38èmes JNBC

16-18 Octobre 2025
Hôtel Radisson Blu- Tunis

New diagnostic strategies in Congenital Disorders of Glycosylation (CDG)

Young scientists session

Tomorrow's biology: new markers, emerging methods and diversified visions

Elodie LEBREDONCHEL

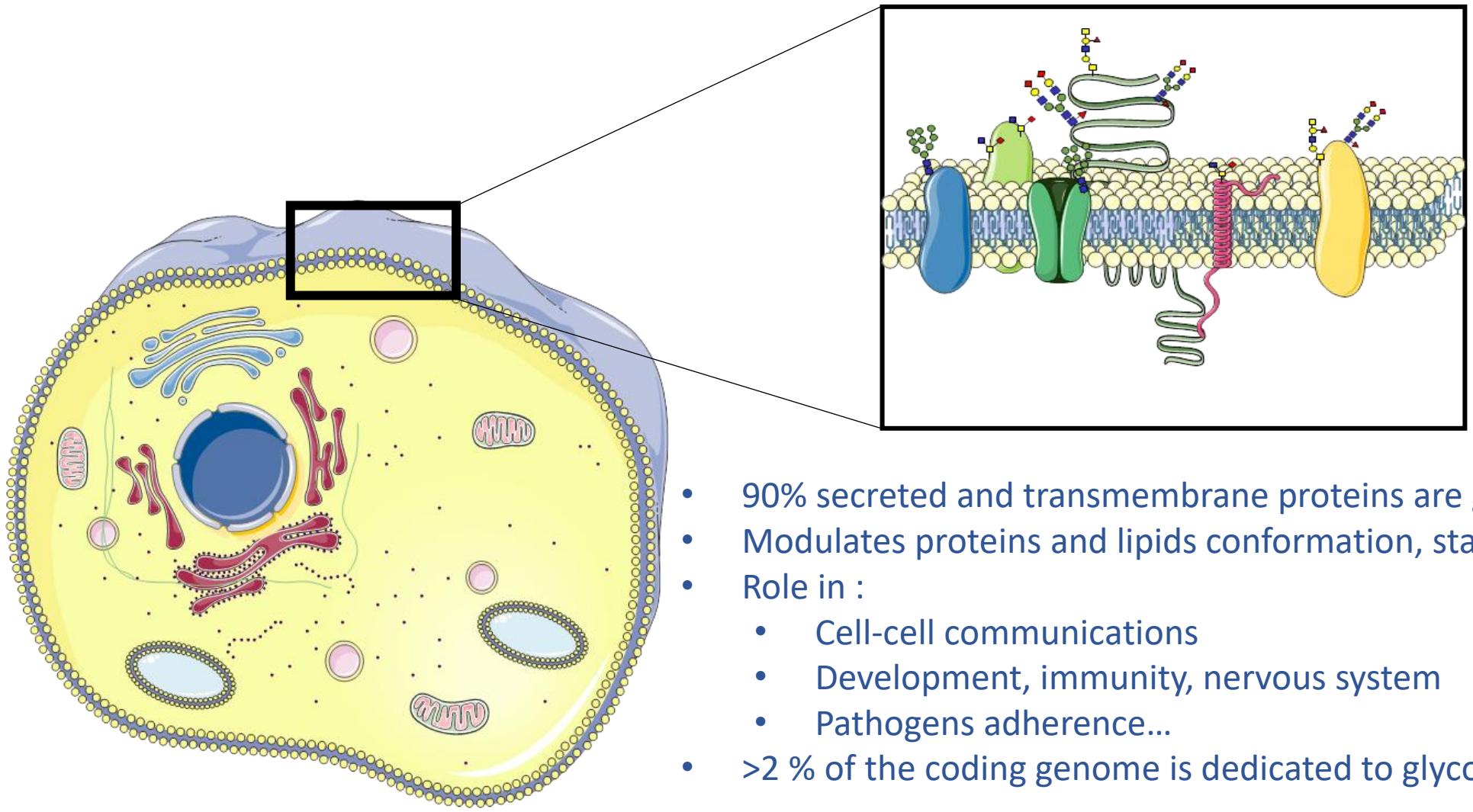
Praticien hospitalier, PharmD, PhD

Laboratoire de Biochimie Métabolique et Nutrition

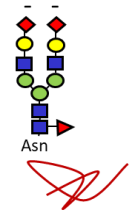
Hôpital Bichat, APHP, Paris, France

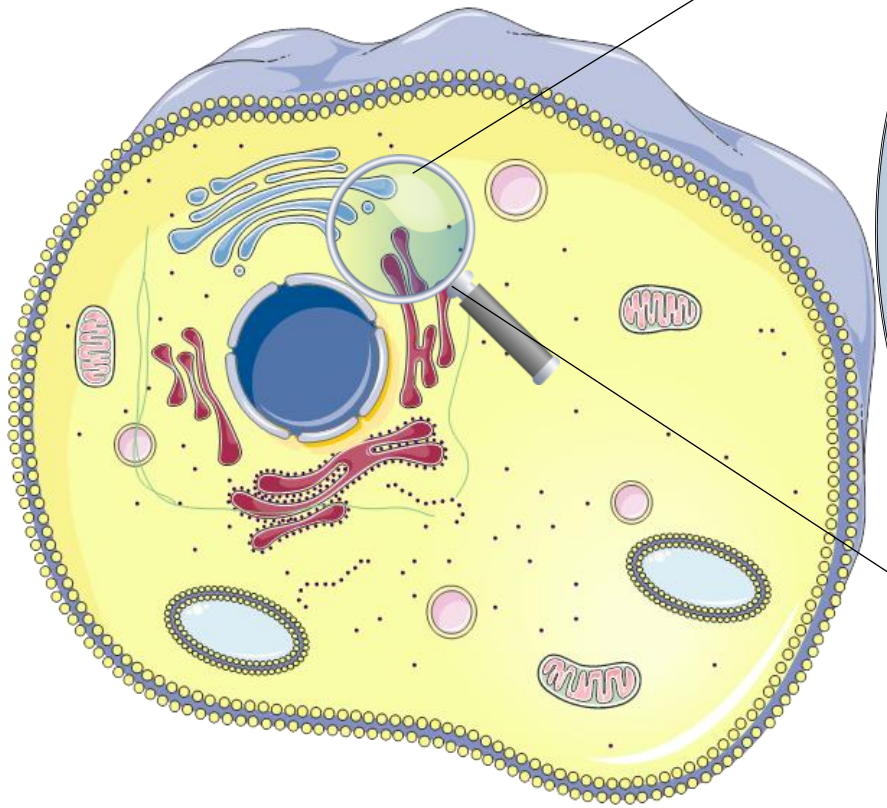
Young Scientist representant of IFCC taskforce on Global Newborn Screening
Representant of the Clinical Biology French Society (SFBC)

Importance of glycosylation

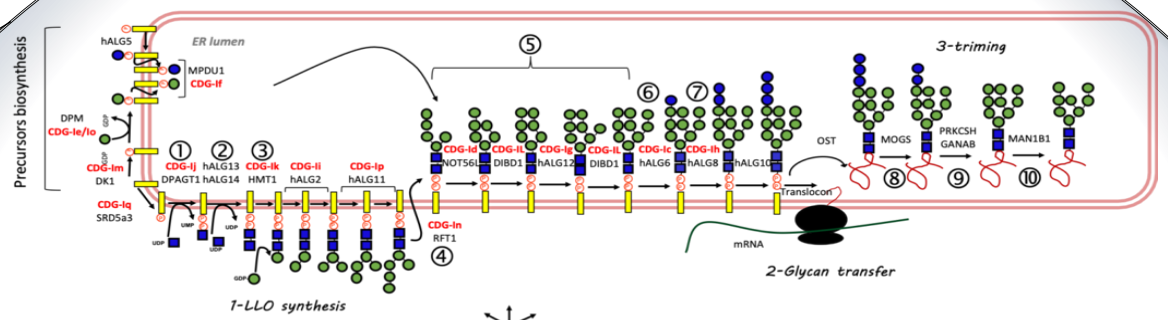


