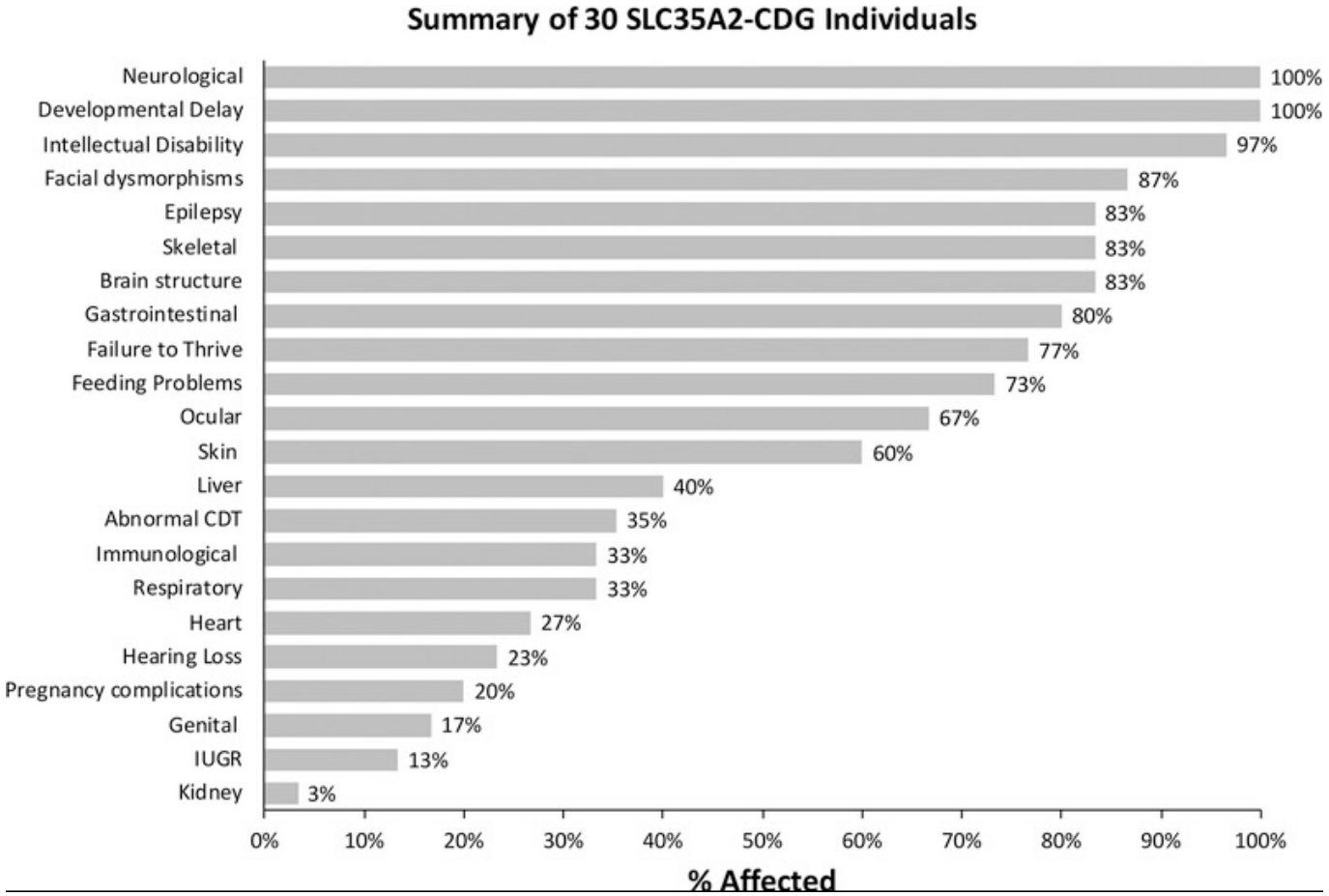
PGM1 was originally categorized as a glycogen storage disorder

- Recent work shows that glycogen in the brain is 20% glucosamine (GlcN), NOT only glucose
- Brain Glycogen contributes to protein glycosylation
- LaFora Disease—a glycogen degradation disorder, shows abnormal N-glycans
- Perhaps other GSD's are also CDG?
- Uncontrolled HFI and Galactosemia patients have abnormal transferrin.

SLC35A2—deficiency in X-linked UDP-Galactose Transporter

Hum Mutat. 2019 Jul;40(7):908-925 Ng et al, 30 cases





Additional cases identified in large epilepsy cohorts

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SLC35A2-CDG

- Ten patients with SLC35A2-CDG were supplemented with oral D-galactose for 18 weeks in escalating doses up to 1.5 g/kg/day.
- Improvements were primarily in growth and development with five patients resuming developmental progress, including postural control, response to stimuli, and chewing and swallowing amelioration.

Clinical and biochemical improvement with galactose supplementation in SLC35A2-CDG. Witters, et al Genet Med. 2020 June ; 22(6): 1102–1107

Clinical Trials not yet recruiting

Sanford Burnham Prebys

FCDGC

SOMATIC MUTATIONS IN SLC35A2 CAUSE EPILEPSY

Possible response to galactose?

Multiple studies now show cases of *de novo* SLC35A2 variants

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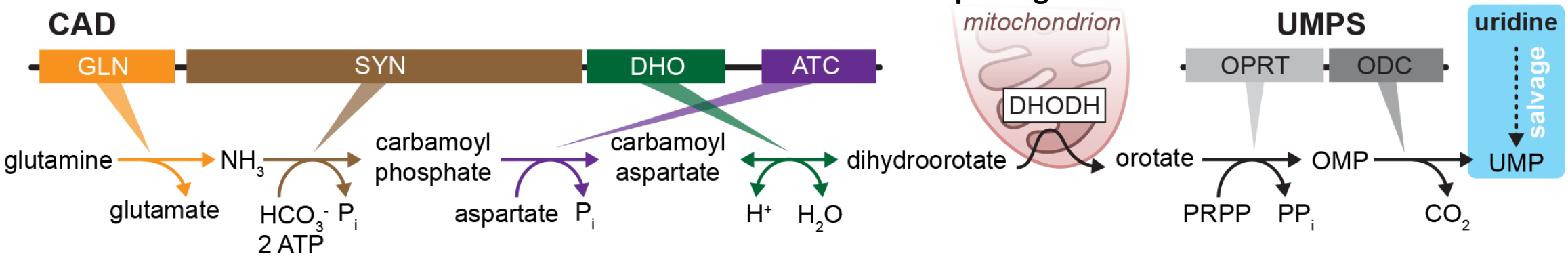
Winower, et al Ann Neurol . 2018 Jun;83(6):1133-1146. Somatic SLC35A2 variants in the brain are associated with intractable neocortical epilepsy

FCDGC

CAD-Dependent Synthesis of Pyrimidines

Francisco del Caño Ochoa, Bobby G. Ng, Hudson Freeze and Santiago Ramon

CAD-2250 aa and 1000 variants. Which are pathogenic?



