Diagnostic value of serum bikunin analysis in congenital disorders of glycosylation (CDG) with liver diseases and inherited proteoglycan defects

20-09-2021 – *Réunion d'unité*

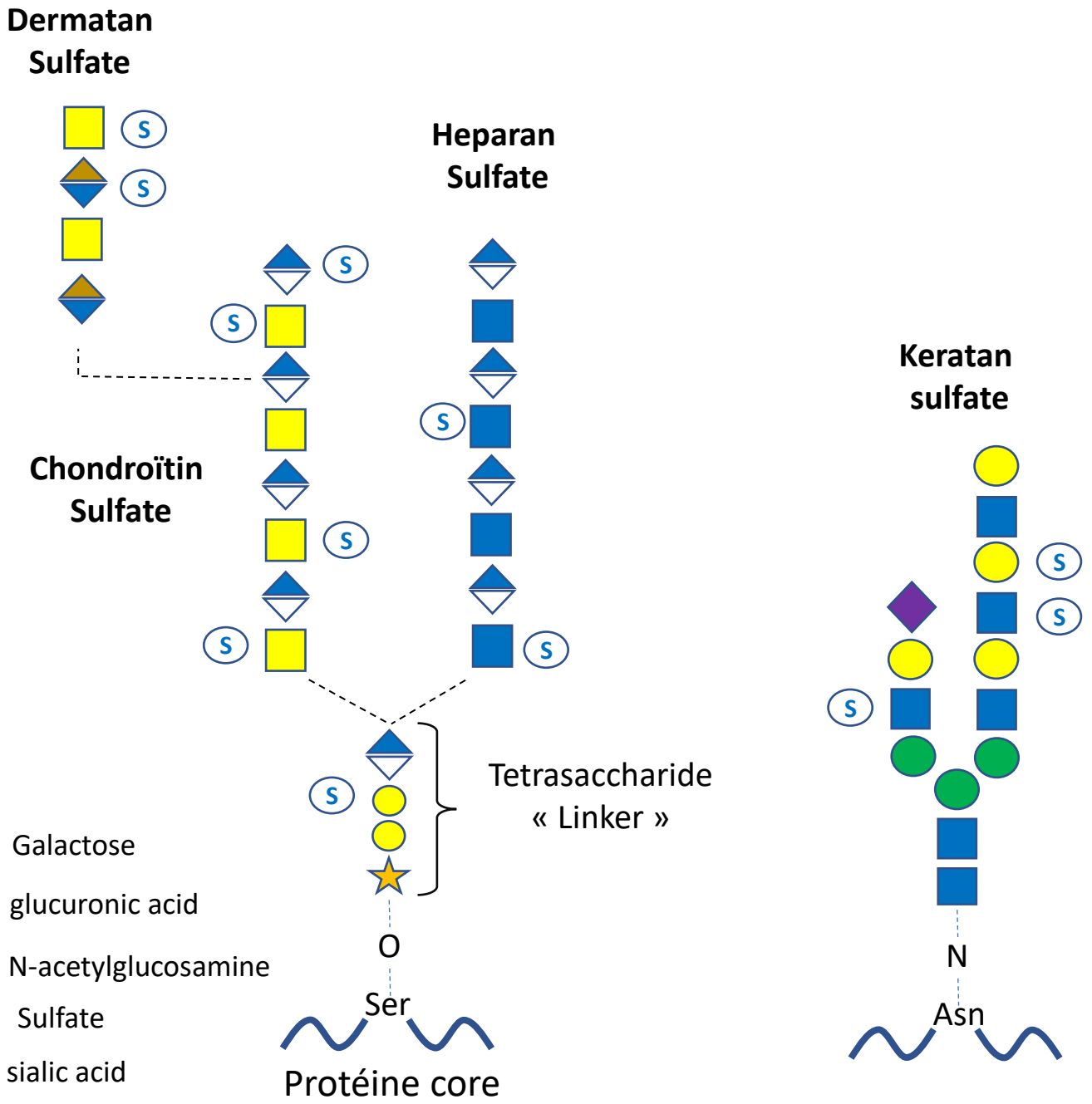
Walid Haouari (PhD student)
Dr Arnaud Bruneel
Pr Christian Poüs

INSERM U 1193 Pathophysiology of liver diseases – Equipe 1
Facuté de pharmacie de Châtenay-Malabry Université Paris-Saclay

Proteglycan biosynthesis defects

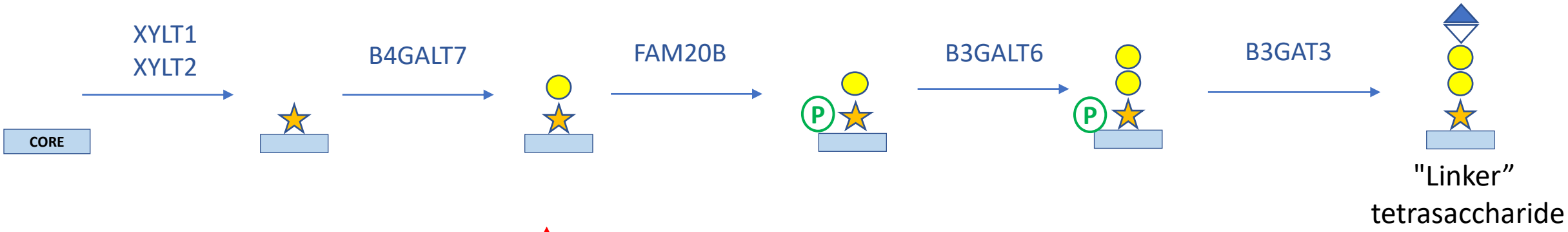
Proteoglycans

- PG = core protein + Glycosaminoglycan chain (GAG)
- ECMs of bones, joints, cartilage, skin
- Immune response

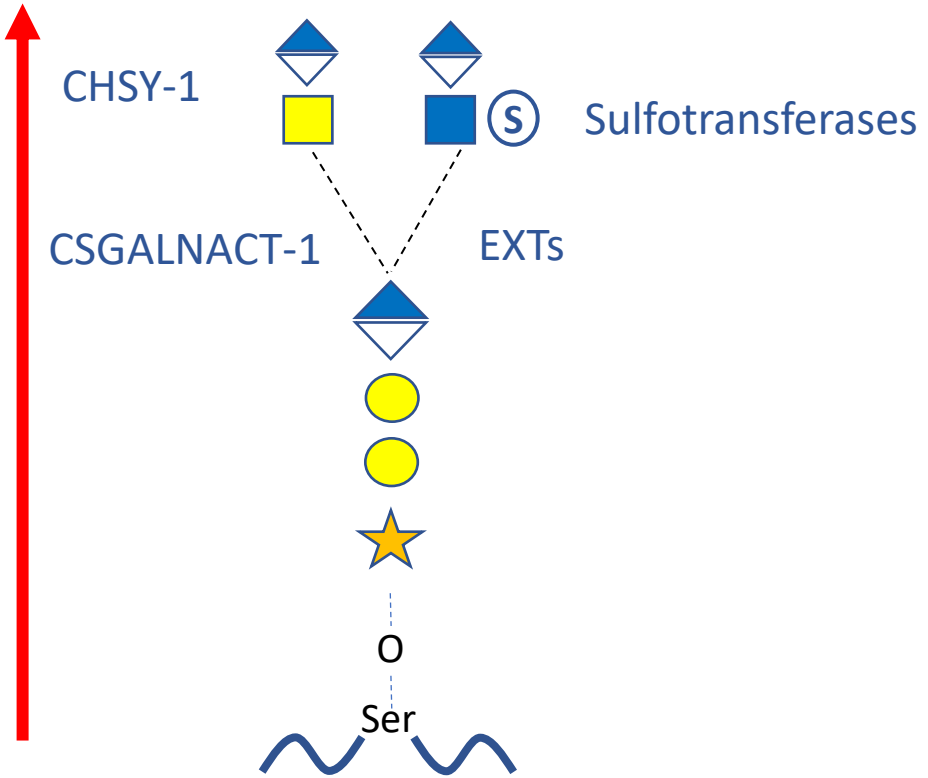


PG biosynthesis defects

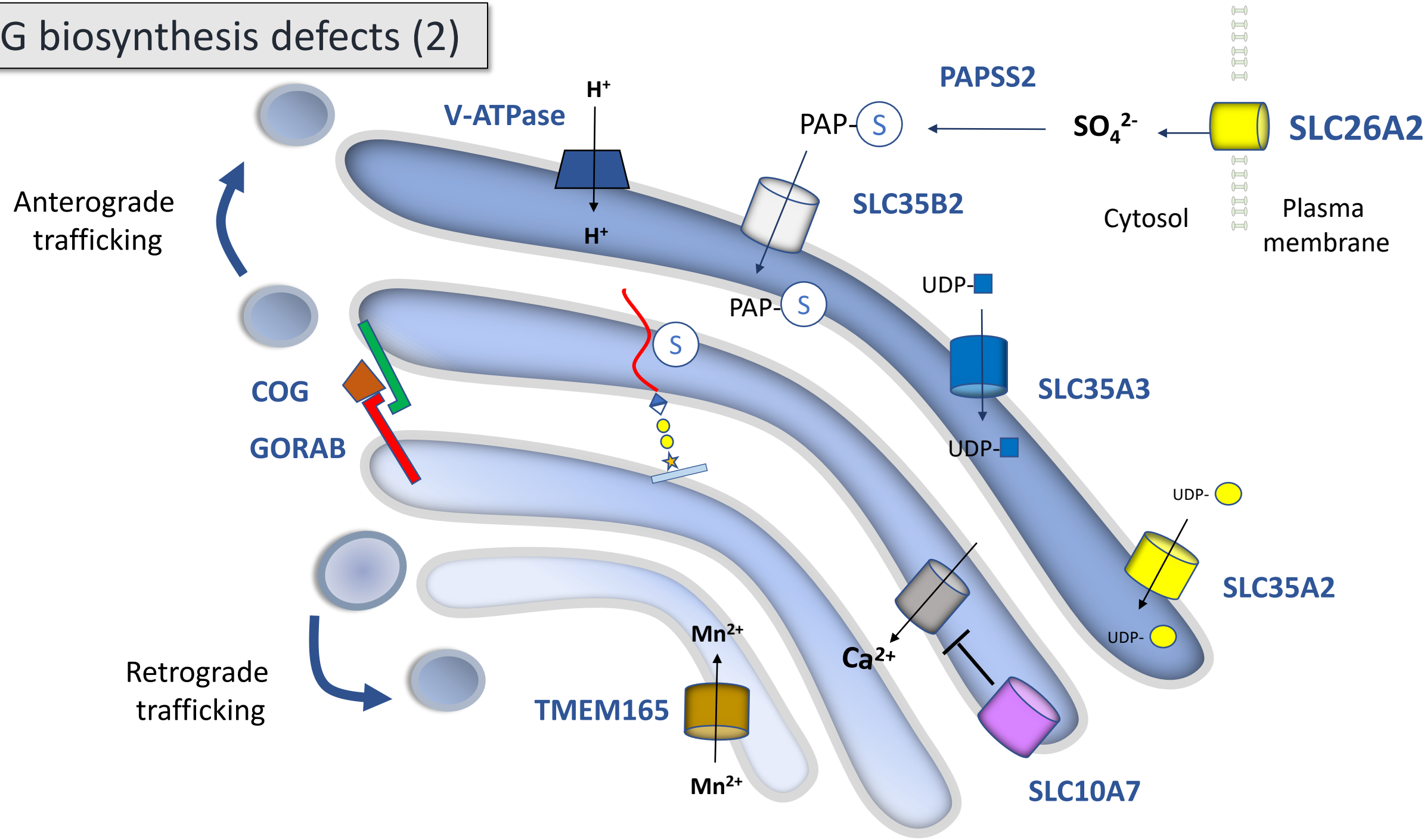
Linkeropathies



Elongation and sulfation defects



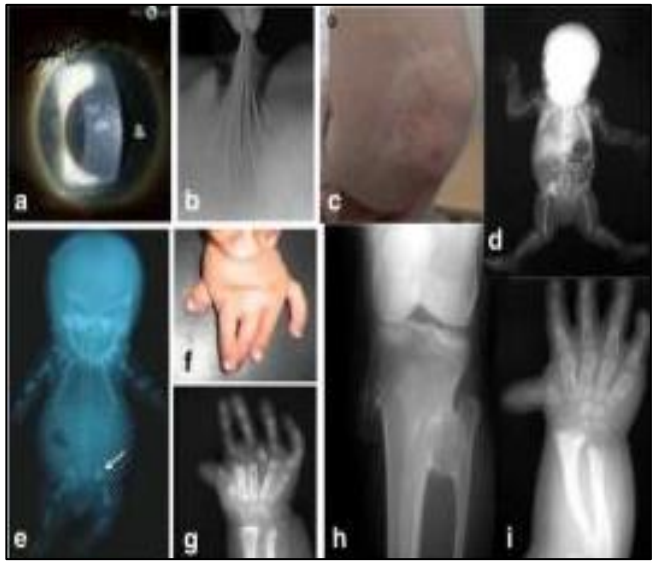
PG biosynthesis defects (2)

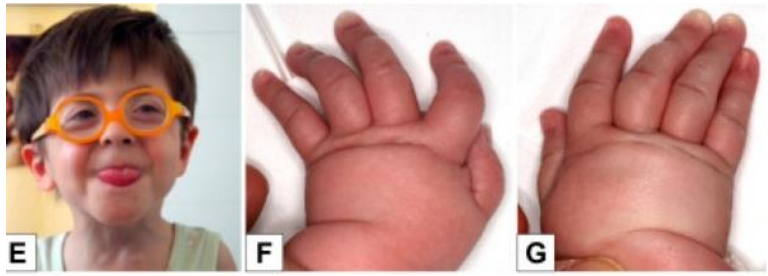


PG-IMD - Clinics

Osteoarticular defects

- Skeletal dysplasia
- Short stature, hand deformities
- Multiple fractures
- Joint dislocations and hyperlaxity





Unspecific symptoms

- Intellectual disabilities
- Skin, ocular, cardiac defects
- Deafness
- Tooth abnormalities