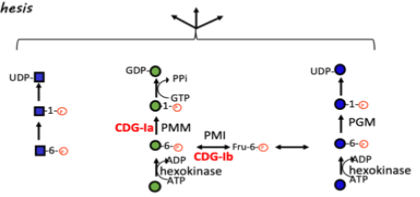
- 90% secreted and transmembrane proteins are glycoconjugates
- Modulates proteins and lipids conformation, stability, and functions
- Role in :
  - Cell-cell communications
  - Development, immunity, nervous system
  - Pathogens adherence…
- >2 % of the coding genome is dedicated to glycosylation actors





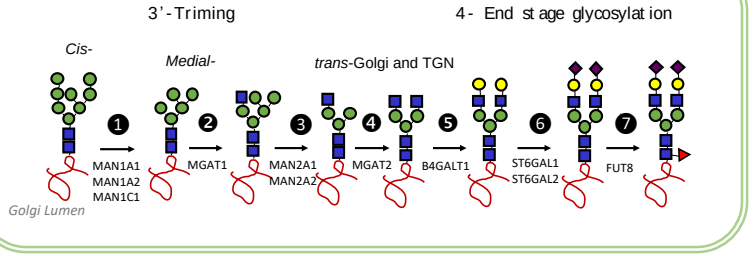
Endoplasmic Reticulum



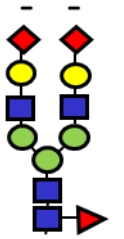


Legend:
- *N*-Acetylglucosamine (GlcNAc)
- Mannose (Man)
- Galactose (Gal)
- Glucose (Glc)
- *N*-acetylneuraminic acid (Neu5Ac)
- Fucose (Fuc)
- Dolichol
- Phosphate
- CDG-xx Localization of the different CDG