ORIGINAL ARTICLE

Biallelic mutations in *CAD*, impair *de novo* pyrimidine biosynthesis and decrease glycosylation precursors

Bobby G. Ng[1,†], Lynne A. Wolfe[2,†], Mie Ichikawa[1], Thomas Markello[2], Miao He[4], Cynthia J. Tifft[2,3], William A. Gahl[2,3] and Hudson H. Freeze[1,*]

[1]Human Genetics Program, Sanford - Burnham Medical Research Institute, 10901 N. Torrey Pines Rd, La Jolla, CA 92037, USA, [2]NIH Undiagnosed Diseases Program, Common Fund, Office of the Director and [3]National Human Genome Research Institute, National Institutes of Health, Bethesda, MD 20892, USA and [4]Department of Pathology and Laboratory Medicine, Children's Hospital of Philadelphia, Philadelphia, PA 19103, USA

BRAIN
A JOURNAL OF NEUROLOGY

REPORT

***CAD* mutations and uridine-responsive epileptic encephalopathy**

Johannes Koch,[1,*] Johannes A. Mayr,[1,*] Bader Alhaddad,[2] Christian Rauscher,[1] Jörgen Bierau,[3] Reka Kovacs-Nagy,[2] Karlien L. M. Coene,[4,5,6] Ingrid Bader,[1] Monika Holzhacker,[1] Holger Prokisch,[2,7] Hanka Venselaar,[5] Ron A. Wevers,[4] Felix Distelmaier,[8] Tilman Polster,[9] Steffen Leiz,[10] Cornelia Betzler,[11] Tim M. Strom,[2,7] Wolfgang Sperl,[1] Thomas Meitinger,[2,7,12] Saskia B. Wortmann[1,2,7,*] and Tobias B. Haack[2,7,†,*]

SBP

Beyond genetics: Deciphering the impact of missense variants in CAD deficiency

del Caño-Ochoa, Ng, ... , Wortmann, Freeze, Ramón-Maiques *(2023)*

Epileptic encephalopathy
Developmental regression
Normocytic anemia
Lethal without treatment

Missense variants found in CAD gene after whole exome sequencing

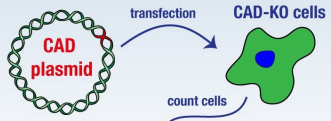
Variants validated as pathogenic or benign for CAD activity

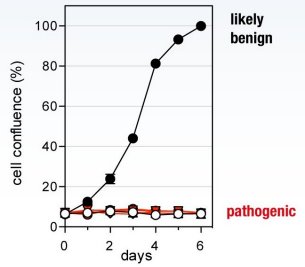
Which is the molecular mechanism of the disease?

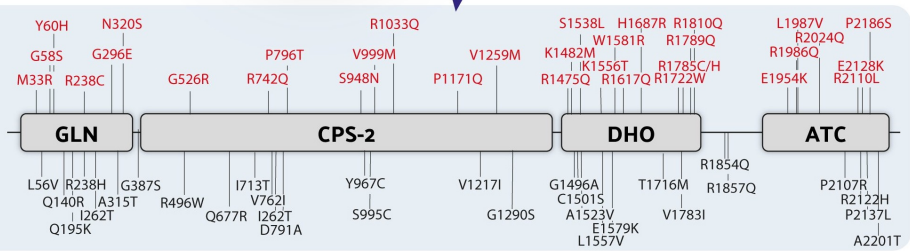
CAD deficiency (OMIM #616457)

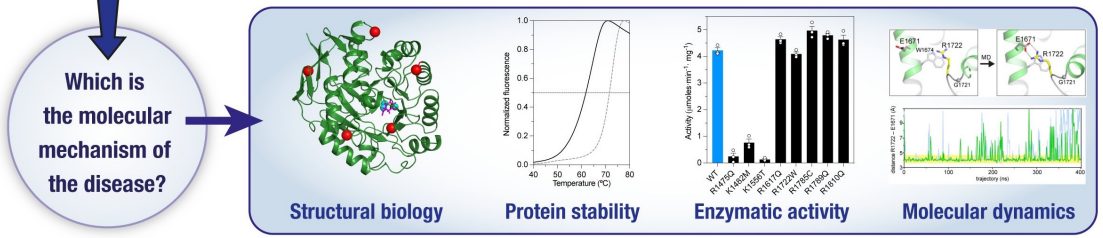
- Simple oral treatment
- > 1000 missense CAD variants
- Rare disease (50 patients in the world)
- Little knowledge about CAD
- No biomarkers
- Defects on pyrimidine synthesis

Cell proliferation assay to classify missense CAD variants





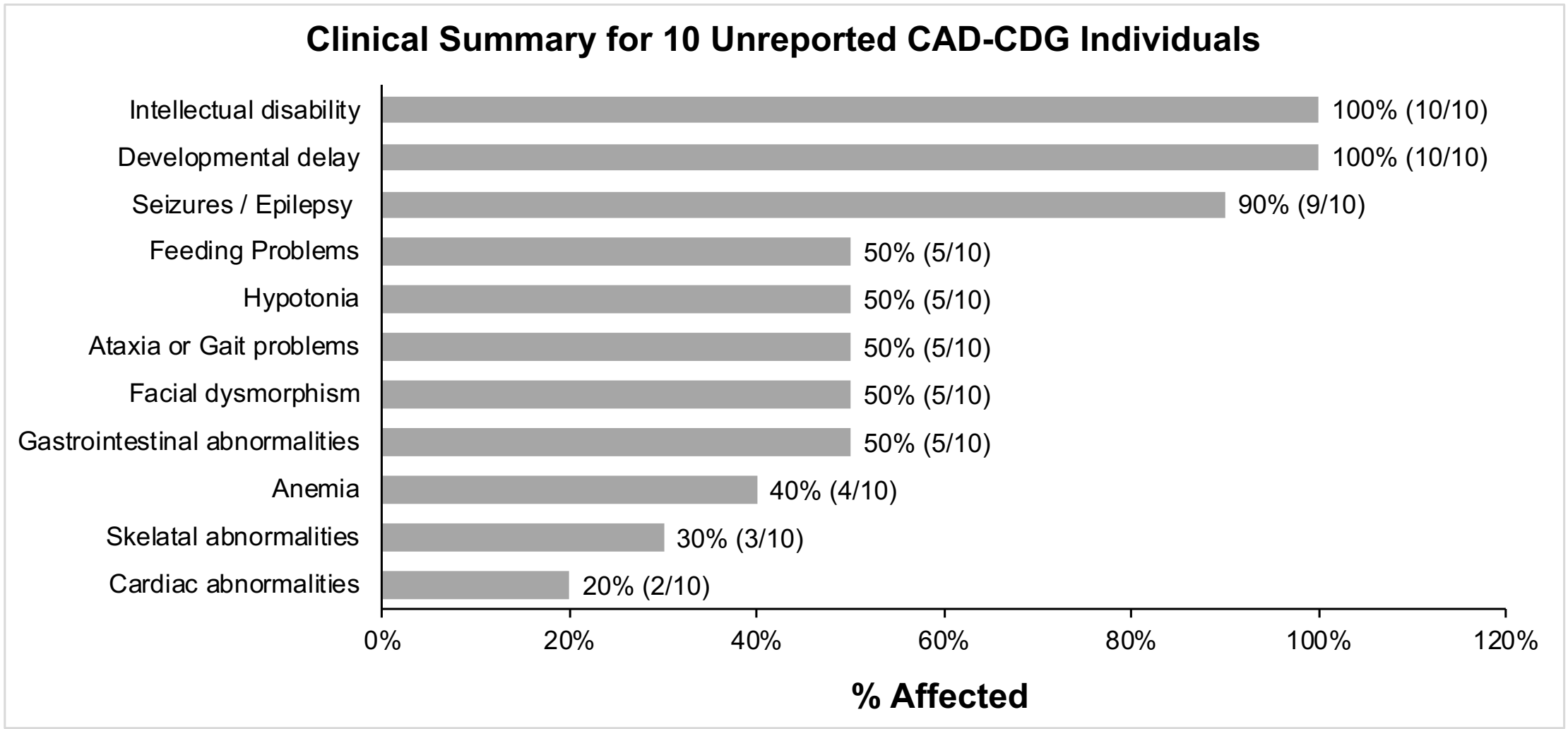




Integrating structural knowledge with clinical and functional genomics can accelerate the diagnosis and treatment of new CAD-deficient patients

https://doi.org/10.1002/jimd.12667

WILEY SSIEM JIMD JOURNAL OF INHERITED METABOLIC DISEASE


Clinical Summary for 10 Unreported CAD-CDG Individuals
Intellectual disability
Developmental delay
Seizures / Epilepsy
Feeding Problems
Hypotonia
Ataxia or Gait problems
Facial dysmorphism
Gastrointestinal abnormalities
Anemia
Skelatal abnormalities
Cardiac abnormalities
100% (10/10)
100% (10/10)
90% (9/10)
50% (5/10)
50% (5/10)
50% (5/10)
50% (5/10)
50% (5/10)
40% (4/10)
30% (3/10)
20% (2/10)
0%
20%
40%
60%
80%
100%
120%
% Affected