Leoni and al., *AJMG,* ***2021***

Sasarman et al, *JIMD,* ***2016***

PG-IMD - Current diagnosis strategy

Genetics

Gene sequencing

- Gene panels
- Whole exome/genome sequencing

Research

Patient fibroblasts

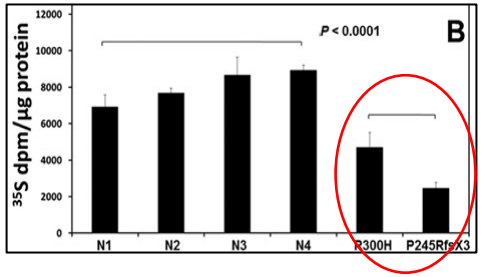
- Labelled substrate incorporation
- PG and GAG quantification
- Functional studies

Blood and urine

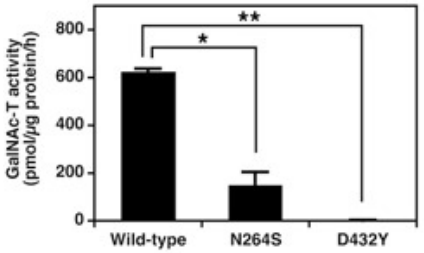
- GAG levels and sulfation by HPLC/MS following chondroitinase/heparitinase

Ex: CSGALNACT-1

PG synthesis in fibroblasts

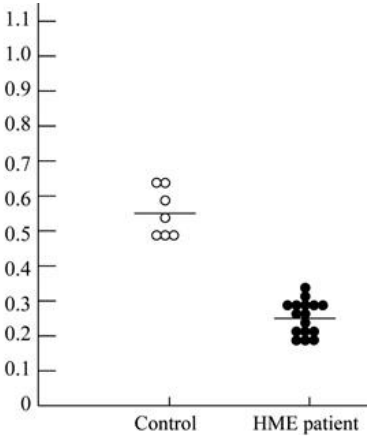


Enzyme activity



Ex: EXTs

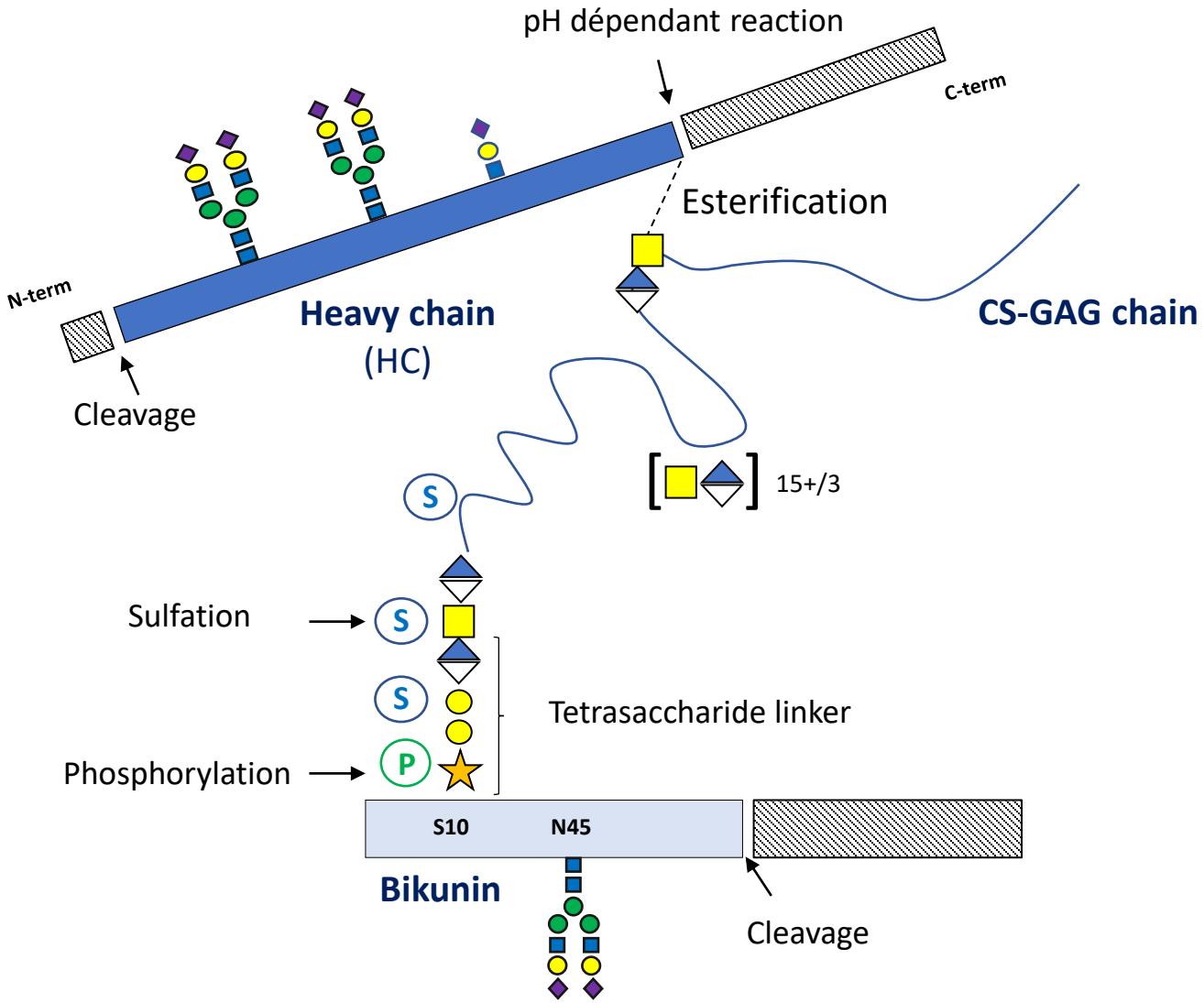
HS/CS disaccharide ratio in serum

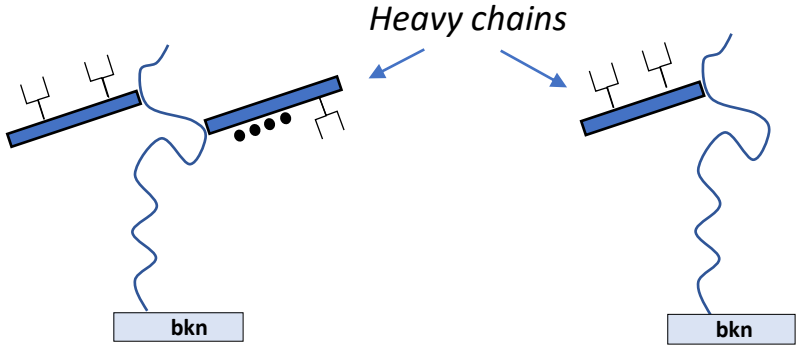


Lack of convenient routine blood biomarkers

Bikunin as a potential biomarker

Liver biosynthesis





Inter-α trypsin inhibitor
(ITI)

Pro-α trypsin inhibitor
(PαI)

Urinary trypsin inhibitor
(UTI)

Bikunin core

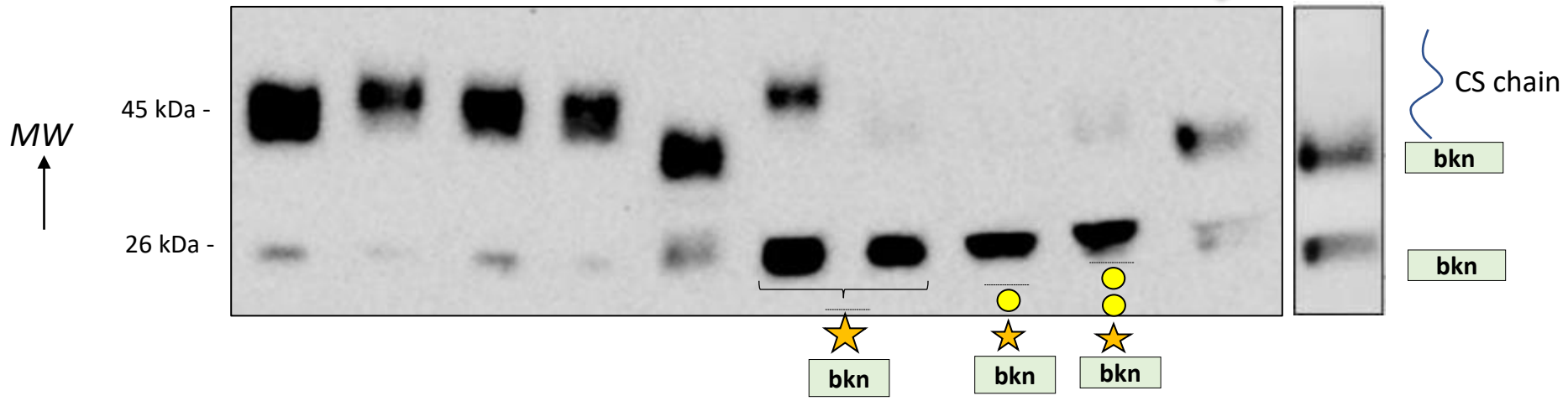
Bikunin analyses in PG defects

Patient serum

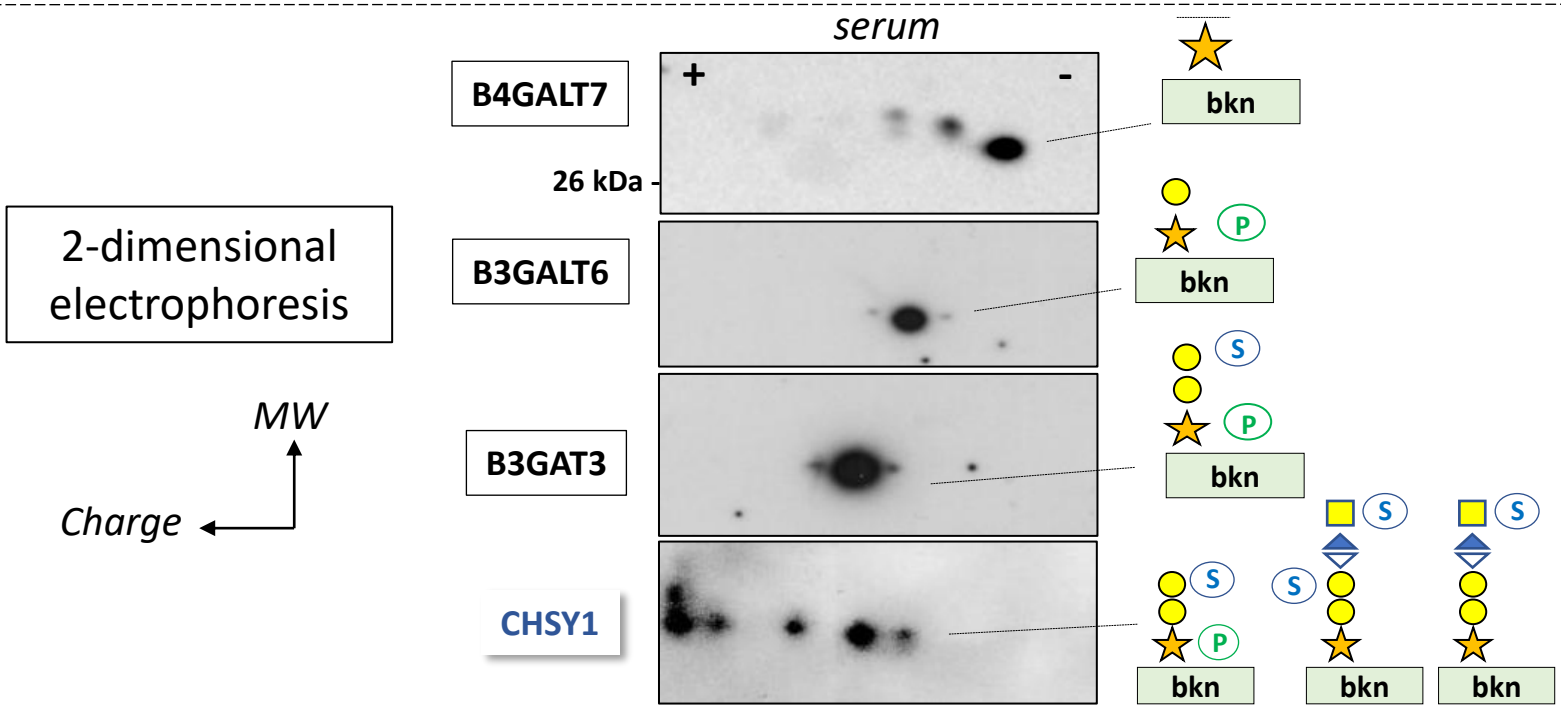
Controls

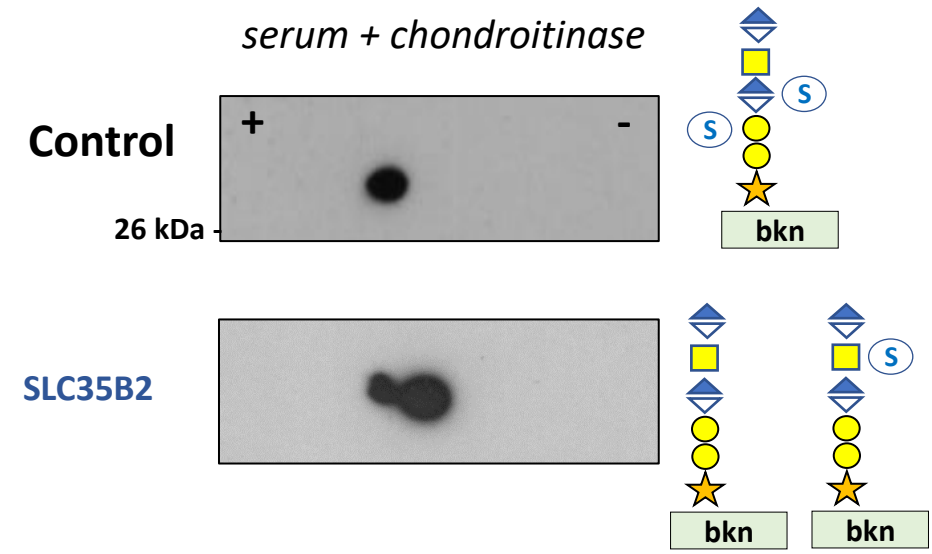
SLC35A2 | SLC35A3 | B4GALT7 (1) | B4GALT7 (2) | B3GALT6 | B3GAT3 | CHSY1 | TMEM165