Golgi

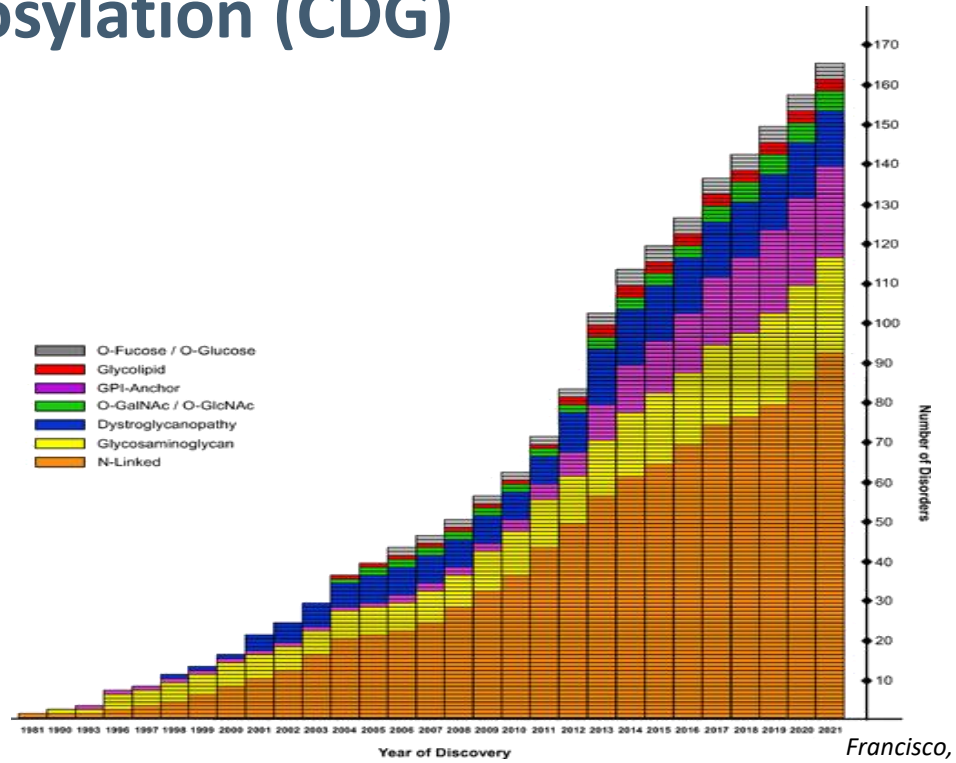


Cytosol



Congenital Disorders of Glycosylation (CDG)

- Rare genetic diseases, estimated 1/20 000 births (Jaeken, 1980)
- Autosomal recessive, rare X-linked cases
- Prevalence increases with consanguinity
- Today, > 160 different CDG *(Dos Reis Ferreira, 2022)*
- Affect glycosylation of proteins /lipids)



Francisco, 2022

Congenital Disorders of Glycosylation (CDG)

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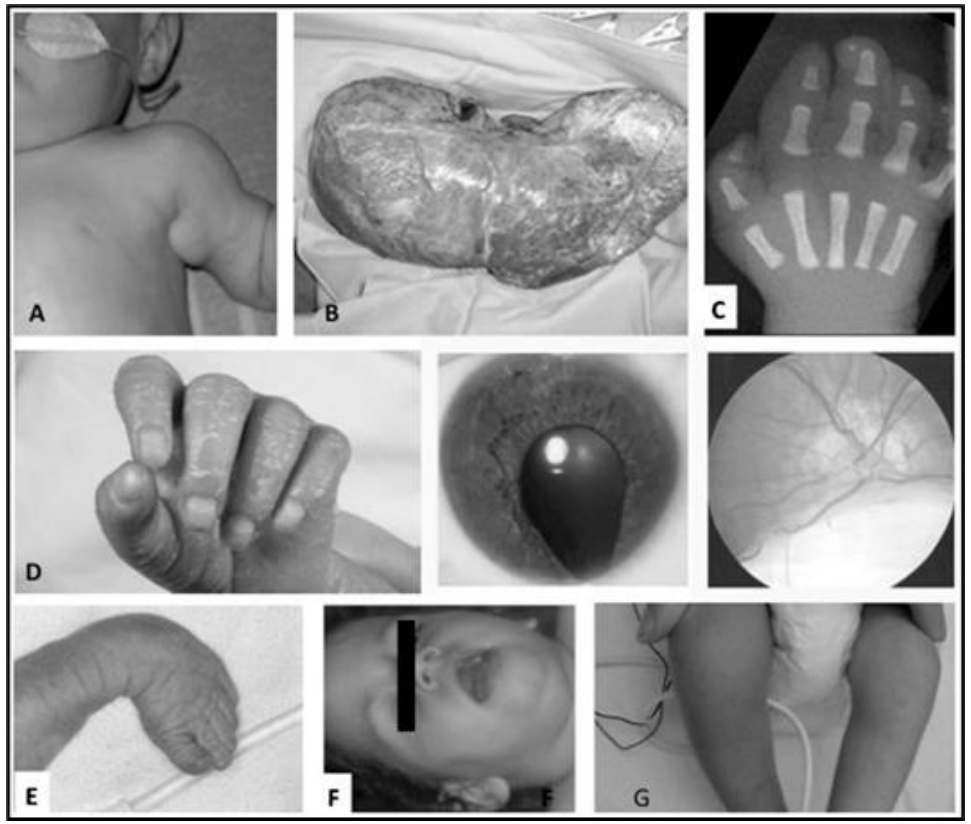
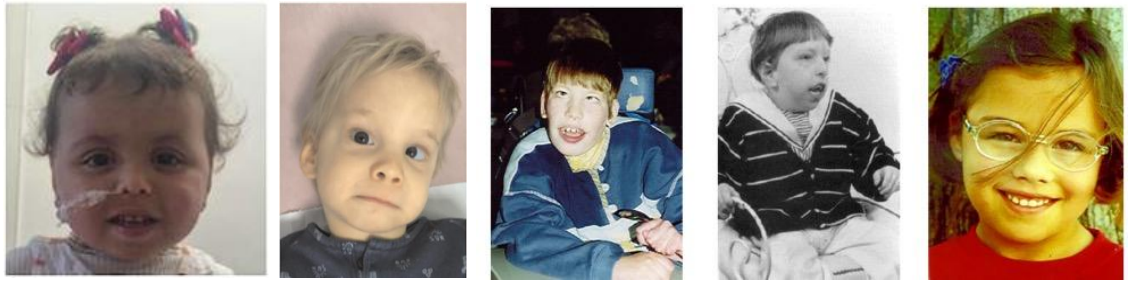
- First described in 1980 by Pr Jaeken
- In 2024, > 160 different CDG (Dos Reis Ferreira, 2022)
- Affect aglycosylation of proteins /lipids)