EFFECTS OF TRIACETYLURIDINE THERAPY

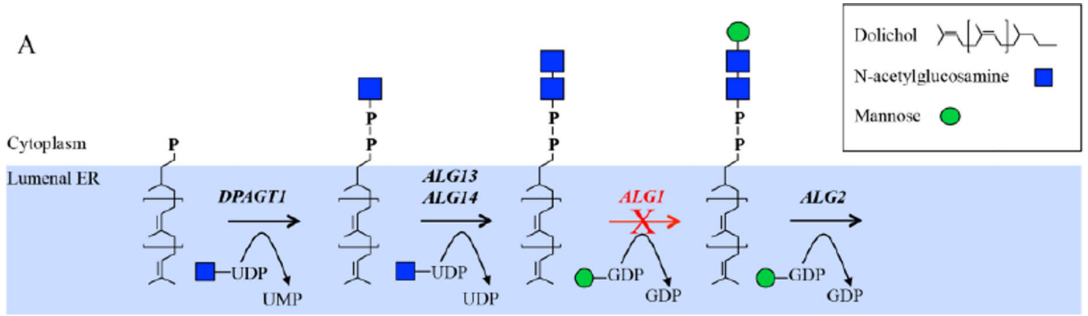
Multiple studies show efficacy of triacetyluridine (Xuriden), uridine, or uridine-monophosphate

Ann Clin Transl Neurol. 2021 Mar;8(3):716-722
Genet Med. 2020 Oct;22(10):1589-1597.
Pediatr Neurol. 2020 Sep;110:97-98

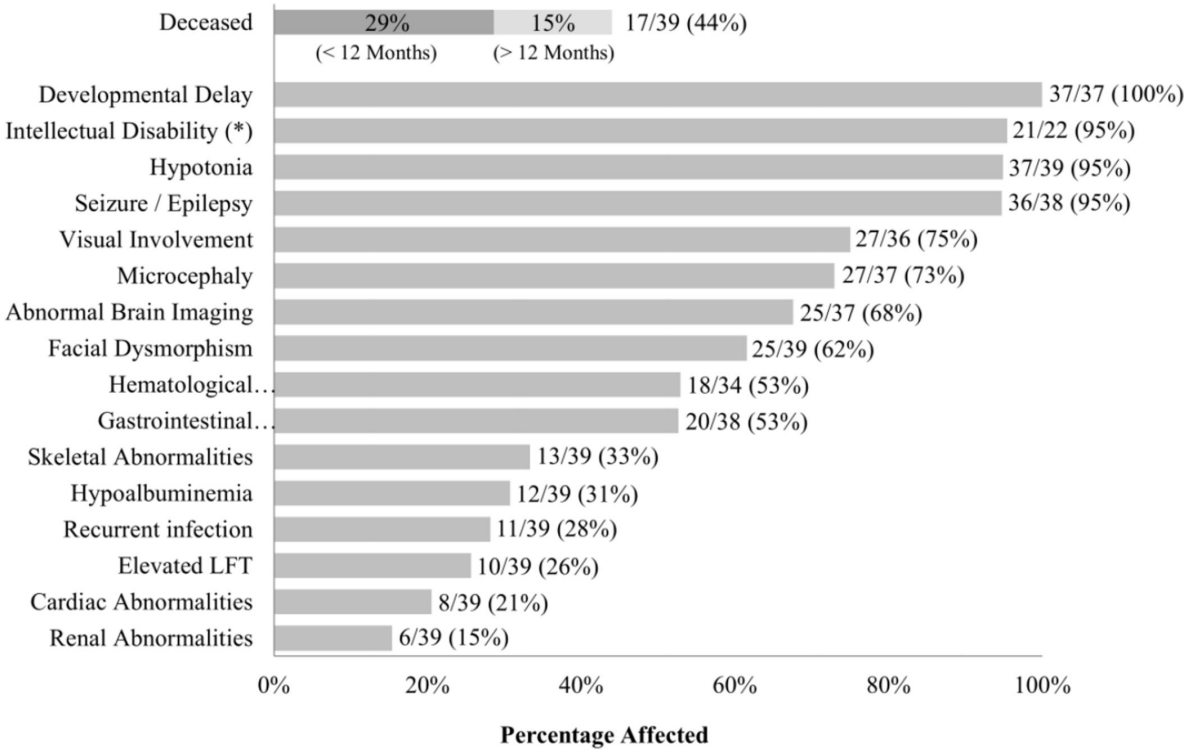
No apparent side effects
Provide Realistic hope