Western blot



2-dimensional electrophoresis

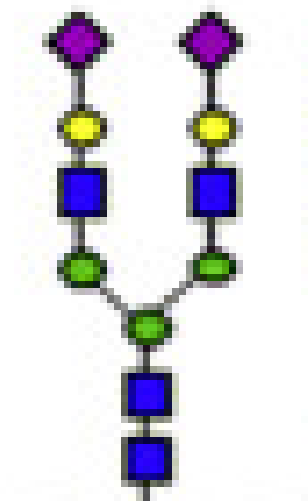




Haouari et al., *JIMD* **2020**

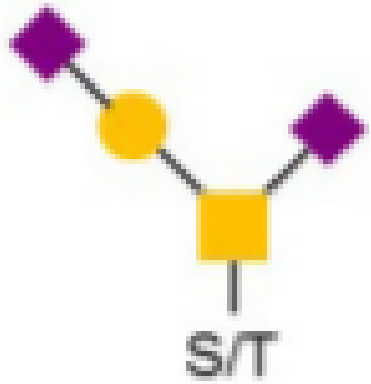
Congenital disorders of glycosylation

N-glycosylation

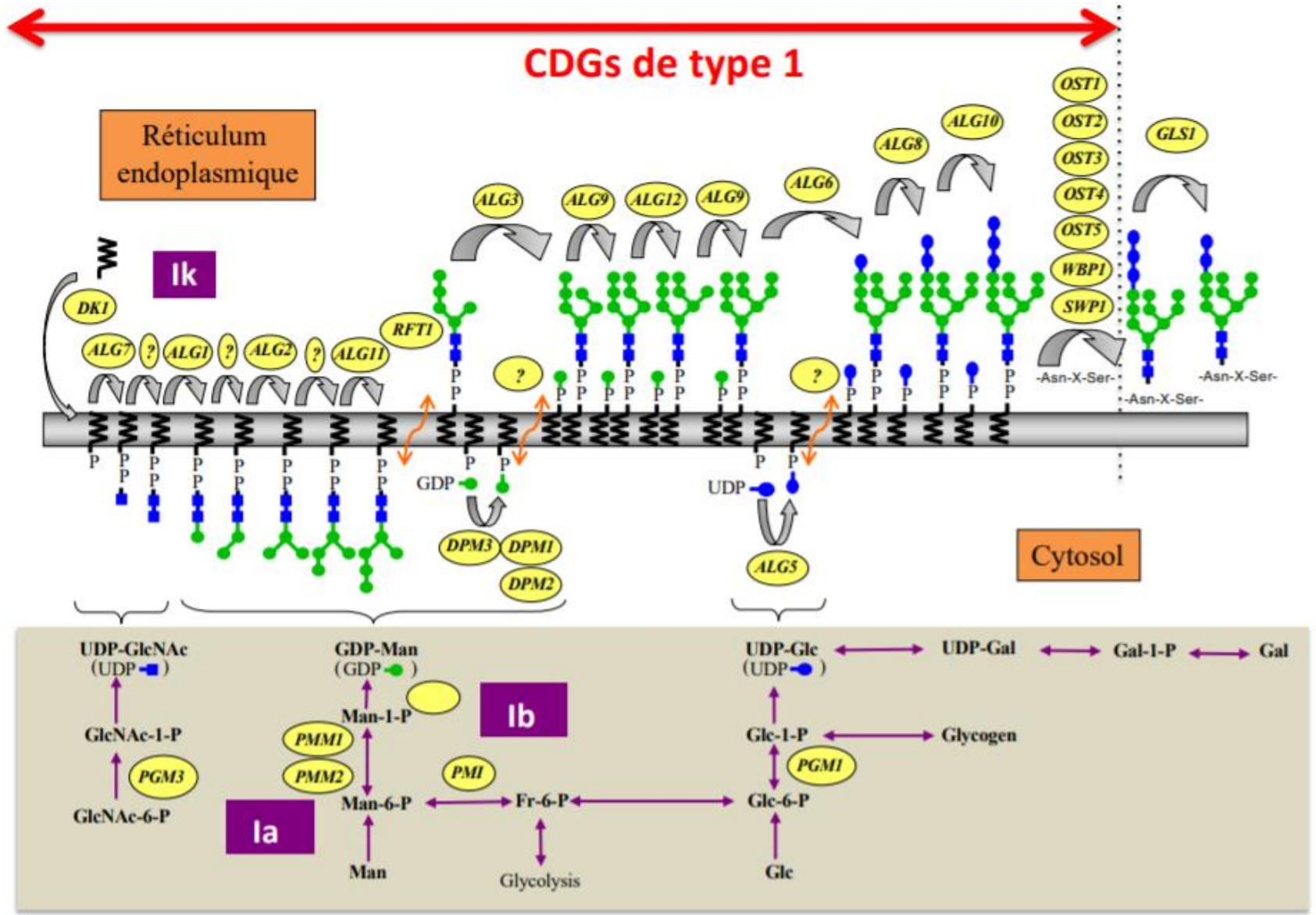


N

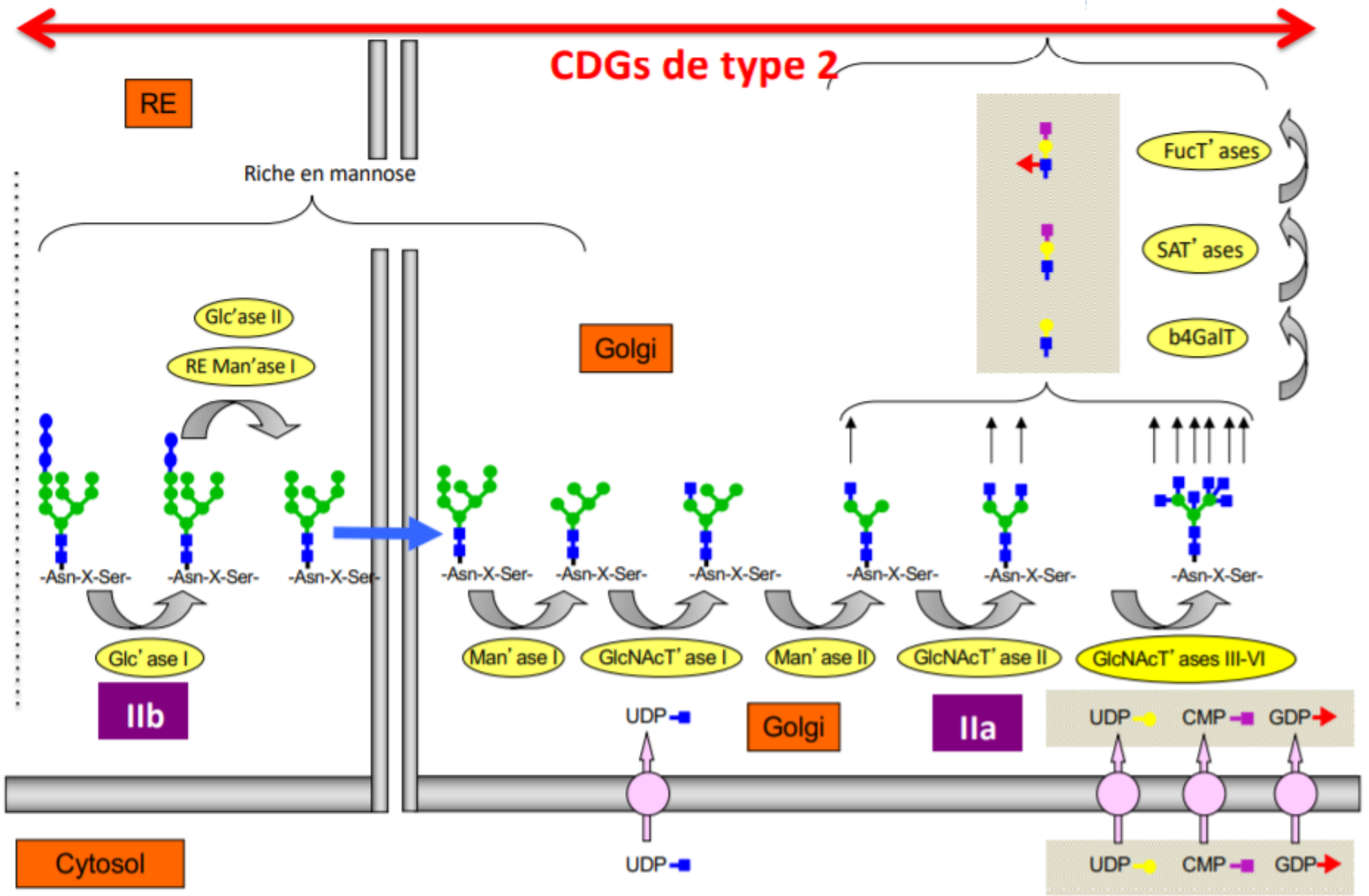
O-glycosylation



N-glycosylation and CDG type 1

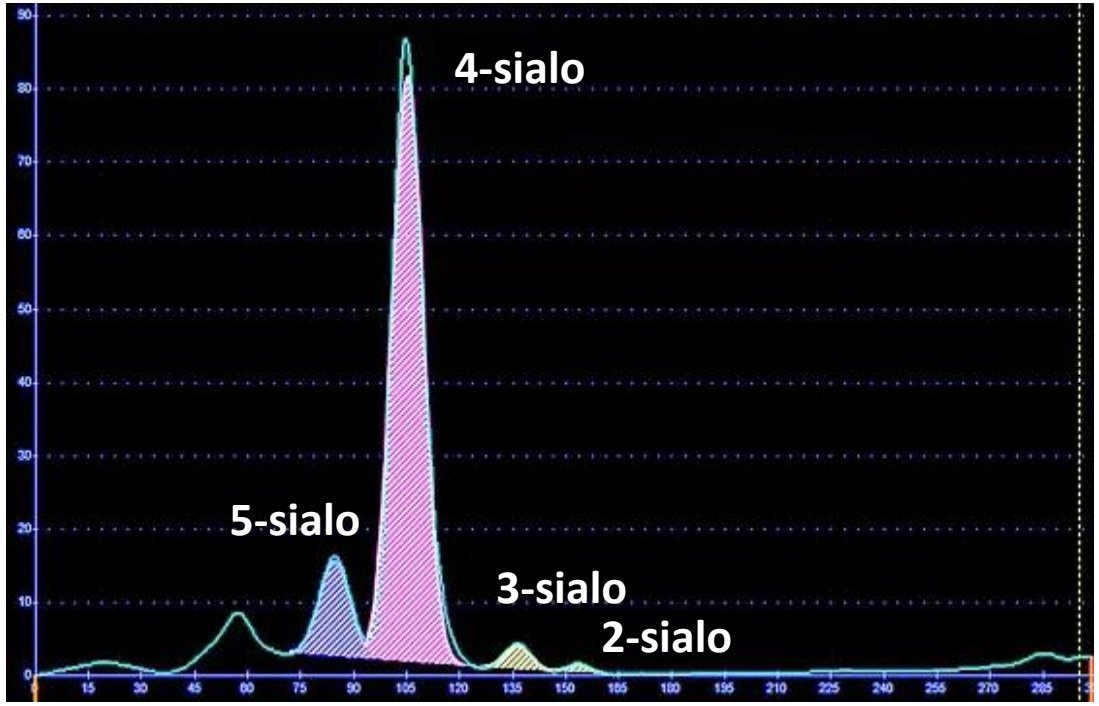


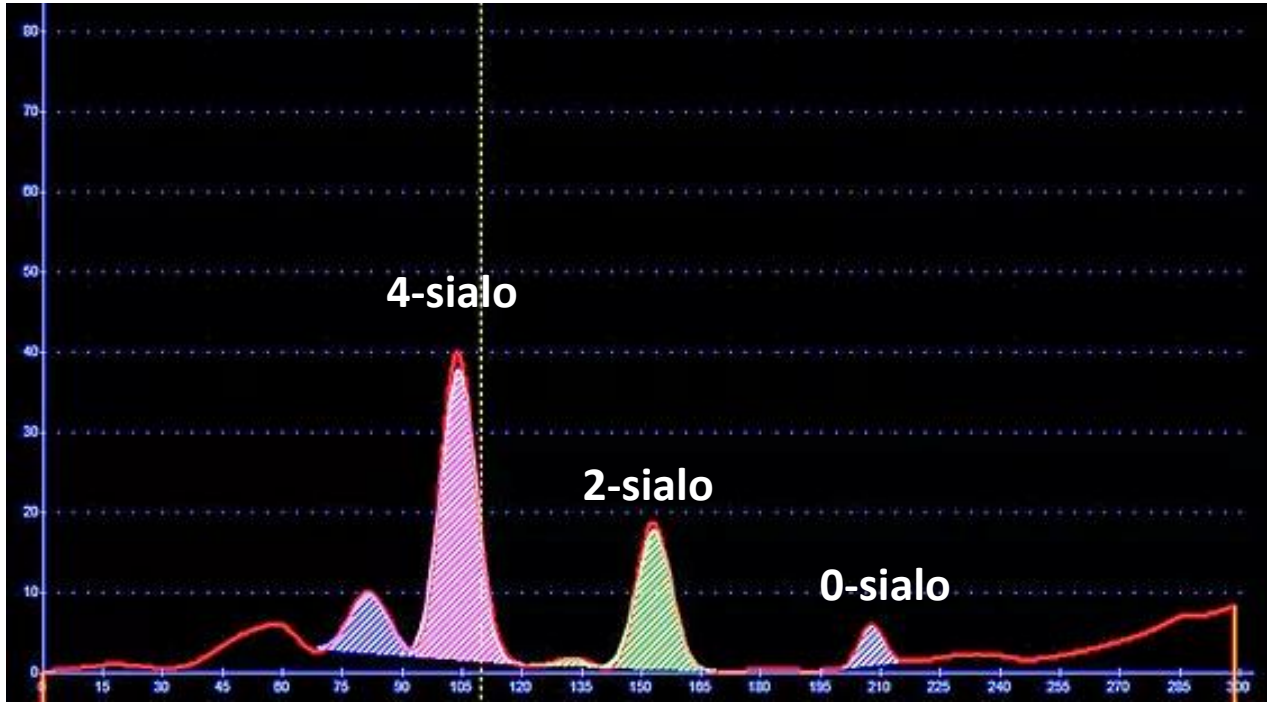
N- glycosylation and CDG type 2



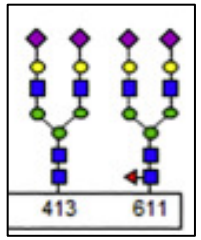
CDG type 1 screening

Capillary-zone electrophoresis (CZE) of serum transferrin (Trf)