- Clinically, 'all is possible in CDG': multiorgan diseases, frequent neurological symptoms
- **Hypotonia**, **developmental delay**, cerebellar ataxia, **intellectual disability**, seizures, encephalopathy, microcephaly, **facial dysmorphism**, lipodystrophy, inverted nipples, growth delay, hepatomegaly, **coagulation disorders**, **hypoglycemia**, cardiomyopathy, **strabismus,** nystagmus, gastrointestinal signs, frequent infections, endocrine disorders (e.g., hypothyroidism, hypogonadism)…



De Lonlay, et al.



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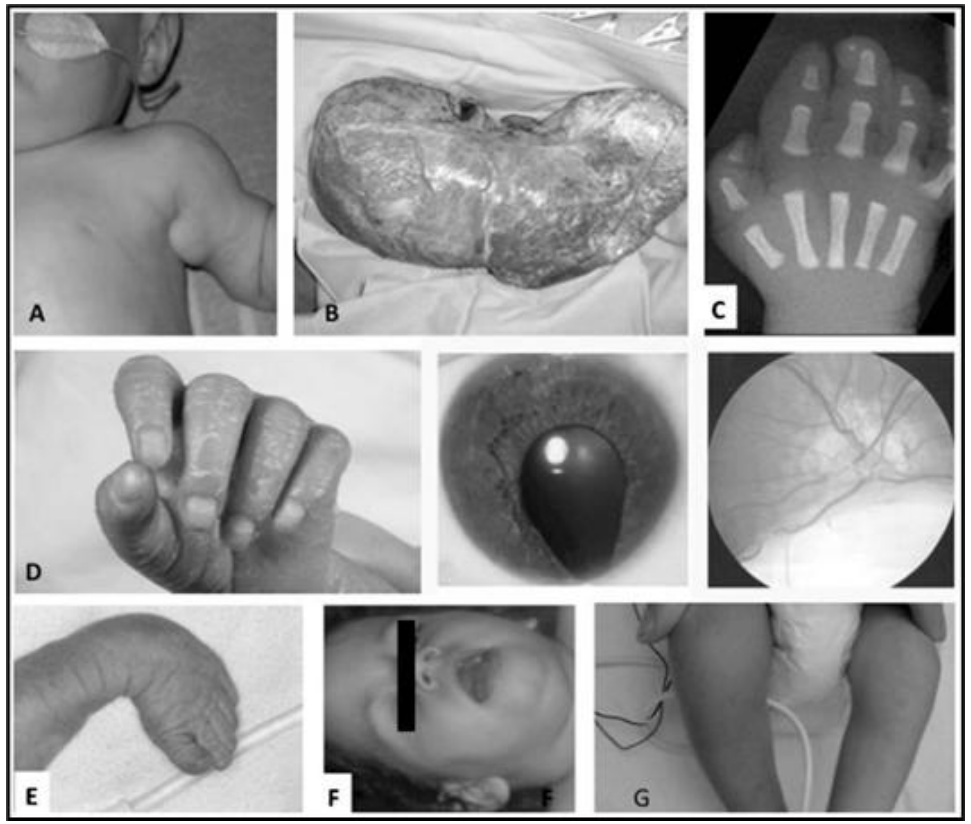
- Hypotonia, developmental delay, cerebellar ataxia, intellectual disability, epileptic seizures, encephalopathy, microcephaly, facial dysmorphism, lipodystrophy, inverted nipples, growth retardation, hepatomegaly, coagulation disorders, hypoglycemia, cardiomyopathy, strabismus, nystagmus, gastrointestinal signs, frequent infections, endocrine disorders (e.g., hypothyroidism, hypogonadism)…