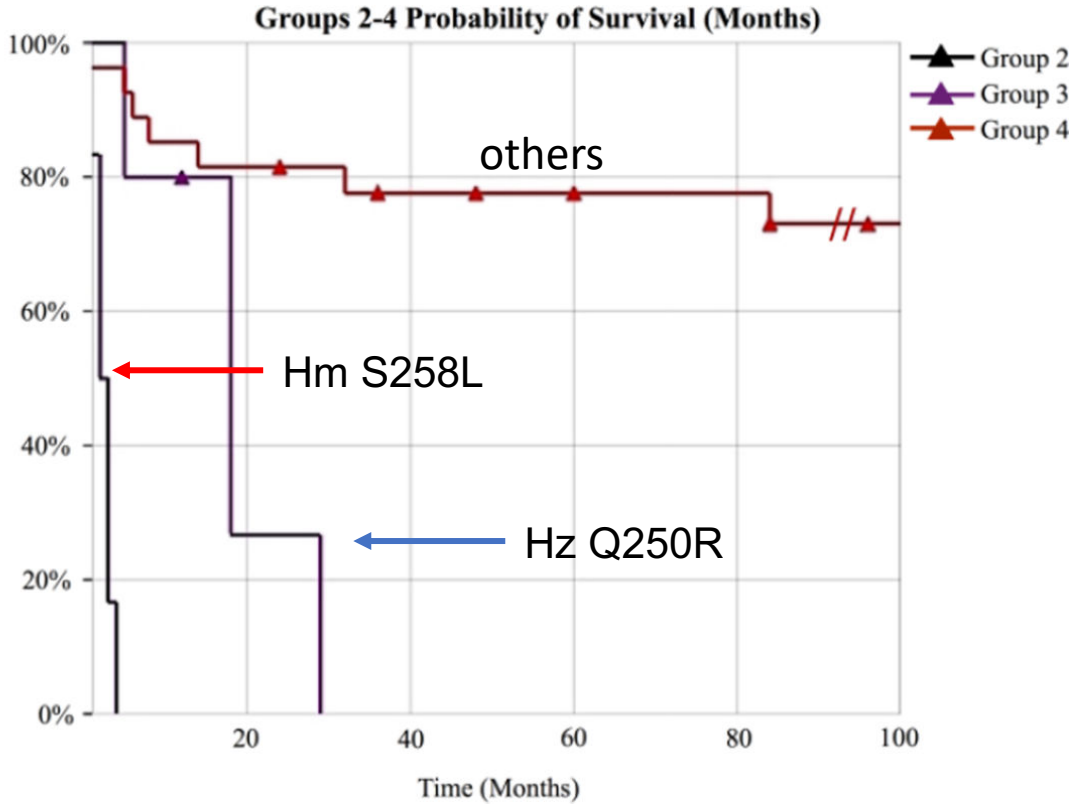
ALG1-CDG

ALG1-CDG: Clinical and molecular characterization of 39 unreported patients



Clinical Summary of ALG1-CDG Cases

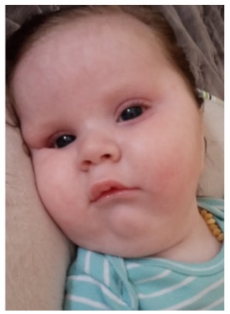




SPECTRUM OF ALG1-CDG CASES

CDG-330S
p.Q50R , p.S258L

CDG-330Z
p.Q50R , p.S258L

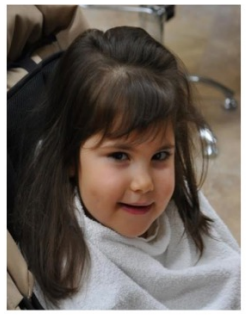


CDG-427
p.C5X , p.Q50R

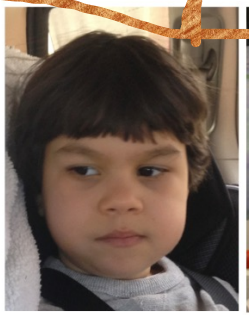
CDG-345
p.Q50R , p.I209S

CDG-247J
p.P98L , p.S258L

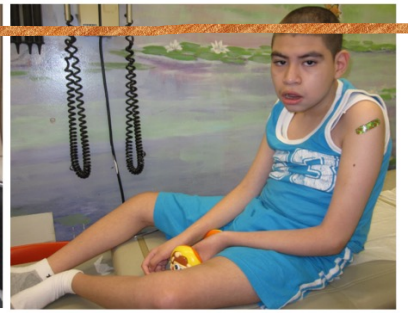
CDG-247A
p.P98L , p.S258L



CDG-286
p.V281F , p.Y353D



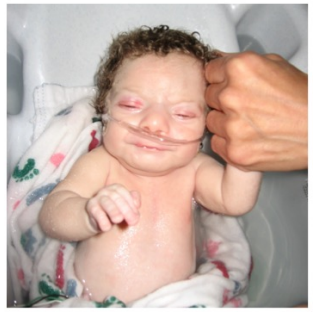
CDG-320
p.T64N, p.S71F,
p.H74L, p.A360V



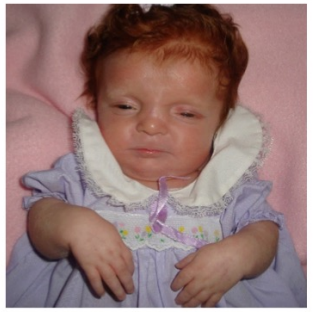
CDG-003
p.D289G , p.R438W

p.P98L , p.S258L

p.P98L , p.S258L



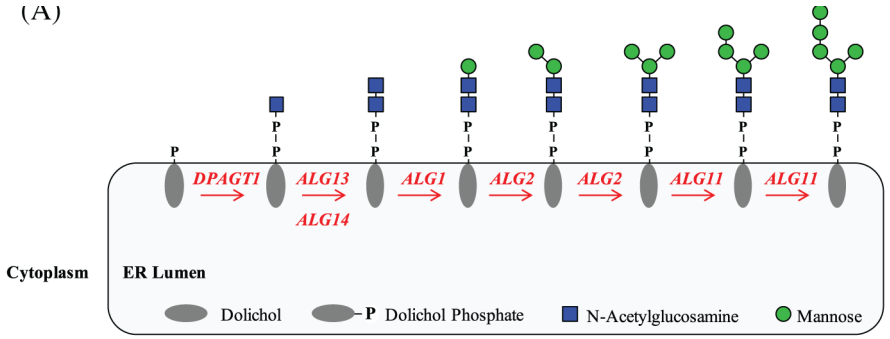
CDG-371
p.S258L , p.S258L

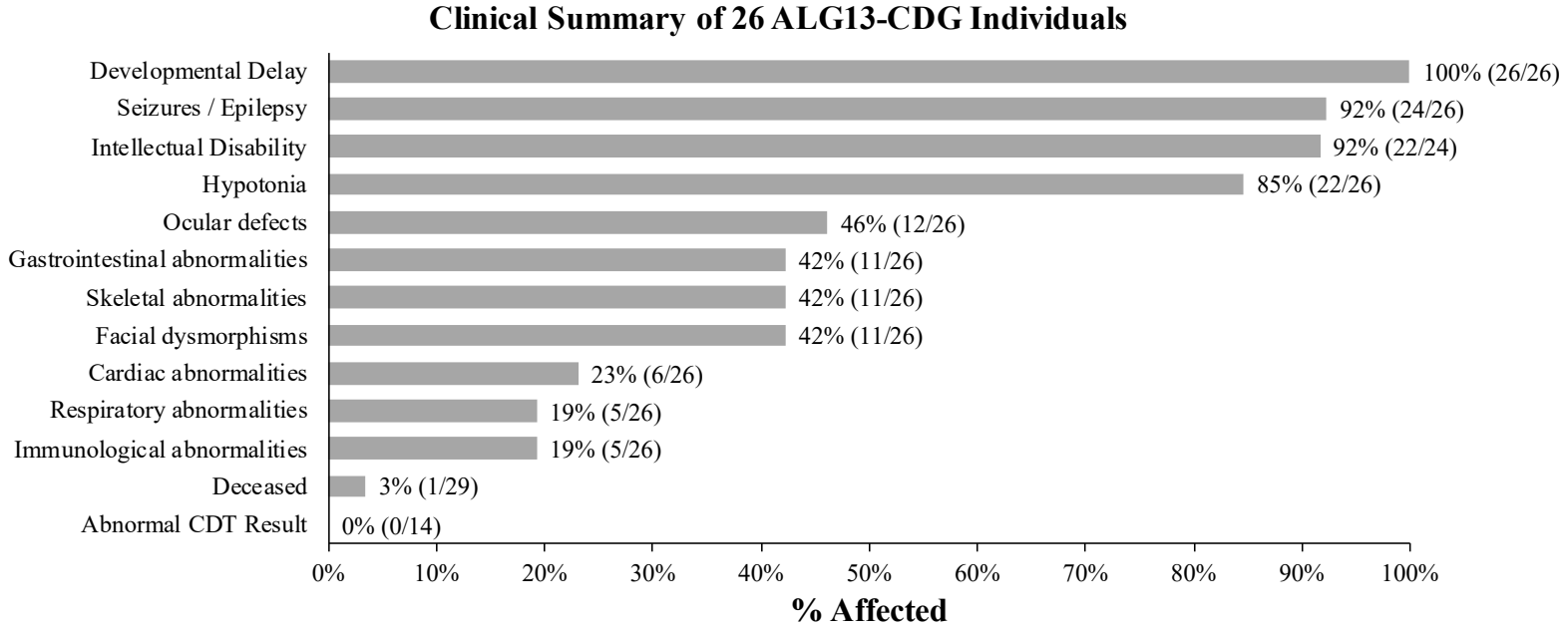


CDG-337
p.S258L , p.S258L

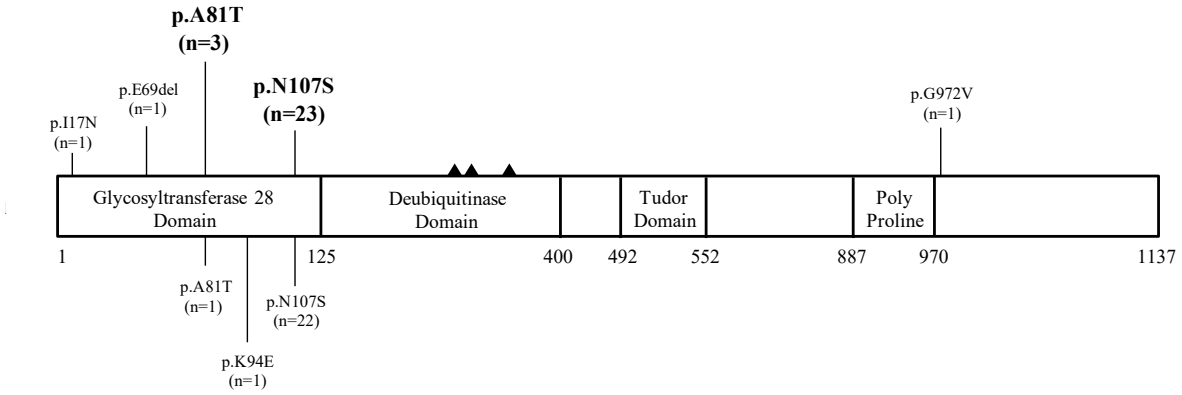
ALG13– X-LINKED *de novo* N107S variant in ~80% of cases