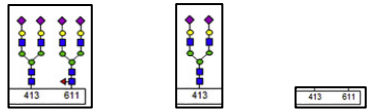


Normal pattern



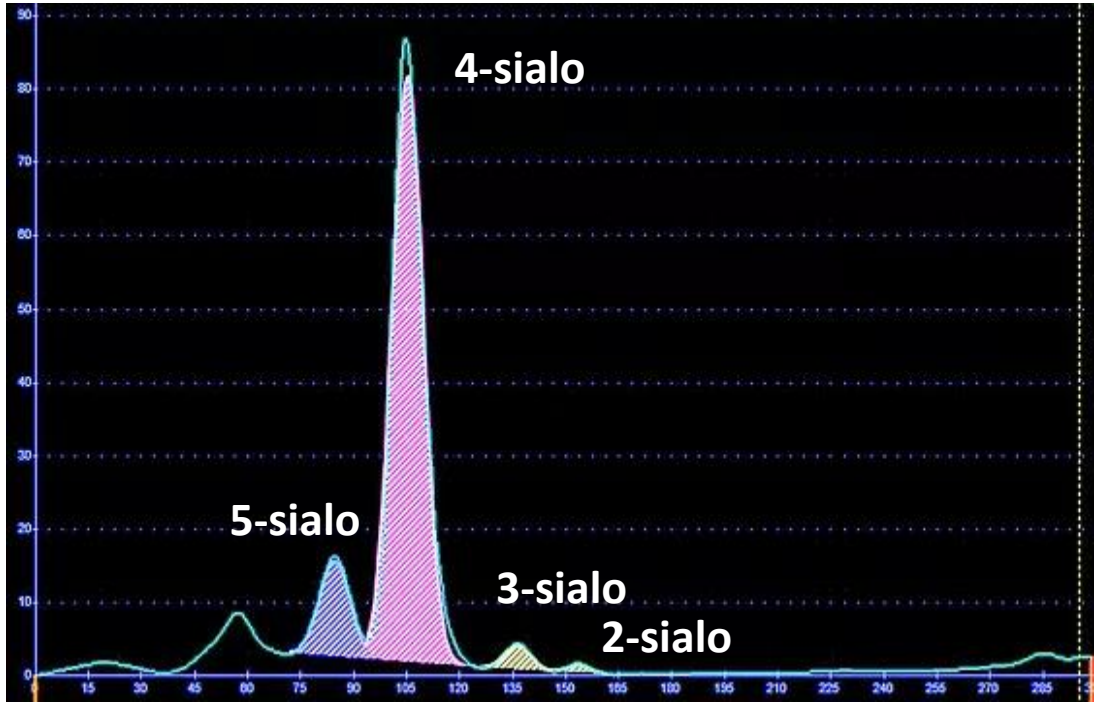
4-sialotransferrin

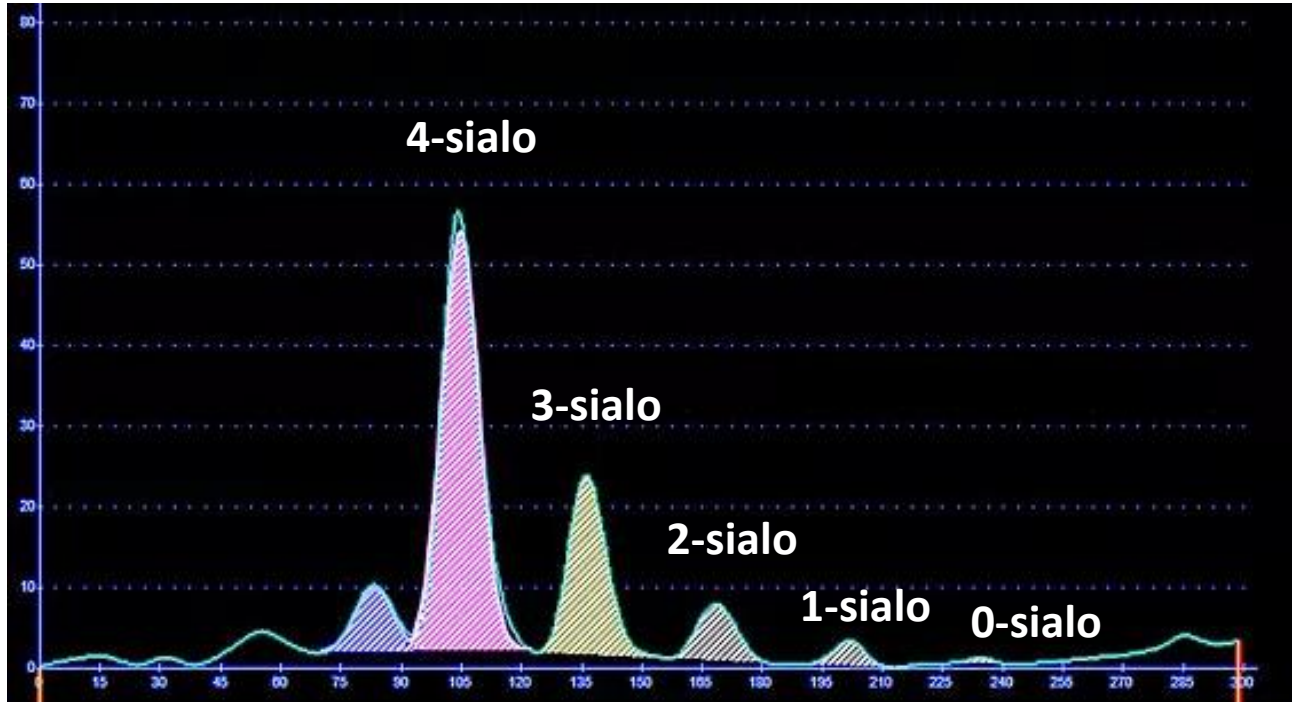
CDG type 1 pattern
4-2-0



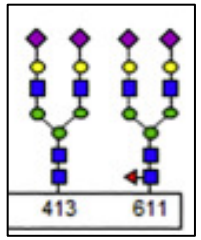
CDG type 2 screening

Capillary-zone electrophoresis (CZE) of serum Trf



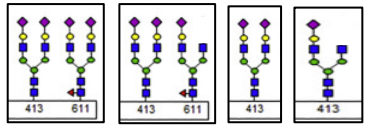


Normal pattern

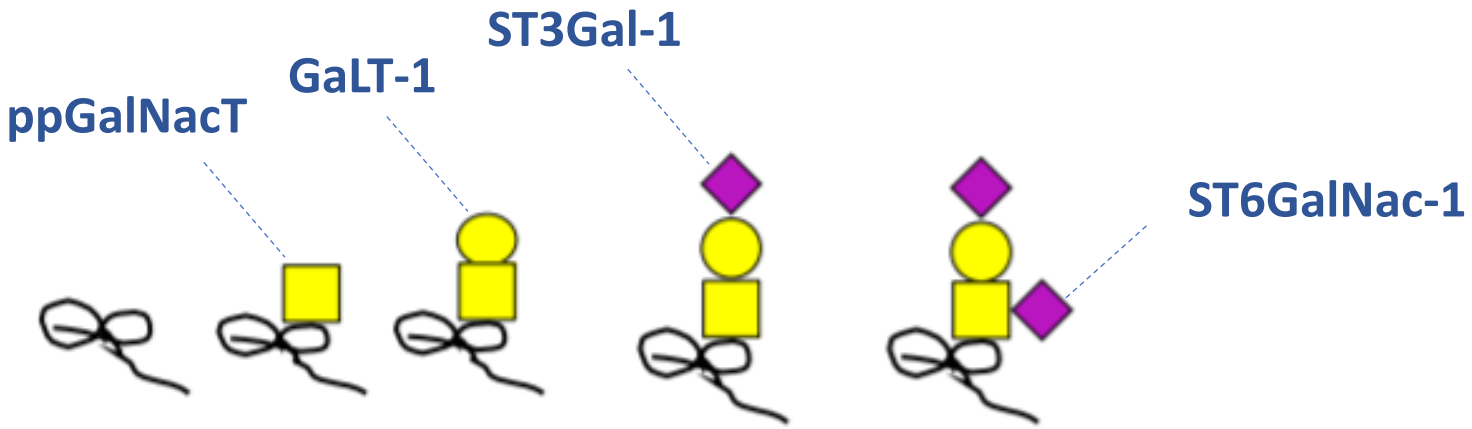


4-sialotransferrin

CDG type 2 pattern
4-3-2-1-0



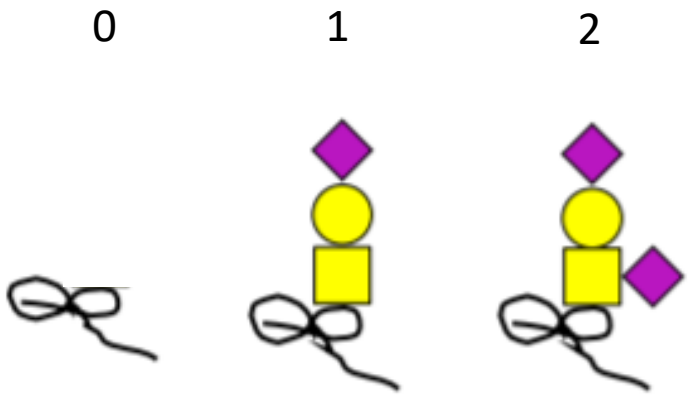
Additional O-glycosylation defects in CDG 2 due to **impaired Golgi homeostasis**



Only in the Golgi apparatus

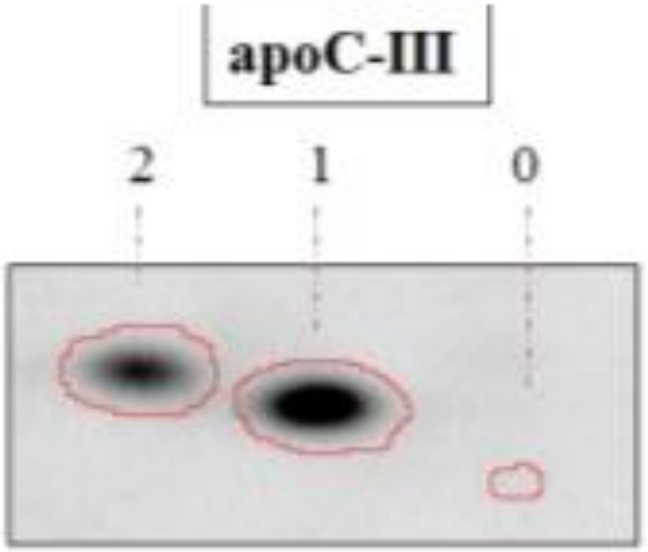
CDG type 2 with impaired Golgi homeostasis

Two dimensional electrophoresis of apoC-III

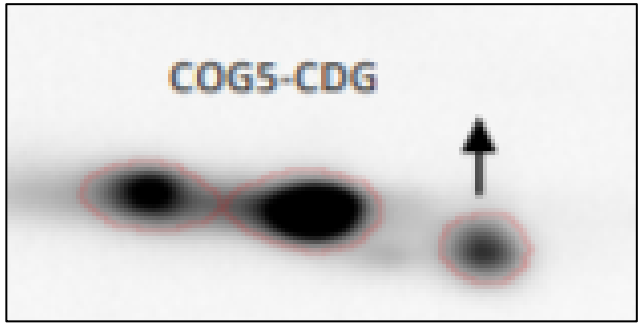


2-DE

ctrl



CDG due to defective Golgi trafficking



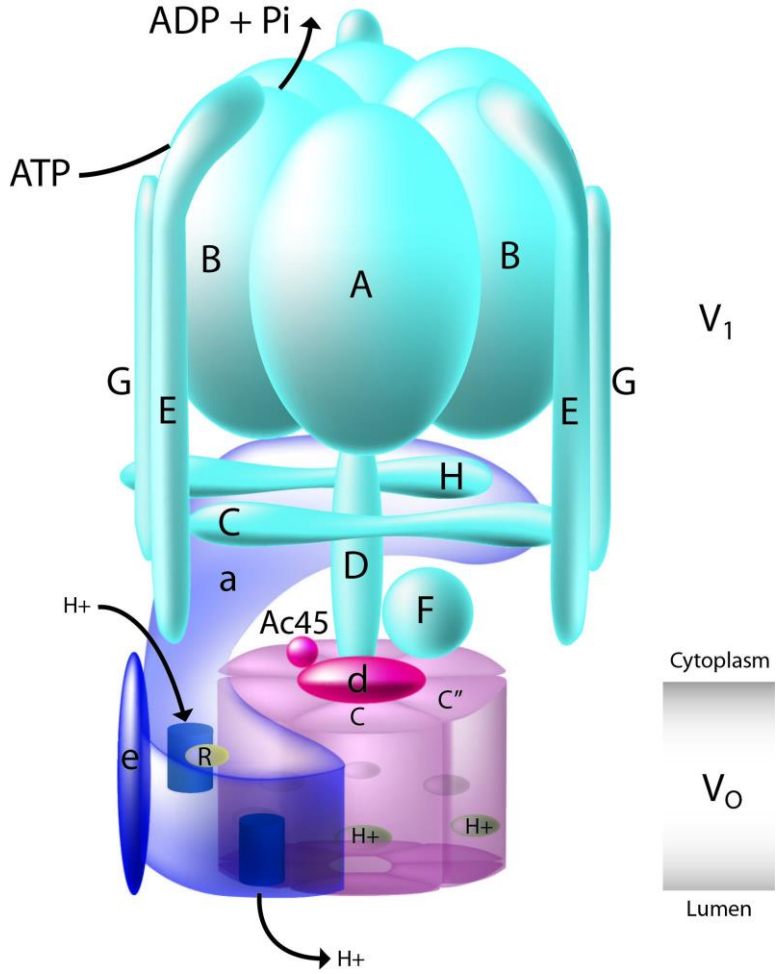
CDG with impaired Golgi V-ATPase and liver diseases