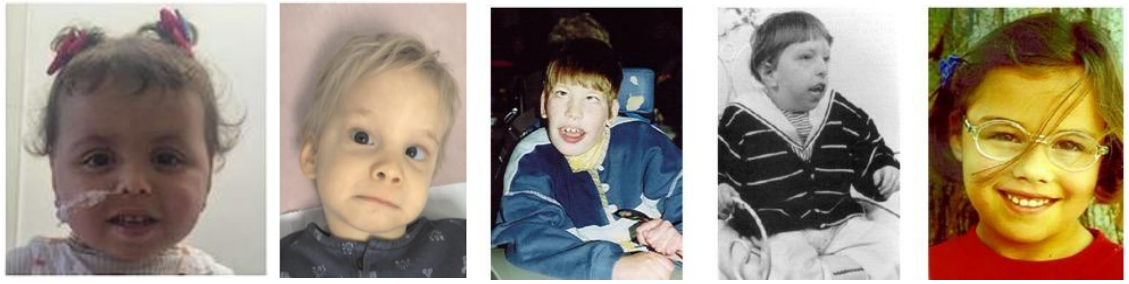


De Lonlay, et al.

- Very few treatable CDG

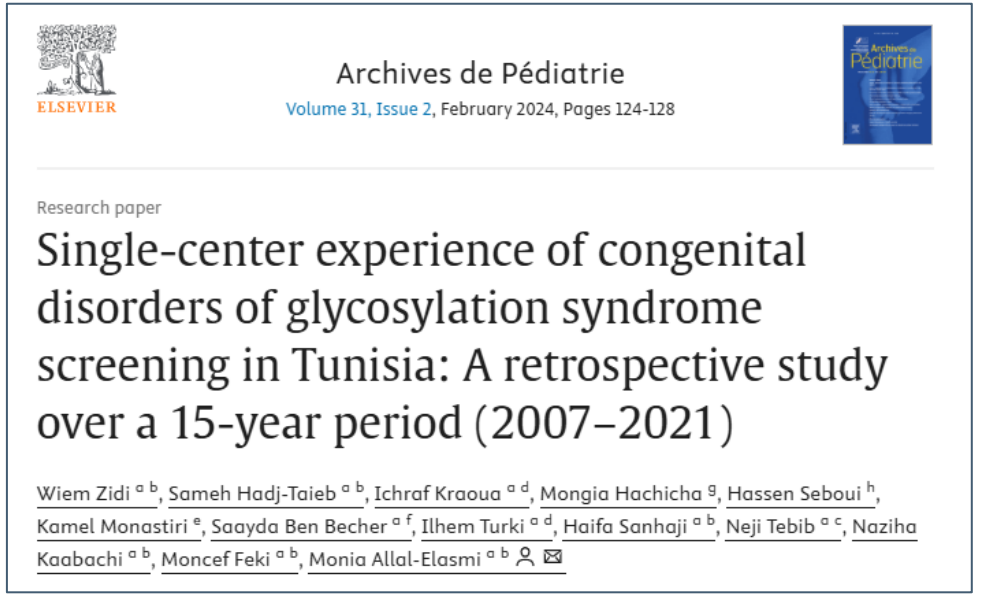
(MPI-CDG, PGM1-CDG, TMEM165-CDG…)

- ~ 100 families in France
- Data missing for most continents
- Tunisia: some reported families



CDG in Tunisia (1)

- Samples from Rabta Hospital (pediatric 46%); National Institute of Neurology (pediatric neurology 40%), other hospital (14%)
- **1055** analysis for CDG suspicion → **23** CDG (2.18% of positive)
- 1 lab in Rabta
- 13 boys, 10 girls
- Between 3 months to 13 years
- 19 **PMM2**-CDG, 3 CDG-II, 1 CDG-Ix
- 91% psychomotor delay, 31% cerebellar atrophy, 48% strabismus, 52% dysmorphic symptoms, 35% *retinis pigmentosa* cataract, 30% hypotonia
- Consanguinity 83%
- Calculated prevalence in Tunisia **1:23 720**



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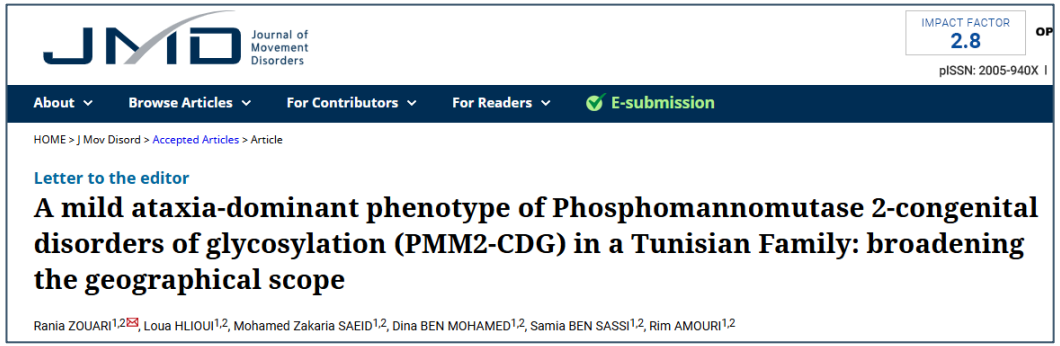
Research paper