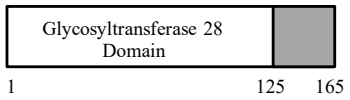
ALG13. Ng et al





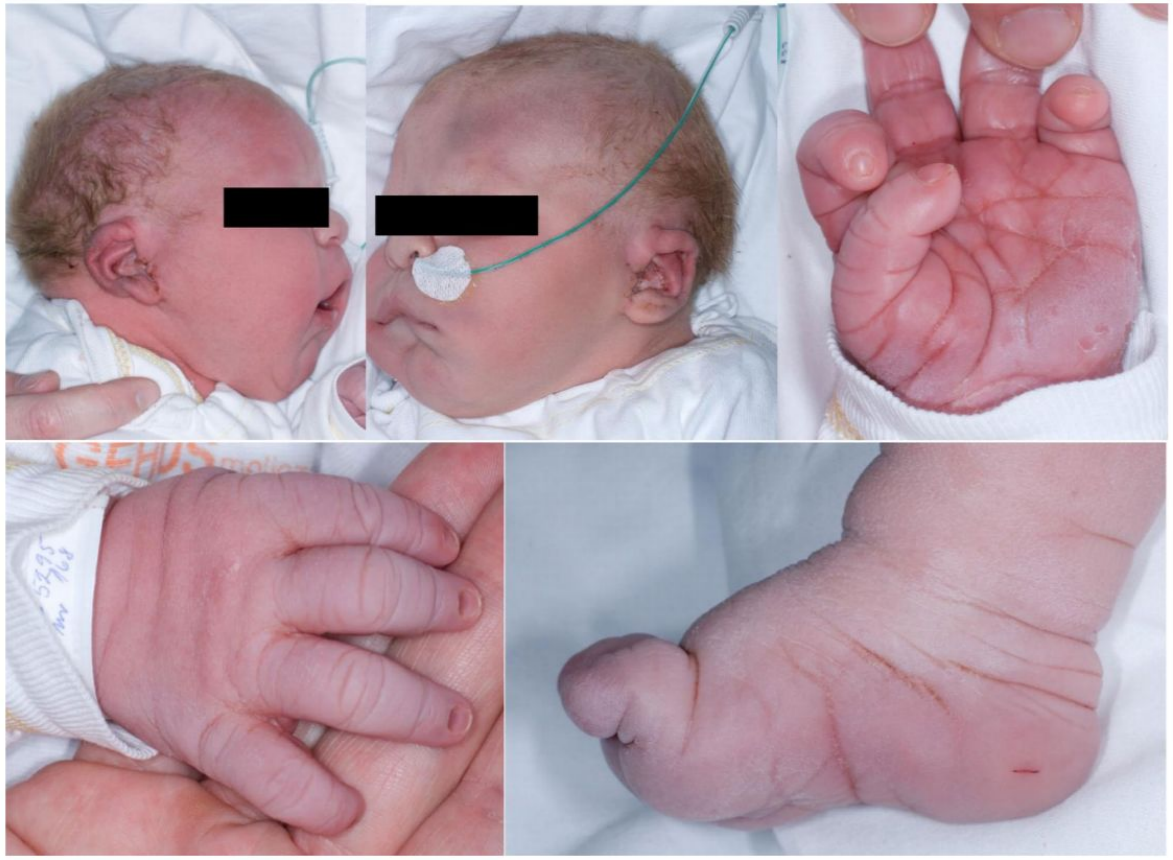
ALG13 PATIENTS DO NOT SHOW THE EXPECTED N-GLYCAN DEFICIENCY AND TRANSFERRIN IS NOT AN INDICATOR





GPI-anchor disorders

- Glycophosphatidylinositol synthesis disorders (*PIGA*, *PIGN, PIGO*, etc)
- GPI-anchors are membrane-embedded lipids that attach to cell-surface proteins via a glycan linker
- Most with severe seizures and developmental delay
- Some with elevated serum alkaline phosphatase,decreased in others.
- Somatic mutations in *PIGA* cause hemolytic anemia

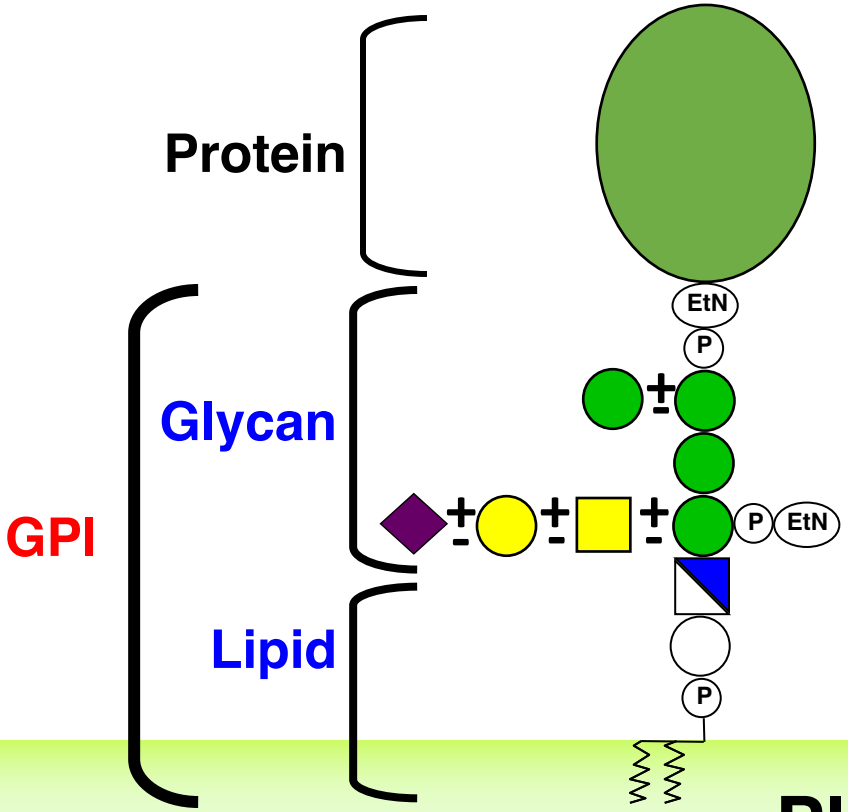


Bayat, Allan, et al. "Lessons learned from 40 novel PIGA patients and a review of the literature." *Epilepsia* (2020).

Courtesy of Austin Larson,

Glycosyl phosphatidyl inositol (GPI) - anchored proteins

>150 various human proteins:



- Enzymes
- Receptors
- Adhesion molecules
- Complement regulators
- Prion protein etc.