- ATP6AP1
- ATP6AP2
- ATP6V1F

V-ATPase subunits

- CCDC115
- TMEM 199

V-ATPase assembling factor



CDG with impaired Golgi V-ATPase and liver diseases - **Clinics**

Chronic liver disease

- Hepatosplenomegaly, ascites
- ↗ AST/ALT, ↗ PAL, normal GGT
- Fibrosis, steatosis, cholestasis
- Cirrhosis, end stage liver failure
- Liver transplantation listing

Wilson-like disease

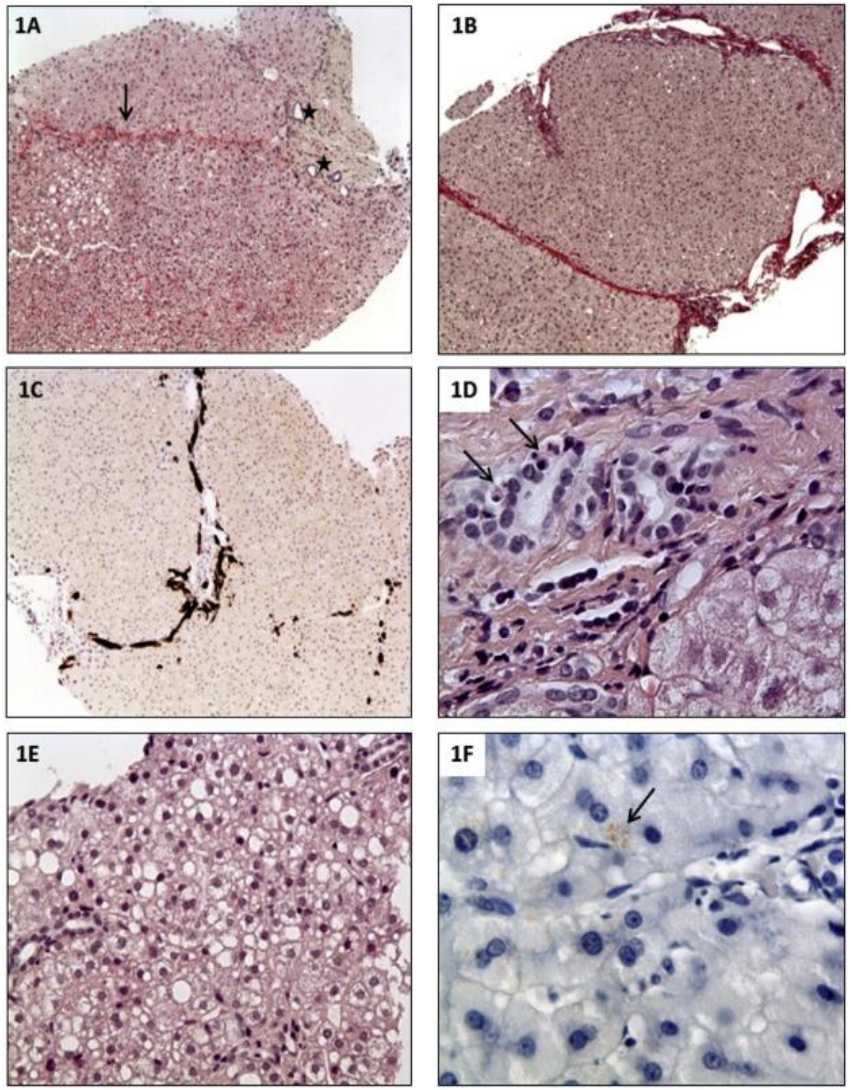
- ↘ serum copper ↗ urinary copper
- ↗ liver [copper]
- ↘ Ceruloplasmin
- ↗ REC
- Absent Kayser-Fleisher ring
- Normal ATP7 status

Other symptoms

- Defective lipid metabolism (↗ Chol.T, ↗ LDL)
- Immunodeficiency with recurrent infections (↘ Ig)
- Neurological symptoms, intellectual disabilities
- Cutis laxa

CDG with impaired Golgi V-ATPase and liver diseases – **liver biopsies**

Ex: CCDC115-CDG



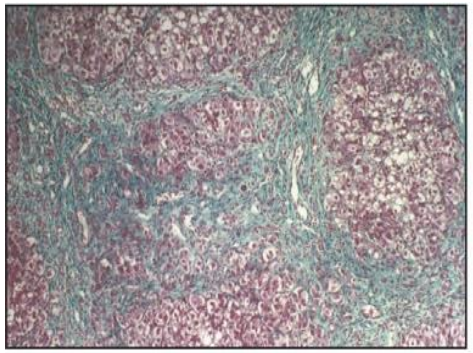
Stage F2 (METAVIR)

Cholangial proliferation

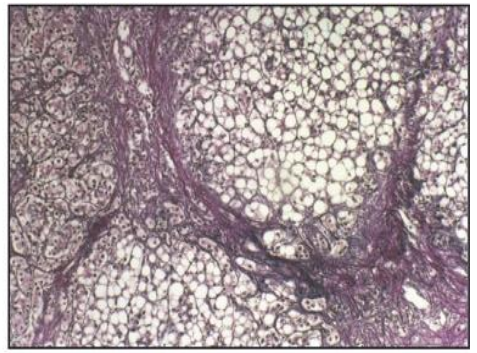
Steatosis and Positive copper staining

Ex: ATPAP2-CDG

Cirrhosis

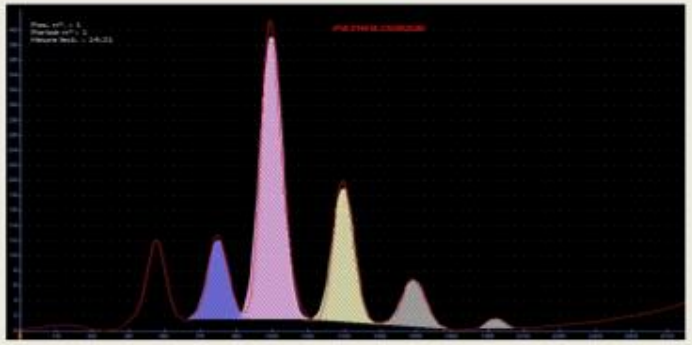


Steatosis



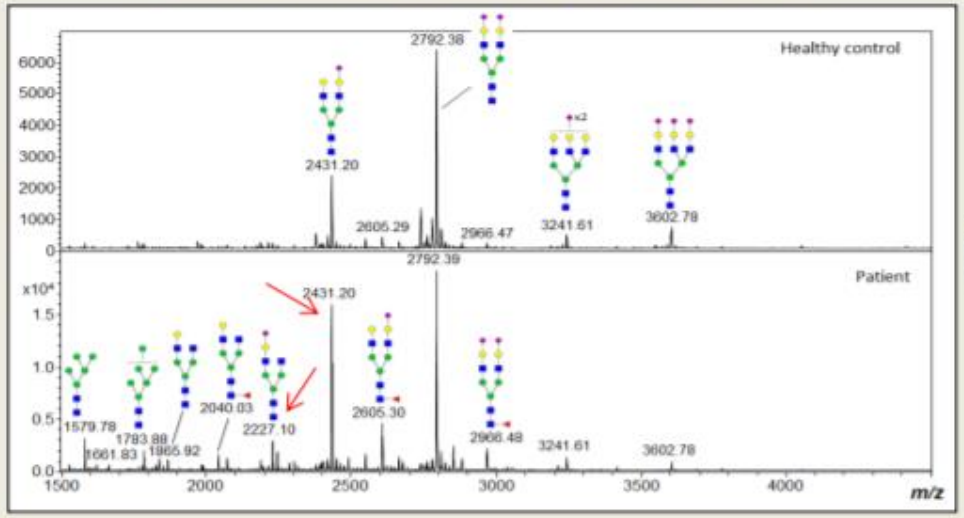
CDG with impaired Golgi V-ATPase and liver diseases – **glycosylation studies**

CCDC115-CDG



CZE Trf

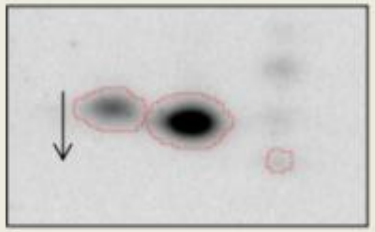
CDG type 2 pattern



MS total N-glycans

Hyposialylation + Hypogalactosylation

2DE apoC-III

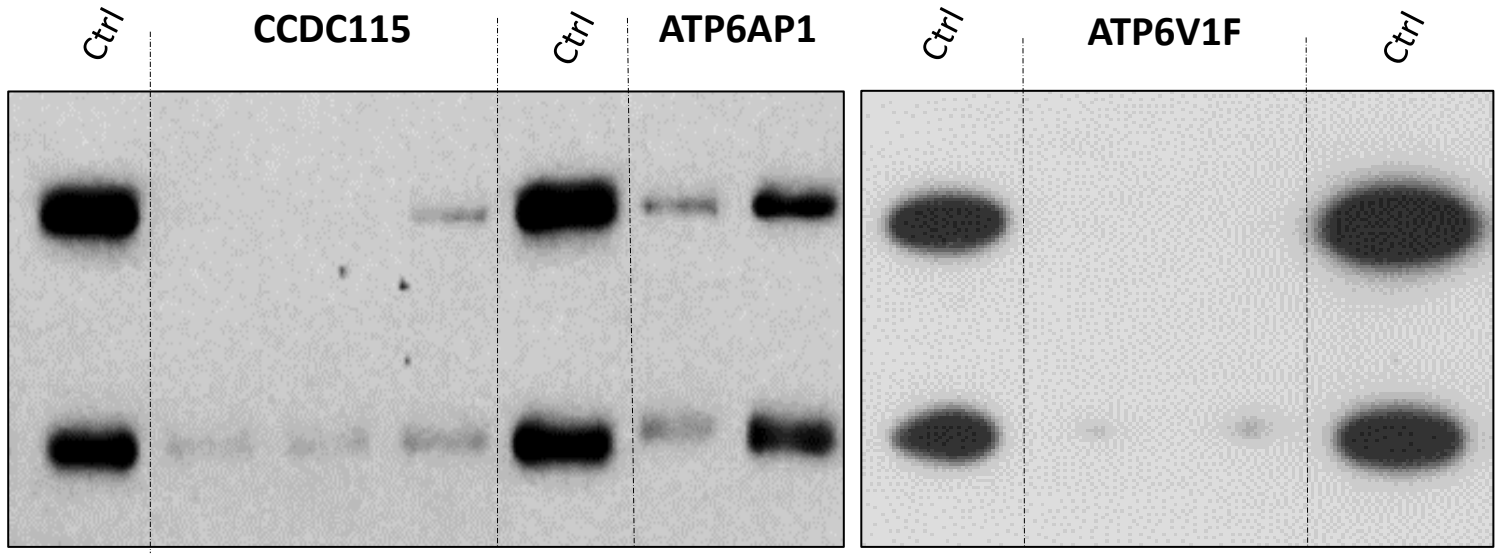


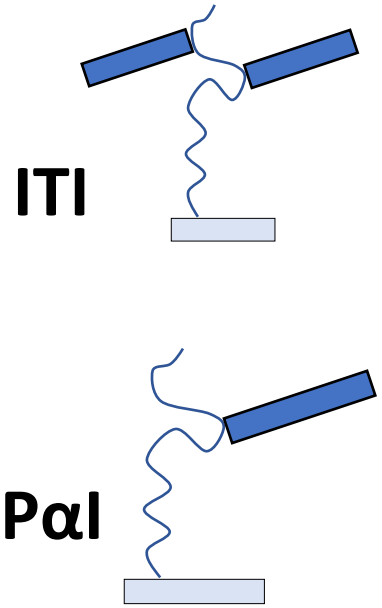
Additional O-glycosylation defect

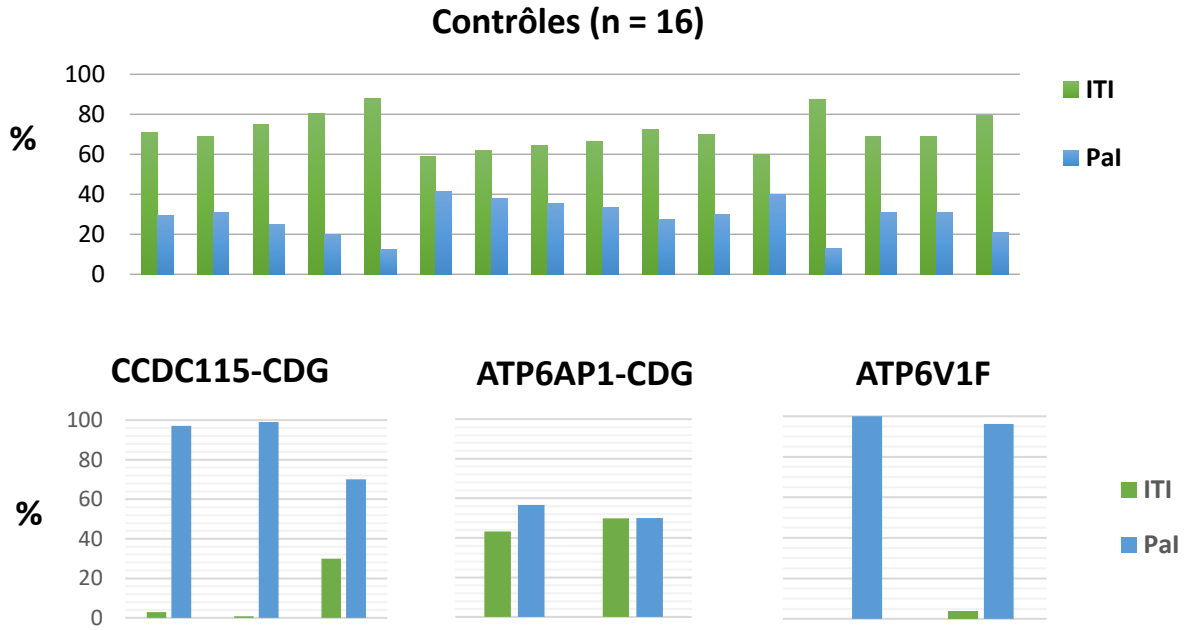
Girard et al., *Mol. Gen. Met.*, ***2018***