Single-center experience of congenital disorders of glycosylation syndrome screening in Tunisia: A retrospective study over a 15-year period (2007–2021)

Wiem Zidi [a b], Sameh Hadj-Taieb [a b], Ichraf Kraoua [a d], Mongia Hachicha [g], Hassen Seboui [h], Kamel Monastiri [e], Saayda Ben Becher [a f], Ilhem Turki [a d], Haifa Sanhaji [a b], Neji Tebib [a c], Naziha Kaabachi [a b], Moncef Feki [a b], Monia Allal-Elasmi [a b]



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Letter to the editor

A mild ataxia-dominant phenotype of Phosphomannomutase 2-congenital disorders of glycosylation (PMM2-CDG) in a Tunisian Family: broadening the geographical scope

Rania ZOUARI[1,2], Loua HLIOUI[1,2], Mohamed Zakaria SAEID[1,2], Dina BEN MOHAMED[1,2], Samia BEN SASSI[1,2], Rim AMOURI[1,2]

A Founder Variant in Tunisian PMM2-CDG Patients: An Integrated Clinical, Radiological, Biochemical, and Genetic Study.

Lilia KRAOUA[1], Thouraya BEN YOUNES[2], Monia EL ASMI[3]. Abir ZIOUDI[2], Hedia KLAA[2], Zouhour MILADI[2]. Hanene BENRHOUMA[2]. Sonia NAGI[4], Sylvie ALGLAVE[8], Arnaud BRUNEEL[5,6], Elodie LEBREDONCHEL[5], Thierry DUPRE[5,7], Sandrine VUILLAUMIER BARROT[5,7,8], *Ichraf KRAOUA[2]*

[1] Department of Congenital and Hereditary Diseases, Charles Nicolle Hospital, Tunis, Tunisia; Faculty of Medicine of Tunis, University of Tunis El Manar, Tunis, Tunisia

- Underdiagnosed or misdiagnosed even in Europe
- Resemblance with neurological disorders
- Unawareness of physicians
- **Biochemical tests are mandatory to complete the clinical diagnosis**

CDG in Tunisia (2)

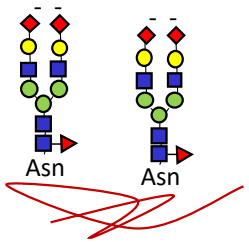
- Samples of 10 Tunisian families studied at Bichat Biochemistry lab

	First name	Birthdate		Sample reception	variant 1	variant 2
Familly 1	Rayen	30/06/2008	PMM2	12/06/2019	I132T	I132T
	Wissem	03/07/2000	PMM2	25/11/2013	I132T	I132T
	Ahlem	06/03/2010	PMM2	05/06/2023	I132T	I132T
	Zakaria	19/12/2022	PMM2	16/05/2024		
Familly 2	Amira	29/05/2015	PMM2	18/10/2007		
	Ayet	29/05/2015	PMM2	18/10/2007		
Familly 3	Chadly		PMM2	13/06/2002	T237M	R141H
	Ghofrane		PMM2	13/06/2002	T237M	R141H
Familly 4	Giovanni	29/01/2001	PMM2	02/07/2002	V231M	F157S
Familly 5	Jasser	01/04/2004	PMM2	25/11/2015		
	Maissa	27/06/1999	PMM2	25/11/2015		
Familly 6	Ranim	16/06/2012	PMM2	20/05/2022		
Familly 7	Faiza	28/01/1998	ALG6	01/04/2003	A333V	A333V
	Hamdi	04/07/1989	ALG6	01/04/2003	A333V	A333V
Familly 8	Intissar	01/10/1995	ALG6	19/04/2001	A333V	A333V
	Manel	18/09/1998	ALG6	19/04/2001	A333V	A333V
	Meriem	18/09/1998	ALG6	19/04/2001	A333V	A333V
Familly 9	Azza	20/06/2005	RFT1 (VUS?)	20/09/2022		
Familly 10	Youssef		CDG-Ix	13/08/2015		

CDG CARE
Where CARE Grows...

Analytical techniques: protein glycosylation study

- First and foremost biomarker: serum transferrin for N-glycosylation
- 1-4 g/L
- ~ 77 kDa N-glycoprotein (two complex N-glycan moieties)
- Initially developed as a biomarker of chronic alcohol abuse (= CDT)