GPI essential for:

- Embryogenesis
- Neurogenesis
- Immune system
- Fertilization

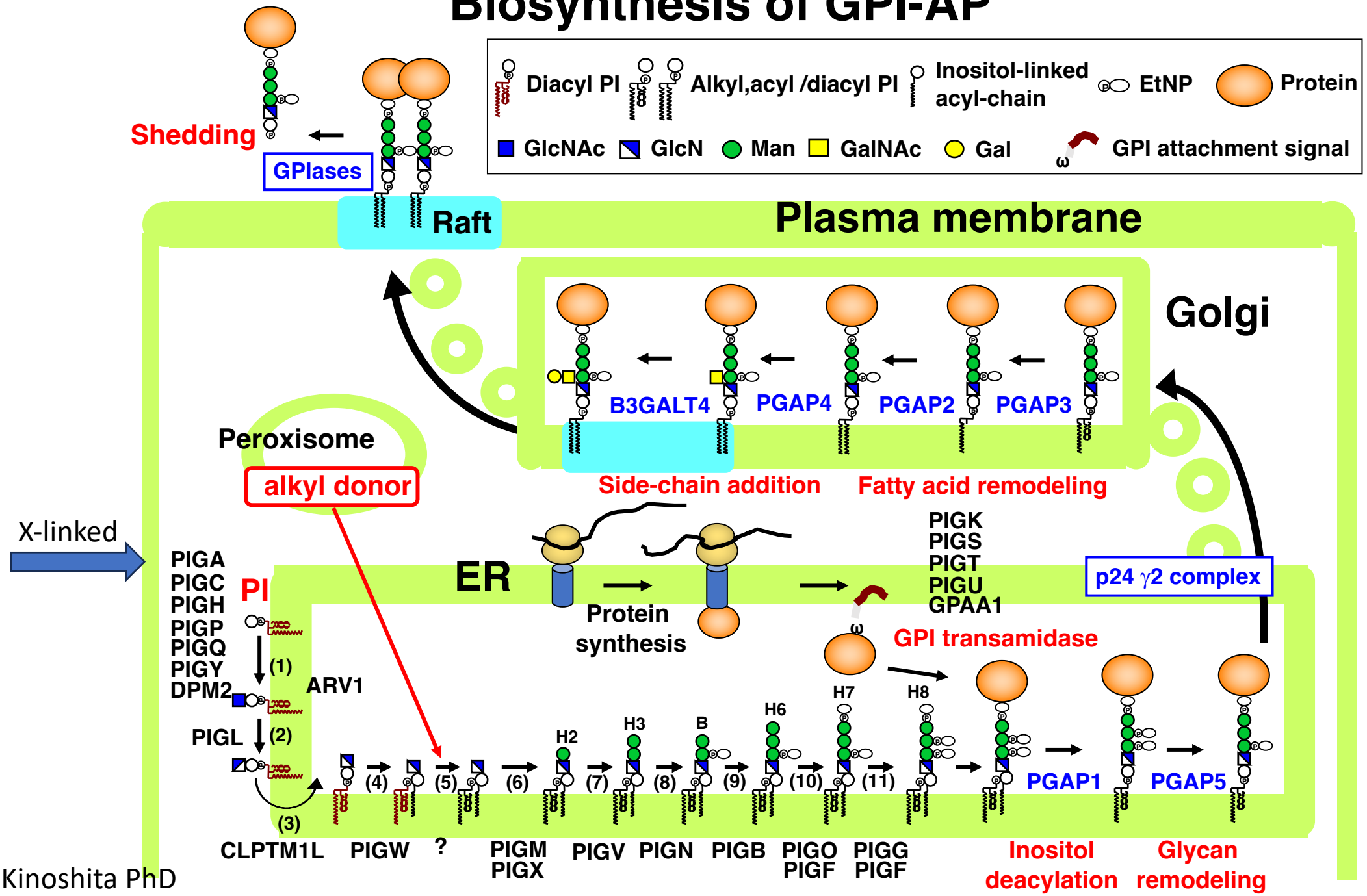
Plasma membrane

Glucosamine | Ethanolamine phosphate | Phosphatidylinositol

Sialic acid | Galactose | N-acetylgalactosamine | Mannose

Taroh Kinoshita PhD

Biosynthesis of GPI-AP



Taroh Kinoshita PhD

Mutated genes vs syndromes

Taroh Kinoshita PhD

Gene	Syndrome
PIGV, PIGO, PIGB, PGAP2, PGAP3	Mabry syndrome / Hyperphosphatasia mental retardation syndrome (HPMRS)
PIGA, PIGN, PIGT	Multiple Congenital Anomalies-Hypotonia-Seizure (MCAHS) syndrome
PIGL	CHIME syndrome
PIGN, PIGV	Fryns syndrome
PIGB	DOORS syndrome
PGAP1	IGD with DD / ID, encephalopathy, CVI
PIGM (promotor)	IGD with seizures and thrombosis

CHIME: coloboma, heart disease, ichthyosiform dermatosis , mental retardation, and ear anomalies

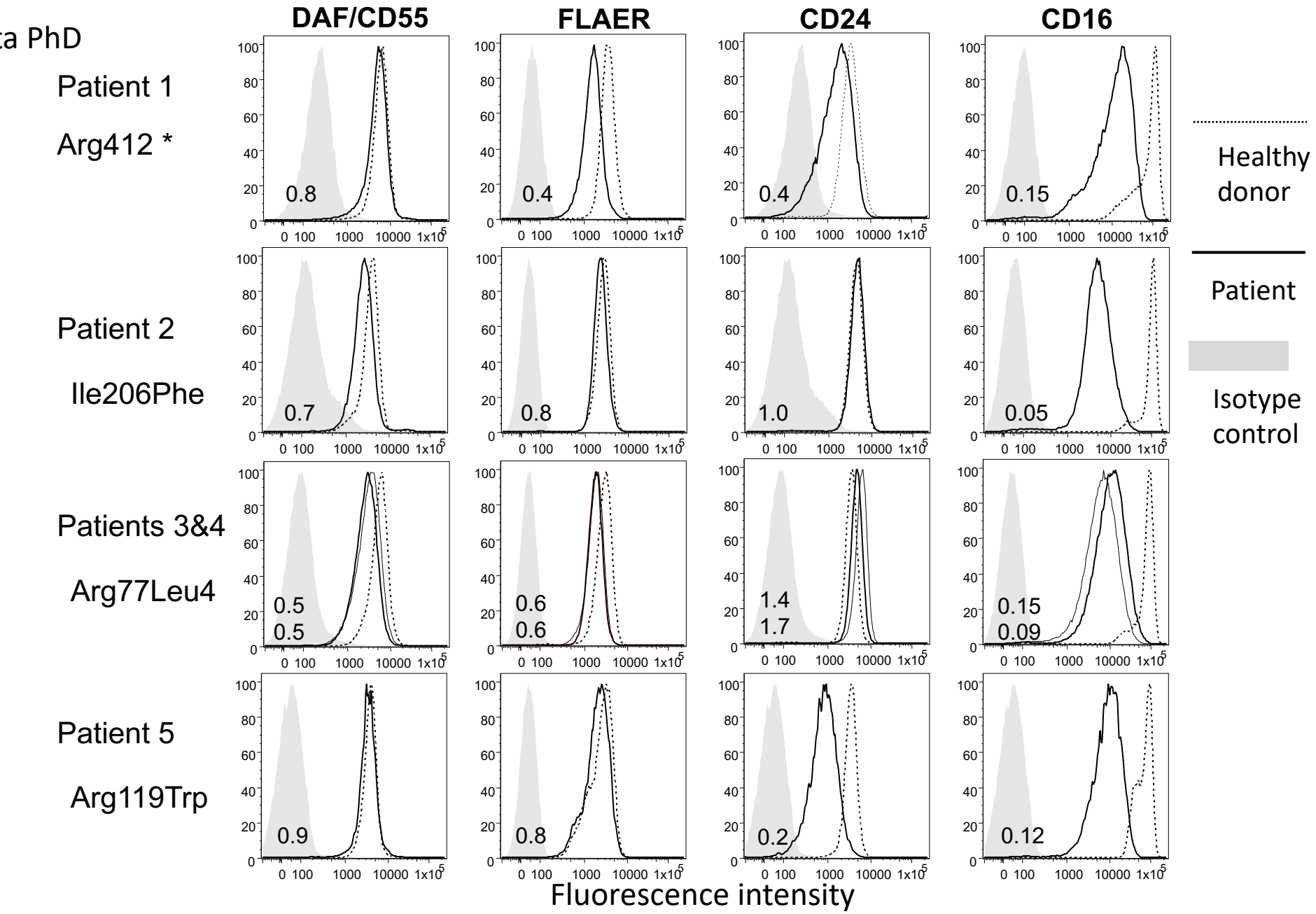
Fryns: dysmorphic facial features, congenital diaphragmatic hernia, pulmonary hypoplasia, and distal limb hypoplasia