CDG type 2 with Golgi homeostasis defect

CDG with impaired Golgi V-ATPase and liver diseases – **Bikunin analysis**







Hallmark of CDG with impaired V-ATPase

- Decreased levels
- Inversion of ITI/Pal ratio
- pH dependent esterification

Haouari et al., JIMD **2020**

The intriguing story of SLC37A4-CDG

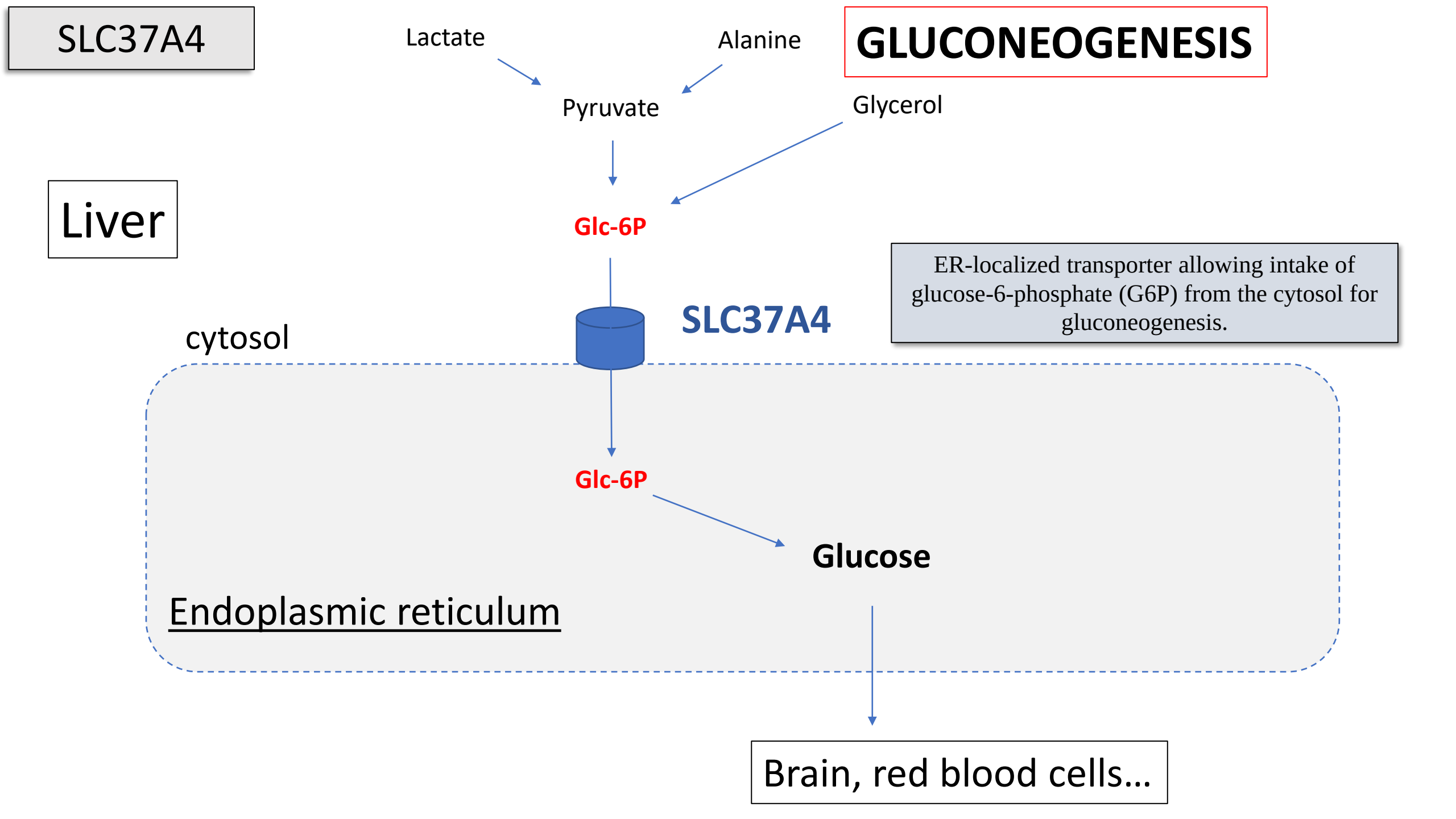
Collaboration with the SBP Medical Discovery Institute (La Jolla - USA)
Pr. Hudson Freeze

Inserm

HÔPITAUX UNIVERSITAIRES PARIS NORD VAL DE SEINE
Bichat - Claude-Bernard

DE LA RECHERCHE À L'INDUSTRIE
cea

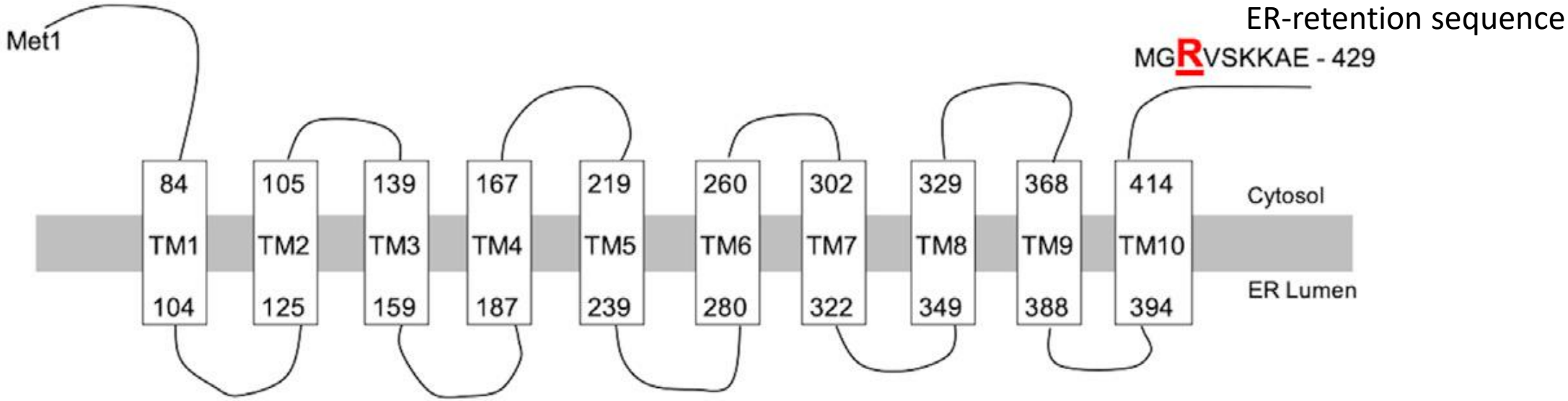
SBP Medical Discovery Institute
SANFORD BURNHAM PREBYS

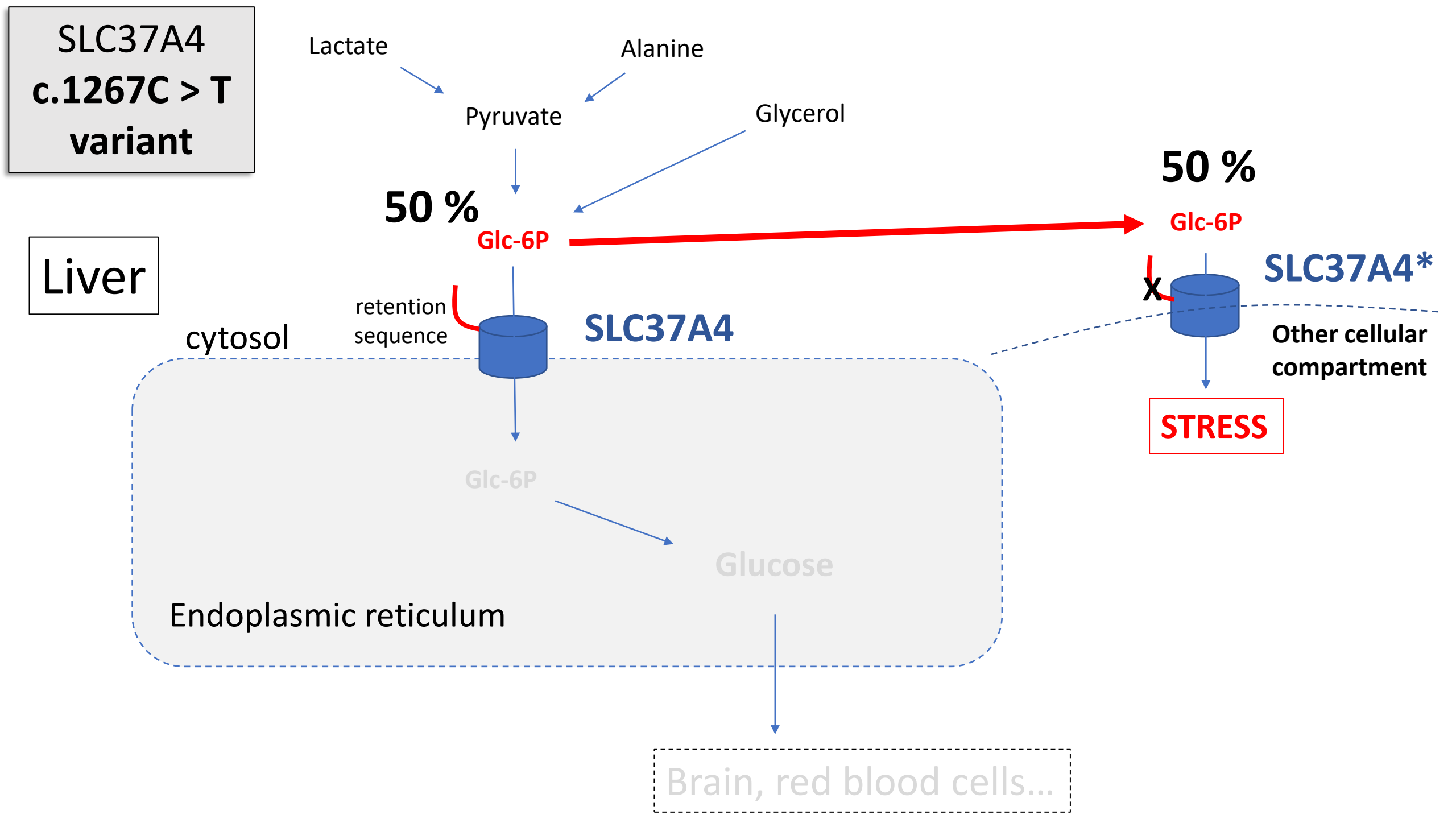

SLC37A4
Lactate
Alanine
GLUCONEOGENESIS
Pyruvate
Glycerol
Liver
Glc-6P
ER-localized transporter allowing intake of glucose-6-phosphate (G6P) from the cytosol for gluconeogenesis.
SLC37A4
cytosol
Glc-6P
Glucose
Endoplasmic reticulum
Brain, red blood cells…

SLC37A4 **c.1267C > T variant**

Bobby G. Ng et al., *AJHG,* ***2021***
Raynord A, Haouari W et al., CCA, ***2021***

Heterozygous SLC37A4 variant (c.1267C > T (R423*)




SLC37A4
c.1267C > T
variant
Liver
Lactate
Alanine
Pyruvate
Glycerol
50 %
Glc-6P
50 %
Glc-6P
SLC37A4*
x
retention
sequence
SLC37A4
cytosol
Other cellular
compartment
STRESS
Glc-6P
Glucose
Endoplasmic reticulum
Brain, red blood cells…

SLC37A4 **c.1267C > T variant – Clinics**

- 9 patients
- **Liver dysfunction**
  ↗ AST (9/9), coagulopathy (9/9)
- Cardiac abnormalities (4/9)
- Scoliosis (3/9)

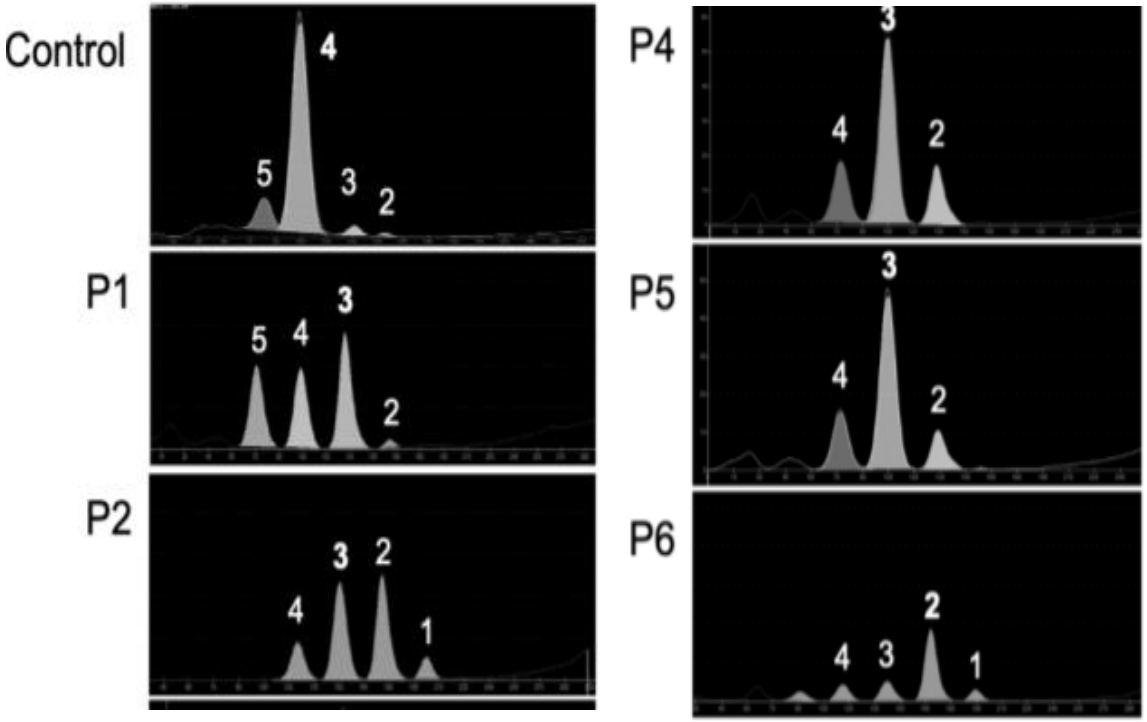
SLC37A4 **c.1267C > T variant - coagulopathy**