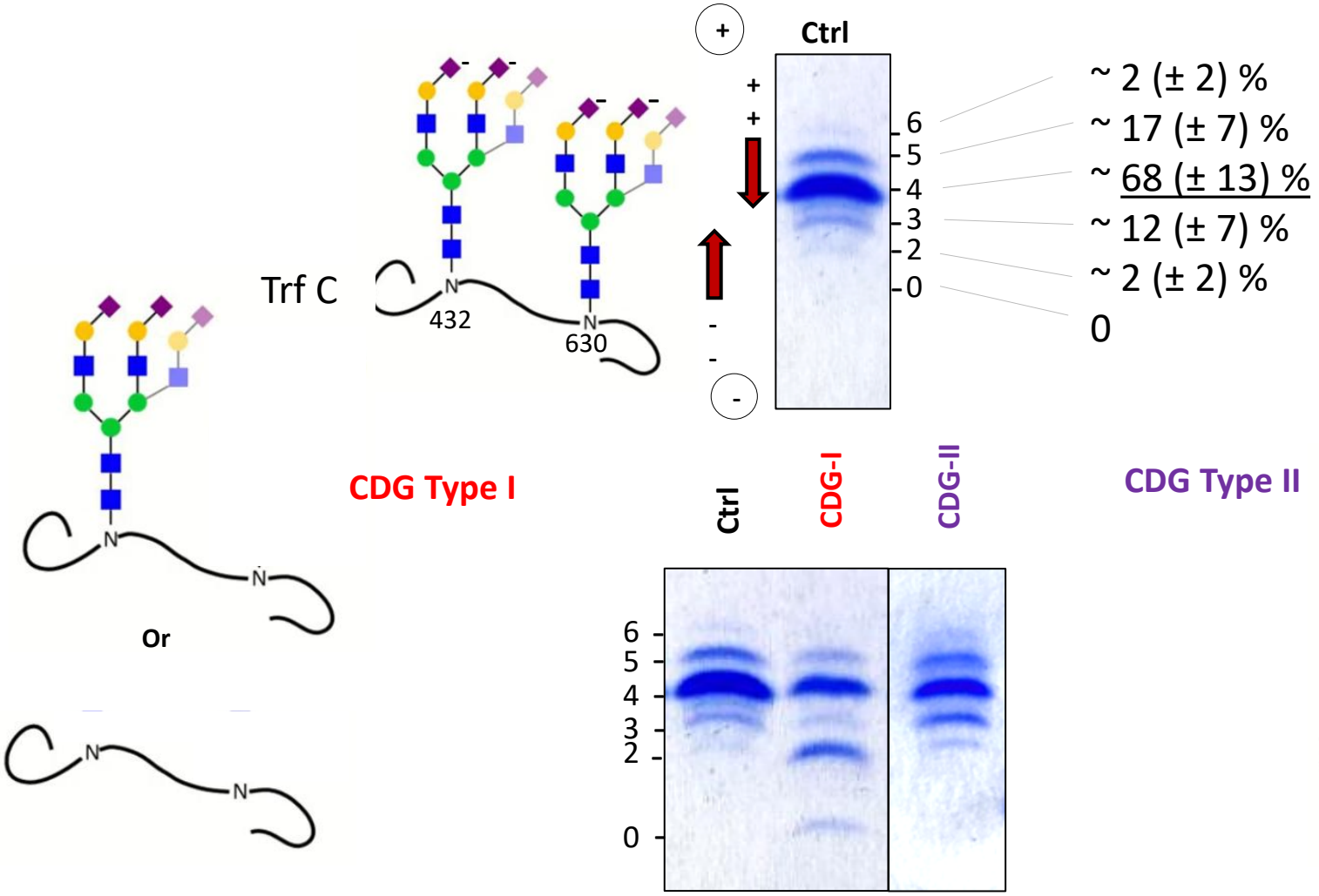


- Hypoglycosylated transferrin observed in the serum and CSF of two sisters suffering from an unknown multisystemic disease (Jaeken, 1984): emergence of CDG as a new group of inherited metabolic diseases
- First CDG classification based on abnormal N-glycosylation (CDG-Ia, CDG-Ib,…, CDG-IIk…), now *[GENE]*-CDG
- Gel-based techniques which separate glycoforms according to charge and/or Mw
- Gold standard: isoelectric focusing (IEF) (van Eijk, 1982), still used in France (Lille) and other countries
- Separation of transferrin glycoforms based on their isoelectric point, which is increased in case of loss of terminal sialic acids

Used in Lille

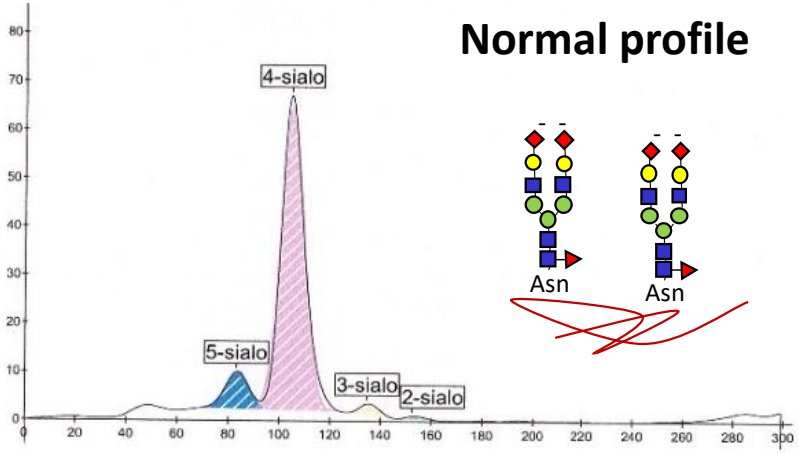
Isoelectric focusing (IEF) principle

- This technique led to the description of two categories of CDG: type 1 (loss of one or two complete glycan chains) and type 2 (loss of sugars among the chain)