DOORS: deafness, onychodystrophy, osteodystrophy, mental retardation, and seizures

DD, developmental delay; CVI, cerebral visual impairment

GPI-AP levels on blood granulocytes from PIGA-IGD patients

Taroh Kinoshita PhD



DYSTROGLYCANOPATHIES

mAb recognizes this glycan

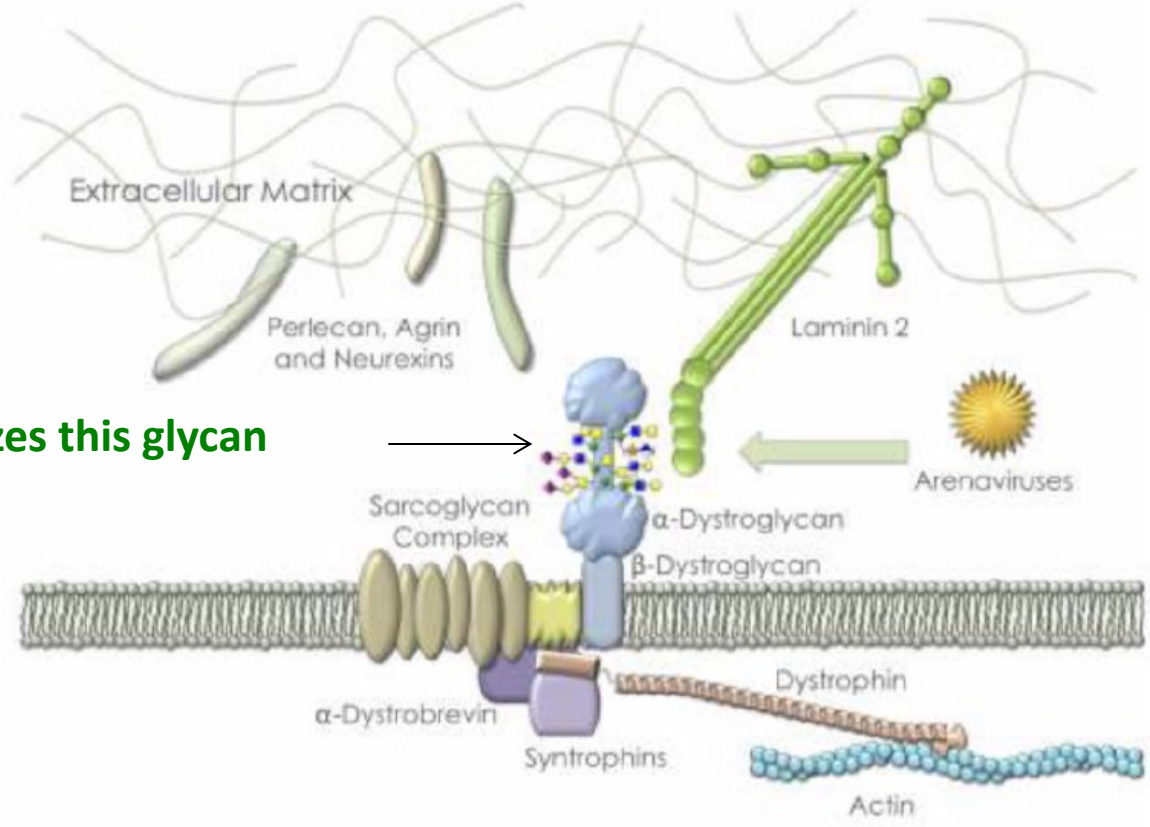
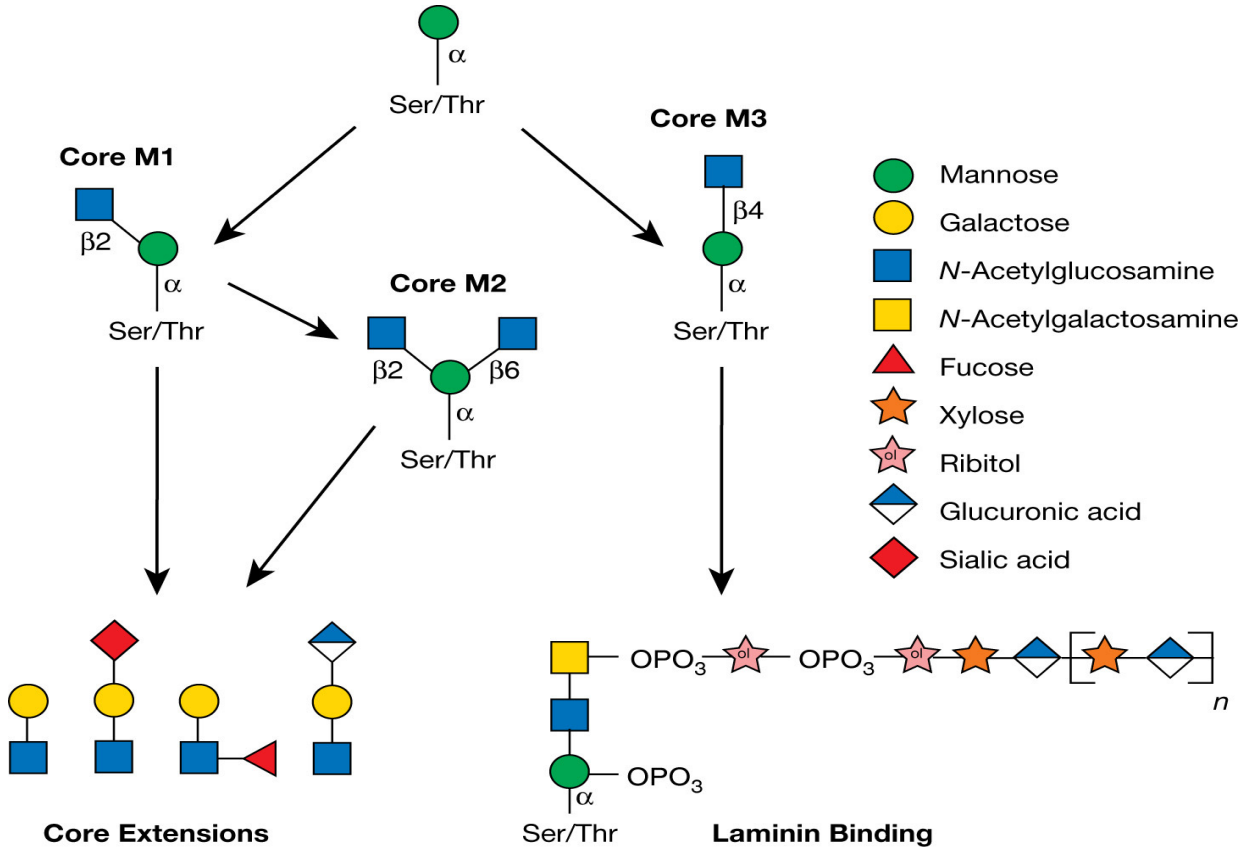


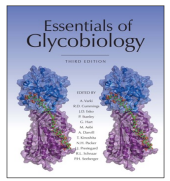
Figure 1. The Dystrophin-Glycoprotein Complex
This multi-protein complex serves to anchor the extracellular matrix (ECM) to actin and other components of the cytoskeleton. O-mannosylated α-DG is a central component of this complex and serves as the binding partner for a number of extracellular matrix proteins (including laminin, perlecan, agrin and neurexin) and also as a receptor for certain members of the Arenaviridae family of viruses. The interaction between α-DG and its binding partners is glycan-mediated and disruption is linked to a number of congenital muscular dystrophies and has been implicated in tumor cell metastasis.