AST (ref. 7–40 U/L)	65 U/L (H)	N/A	50 U/L (H)	77 U/L (H)	91 U/L (H)	147 U/L (H)	80 U/L (H)	228 U/L (H)	522 U/L (H)	6/6
F2 (ref. 60%–140%)	31 (L)	22 (L)	57 (L)	30 (L)	20 (L)	18 (L)	27 (L)	5.5 (L)	N/A	7/7
F5 (ref. 60%–140%)	51 (L)	52 (L)	64	40 (L)	39 (L)	50 (L)	29 (L)	38.5 (L)	N/A	6/7
Fg (ref. 1.5–3.5 g/L)	1.7 g/L	1.7 g/L	2.8 g/L	1.8 g/L	1.3 g/L (L)	1.7 g/L	1.5 g/L	0.4 g/L (L)	0.1 g/L (L)	1/7
F8 (ref. 60%–150%)	117	N/A	165	130	144	109	85	N/A	N/A	0/6
F9 (ref. 60%–140%)	63	N/A	85	58 (L)	42 (L)	55 (L)	44 (L)	N/A	N/A	4/6
F11 (ref. 60%–140%)	59 (L)	N/A	55 (L)	31 (L)	34 (L)	22 (L)	33 (L)	N/A	normal	6/6
SERPINC1 (ref. 80%–120%)	28 (L)	34 (L)	60 (L)	37 (L)	32 (L)	19 (L)	32 (L)	0 (L)	normal	7/7
PROC (ref. 50%–120%)	110	49	97	72	73	59	94	N/A	normal	0/7
PROS1 (ref. 60%–120%)	41 (L)	48 (L)	70	43 (L)	81	35 (L)	35 (L)	N/A	normal	5/7

SLC37A4-CDG – **Impaired serum protein glycosylation**

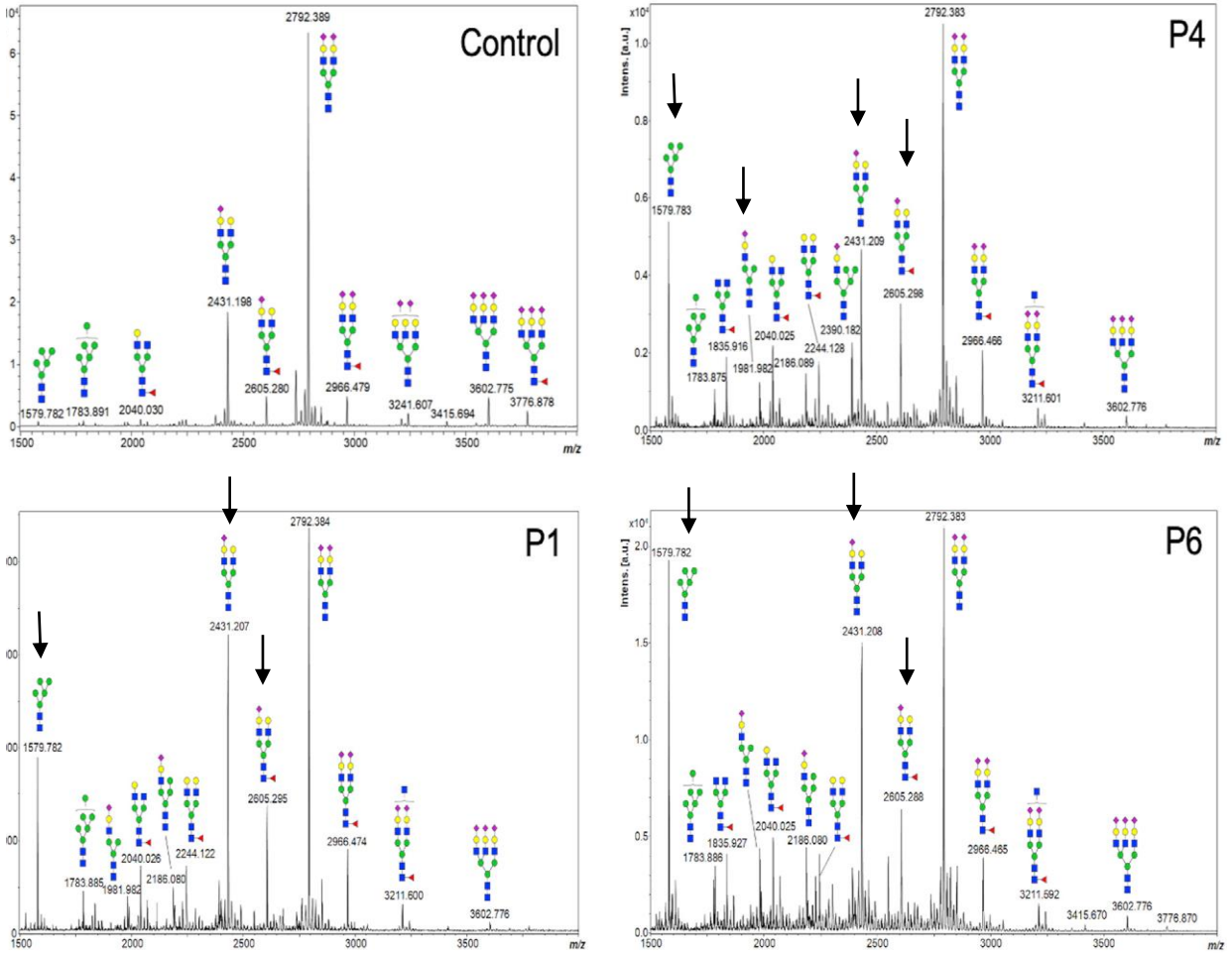
CZE Trf

CDG type 2 pattern



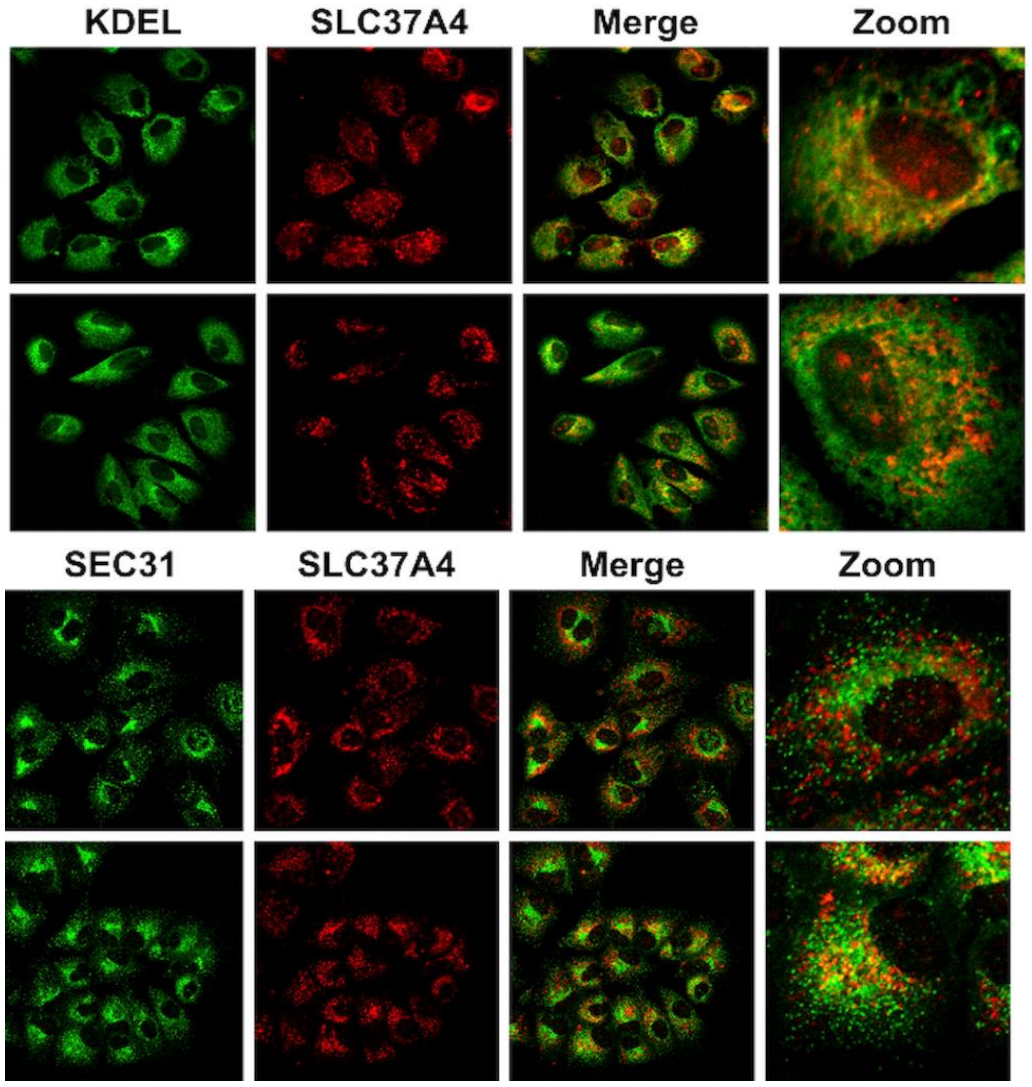
MS total N-glycan

Hypo-sialylation, High-mannose N-glycans



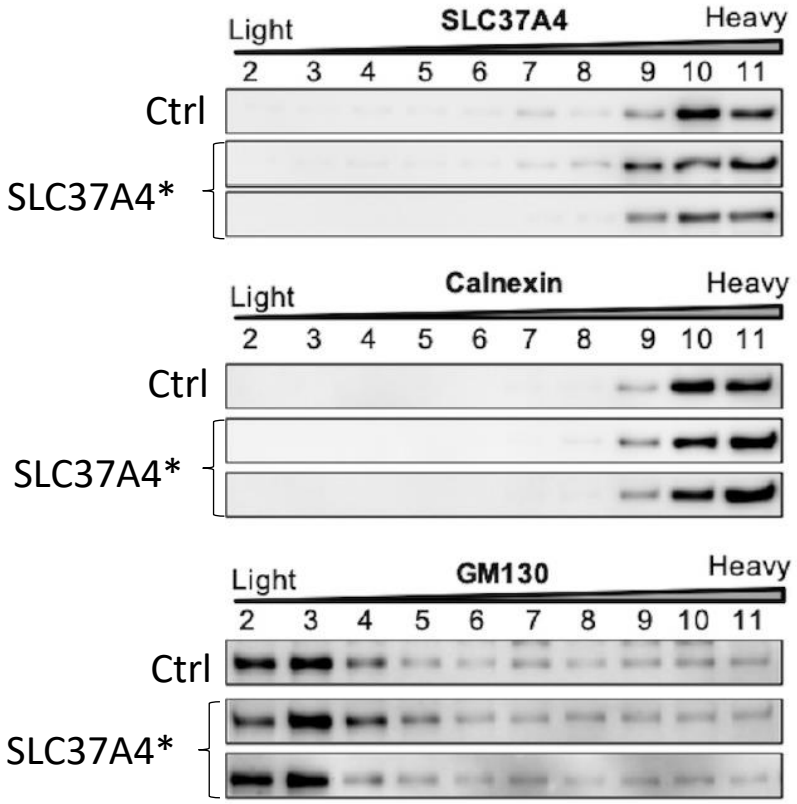
SLC37A4-CDG: Functional studies

HuH7



Bobby G. Ng et al., *AJHG,* ***2021***

Subcellular fractionation



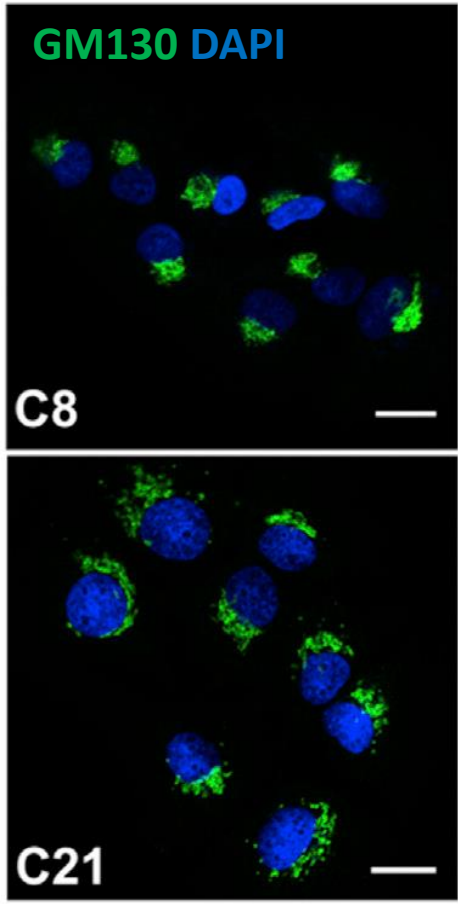
SLC37A4 mutation induced partial (half ?) mislocalization of the transporter to ER-Golgi intermediates