Biosynthetic Pathway for O-Mannose Glycans



Chapter 13, Figure 5. Essentials of Glycobiology, Third Edition

Symbol Nomenclature for Glycans (SNFG)



Dystroglycanopathies

- Dystroglycan is a protein that links the muscle membrane with the extracellular matrix
- If it dysfunctions, then the result is muscular dystrophy
- Dystroglycan is heavily O-mannosylated
- Dysfunction of the O-mannosylation pathway leads to dystroglycanopathy
- Milder disease is called limb-girdle muscular dystrophy
- Severe disease is called Muscle-Eye-Brain Disease, Walker-Warburg or Congenital Muscular Dystrophy

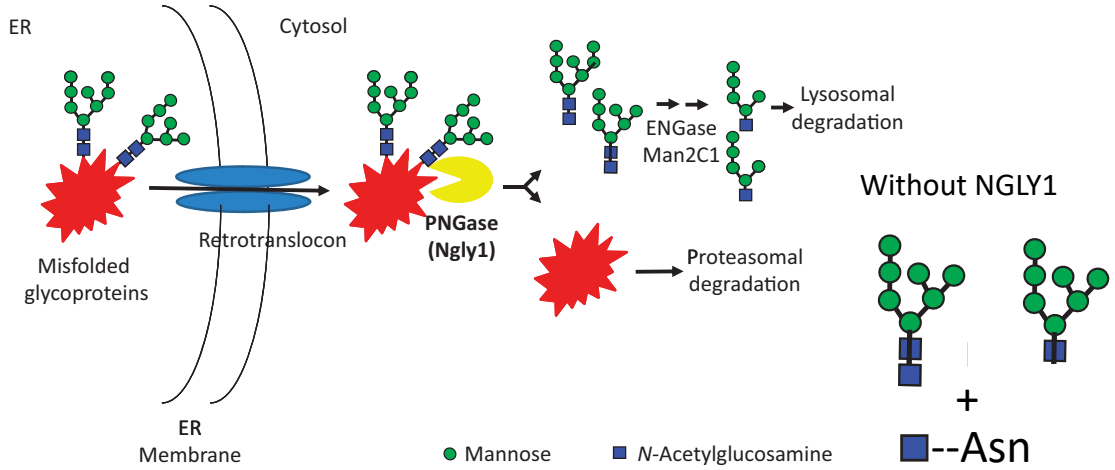
Unique Features

- Glycan specific mAb
- Glycan Contains ribitol-5-P
- FKRP is most common LGMD
- Model systems show improvement treating with ribitol supplements
- Adenovirus treatment ISPD gene therapy shows positive effects
- Combination is synergistic

Sanford Burnham Prebys

FCDGC

NGLY1—A CONGENITAL DISORDER OF DEGLYCOSYLATION



- N-glycanase enzyme removes N-glycans from proteins in the ER prior to degradation
- A congenital disorder of deglycosylation (CDDG)
- Clinical features are: developmental delay, microcephaly, seizures, alacrima, LFTs normalize with age

Suzuki T, Huang C, Fujihira H. The cytoplasmic peptide:N-glycanase (I Structure, expression and cellular f Gene. 2016 Feb 10;577(1):1-7.

NGLY1

Lam, C., Ferreira, C., Krasnewich, D. *et al.* Prospective phenotyping of NGLY1-CDDG, the first congenital disorder of deglycosylation. *Genet Med* **19,** 160–168 (2017)

Aspartylglycosamine is a biomarker for **NGLY1**-CDDG, a congenital disorder of deglycosylation.
Haijes HA, et al
Used dried BS. Now confirmed in rodent models
Mol Gen Metabolism 127 368-72 2019

N-glycanase **NGLY1** regulates mitochondrial homeostasis and inflammation through NRF1.
Yang K, Huang R, Fujihira H, Suzuki T, Yan N.
J. Ex Med 2018 215, 2600-2016
Lehrbach NJ, Breen PC, Ruvkun G. Cell. 2019 Apr 18;177(3):737-750

Drug screens of **NGLY1** deficiency in worm and fly models reveal catecholamine, NRF2 and anti-inflammatory-pathway activation as potential clinical approaches. Iyer S, Mast JD, Tsang H, Rodriguez TP, DiPrimio N, Prangley M, Sam FS, Parton Z, Perlstein EO. Dis Model Mech. 2019 Nov 4;12(11): 31615832

Sanford Burnham Prebys

FCDGC

VIEWED FROM A NEUROLOGICAL PERSPECTIVE

PMM2-CDG-

psychomotor retardation/intellectual disability (ID) (90–96%)
- ataxia/cerebellar syndrome (96%)
- cerebellar atrophy (95%)
- hypotonia with frequent hyporeflexia (92%)
- strabismus (84%)
-abnormal EEG findings (69%)
- peripheral neuropathy (53%)
- retinitis pigmentosa (22%)
- nystagmus (9.5%)

ALG6-CDG

>90% experience epilepsy, ataxia and proximal muscle weakness. Behavioral changes, autistic features, depressive symptoms quite frequent

ALG13-CDG developmental delay, clinical spectrum apart from epilepsy covers (100%), intellectual disability (92%), hypotonia (85%)

Epileptic Spasms (ESp)/West Syndrome
ALG1, ALG3, ALG11, ALG13.DOLK, DPAGT1,MPDU1, ST3GAL3, SLC35A2, RTF1, PIGA, PIGV, PIGN, ST3GAL5

Early Myoclonic Encephalopathy of Infancy (EMEI)
PIGA, ALG3, ALG6, DPM2, ALG1

Epilepsy of Infancy with Migrating Focal Seizure
ALG1, ALG3, RFT1

Cerebellar anomalies and ataxia
ALG1, ALG3, ALG6,ALG8, ALG9, COG8, DPM1, FKRP, FKTN, PIGN, PMM2, SLC35A2, SRD5A3, STT3A, STT3B, TRAPPC11, POMT1, POMT2, VPS13B

Hyperkinetic movement
MGAT2, DPAGT1, DDOST, COG5, MOGS, SRD5A3

Retinitis pigmentosa
PMM2, ALG6, POMGNT1, DHDS

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FCDGC

Congenital Disorders of Glycosylation from a Neurological Perspective
Paprocka, et al Brain Sci. 2021 Jan; 11(1): 88.

TAKE HOME MESSAGES

- Over 170 types of CDG, covering all glycosylation pathways
- Resources: NIH-funded clinical consortium (FCDGC) and Patient organization CDG CARE
- Therapies are emerging for the most common form, PMM2-CDG
- Clinical trials of monosaccharides, acetazolamide, epalrestat are underway
- Biomarkers are available, others being developed
- Diagnosis: Exome/genome + biochemical confirmation

FCDGC

RARE DISEASE DAY 2018: CDG-CARE AND NGLY1.ORG

March 1-3, 2024 San Diego, CA

CONGENITAL DISORDERS OF GLYCOSYLATION SCIENTIFIC & FAMILY CONFERENCE

Models and therapy