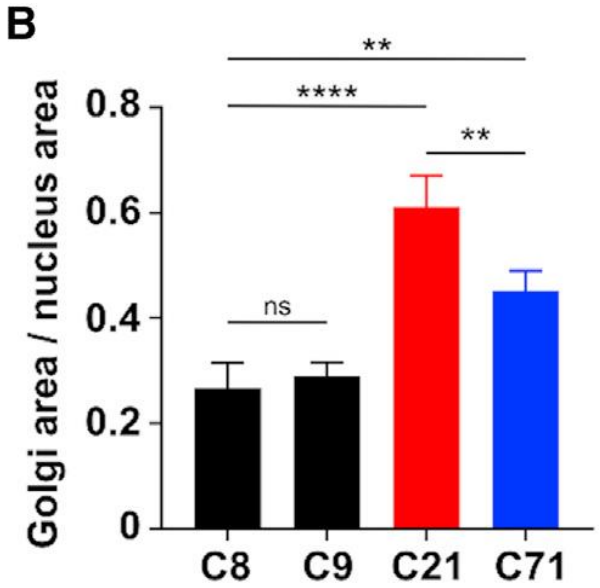
SLC37A4-CDG: Functional studies (2)

HuH7

Ctrl

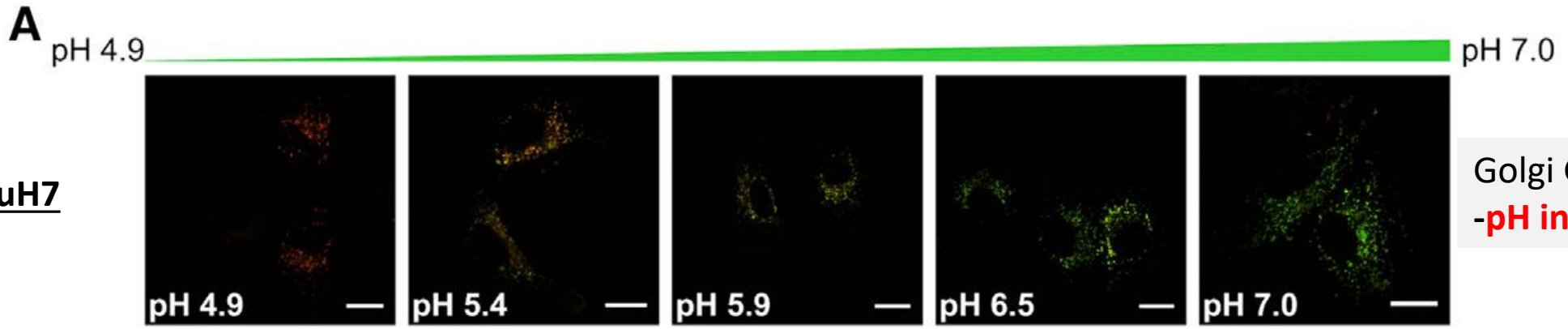
SLC37A4 pArg423*





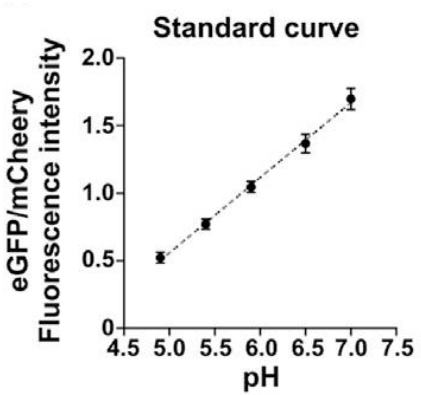
SLC37A4 mutation induced abnormal Golgi morphology

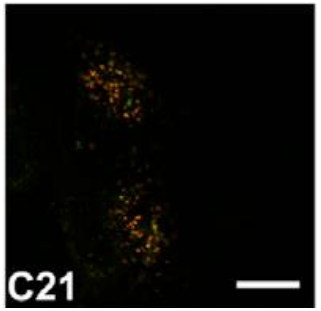
SLC37A4-CDG: Functional studies (3)



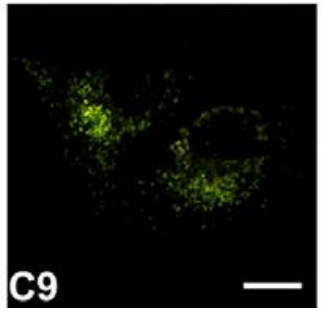
HuH7

Golgi GalT- **pH sensitive GFP**
-pH independent mCherry

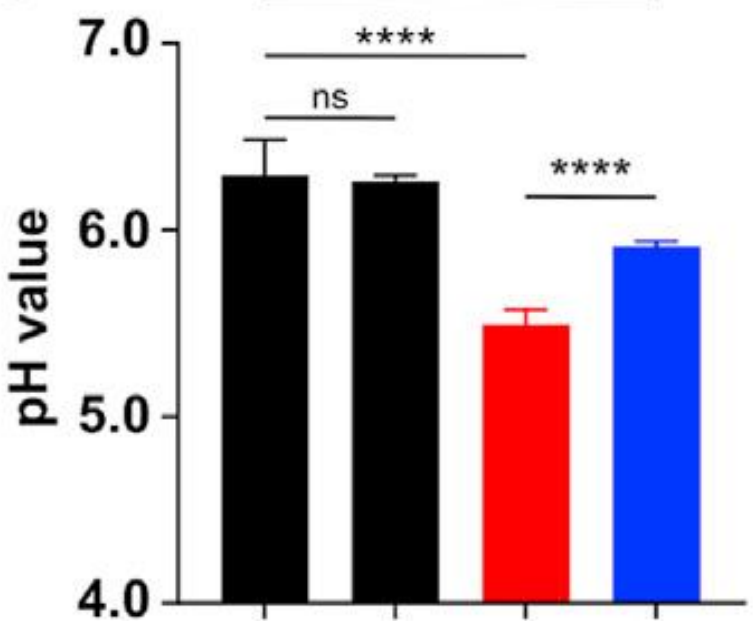




SLC37A4 pArg423*



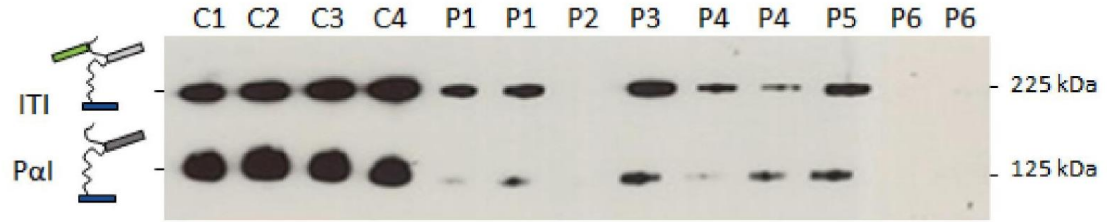
Ctrl



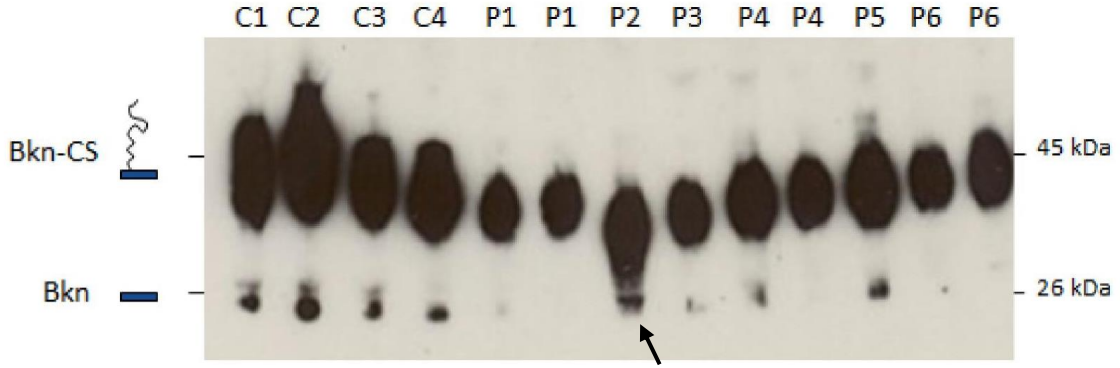
SLC37A4 mutation decreased Golgi pH

SLC37A4-CDG – Bikunin analysis

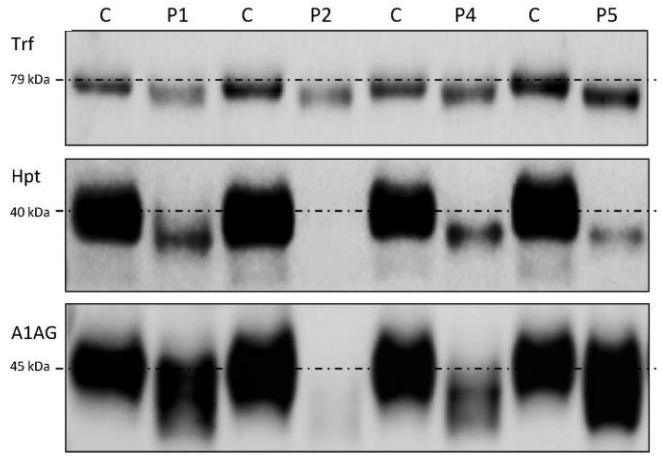
Haouari W, Raynord A et al., CCA, *2021*



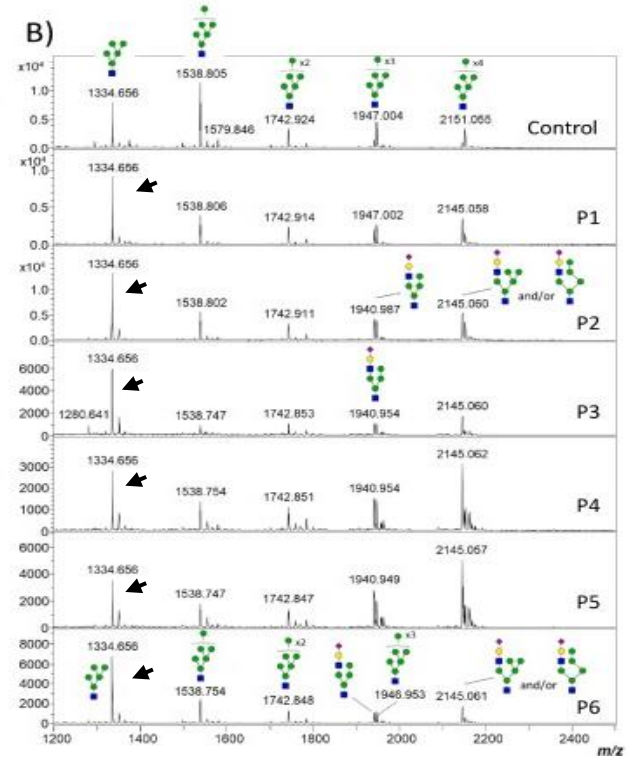
Impaired CS-HC esterification (6/6)
Normal ITI/PaI ratio



Normal CS elongation (5/6)
PG defect in one patient (most severe phenotype)



Impaired N-glycoproteins' WB profile



high-mannose N-glycans

SLC37A4-CDG: **Fast screening strategy**

- ↗ AST, decreased coagulation factors level F2, F11, AT
- CDG-type 2 transferrin pattern, abnormal WB of glycoproteins
- MS pattern with high-mannose N-glycans
- Abnormal bikunin profile

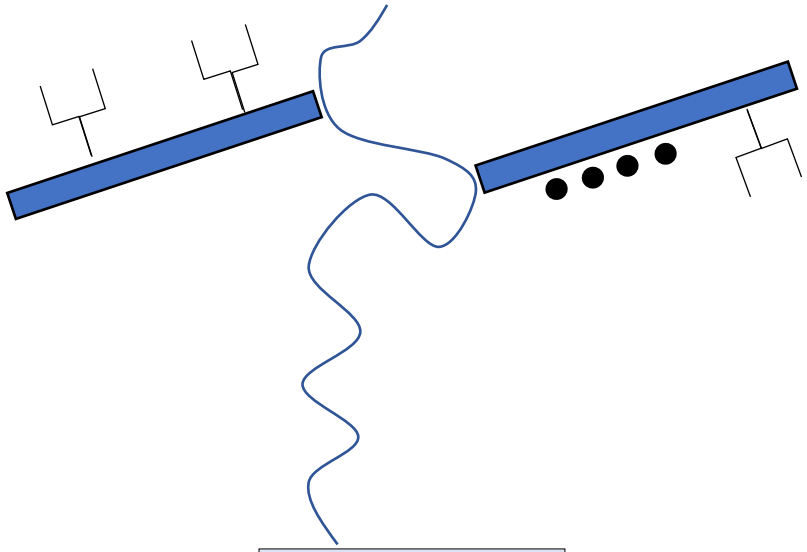
Conclusion

Versatile biomarker

Proteoglycan defects

CDG with impaired golgi homeostasis

Sérum/Plasma/Guthrie

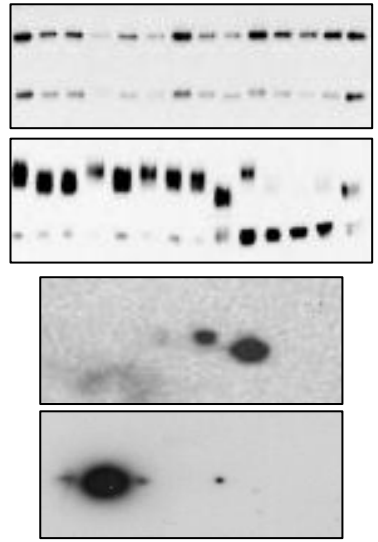


Bikunin

Convenient analysis

Western-blot

Électrophorèse 2D



Acknowledgments

Coordination

Arnaud BRUNEEL

Christian POÜS

Collaborations

François FOULQUIER

François FENAILLE

Bobby NG, Hudson FREEZE

Valérie CORMIER-DAIRE

université PARIS-SACLAY

INSERM U1193

"Pathophysiology of liver diseases"

Arnaud Bruneel

Antoine PILON

Anita BAILLET

Béatrice BENOIT

Daniel PERDIZ

Elise JACQUIN

Najet CHAREF

Bruno BAUDIN

Christian POÜS

Samra OUARAS

Alexandre RAYNORD

Sara MERABET

Isabelle CANTALOUBE

Ameetha RATIER