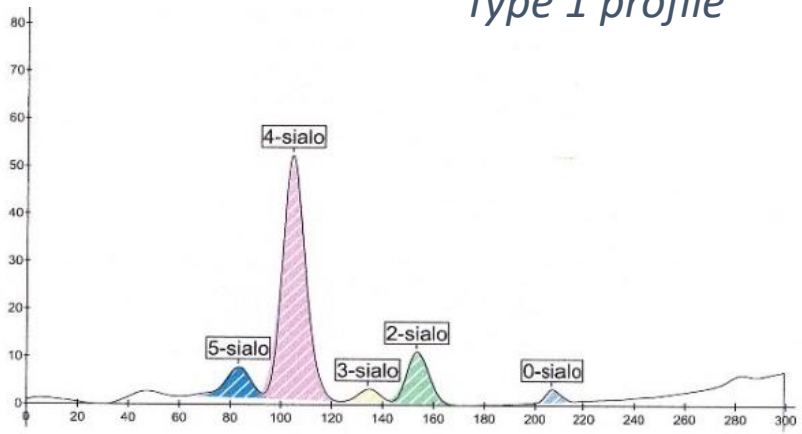
Used in Bichat, Paris

Capillary Electrophoresis (CE) of transferrin

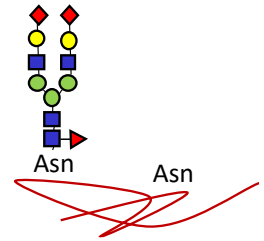


- Nowadays, capillary electrophoresis (CE) allows high-throughput study of transferrin glycoforms
- The advantage of these analyzers is that they can also be used for other tests, such as the separation of other proteins (SPEP, hemoglobinopathy) and the detection of chronic alcohol consumption (CDT).

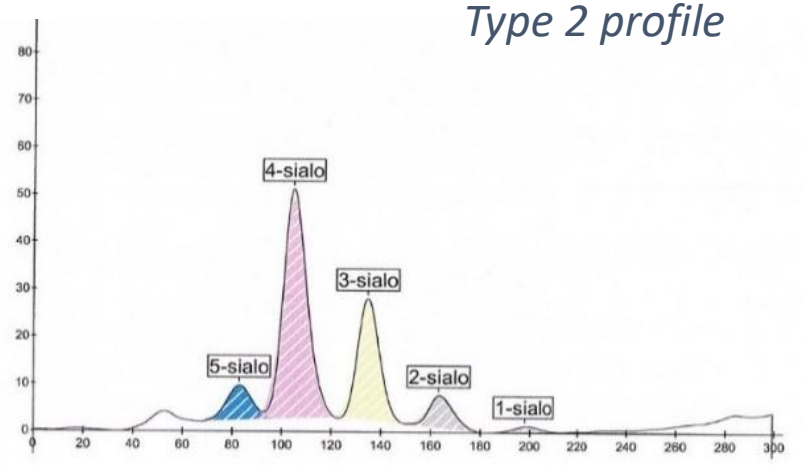
Type 1 profile



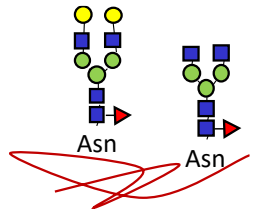
→ Abnormal glycan precursor synthesis or transfer (ER)



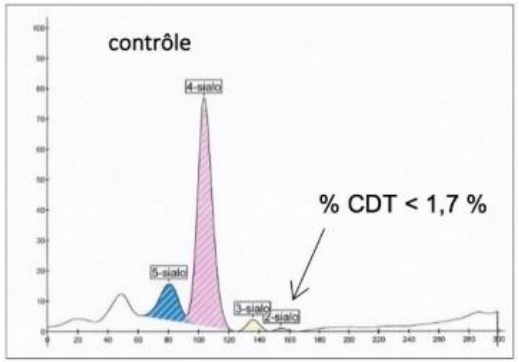
Type 2 profile

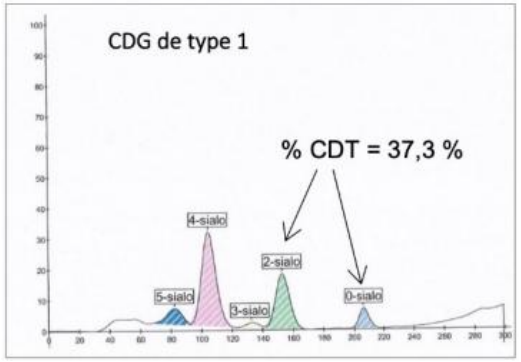


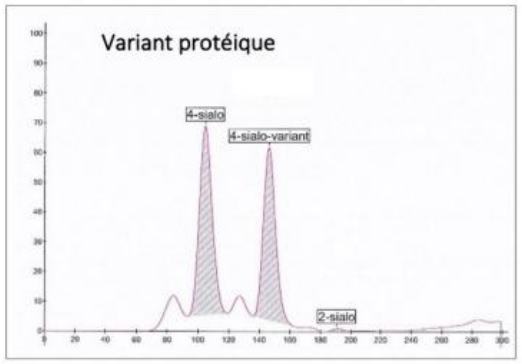
→ Abnormal glycan maturation (ER, Golgi)



Main limits



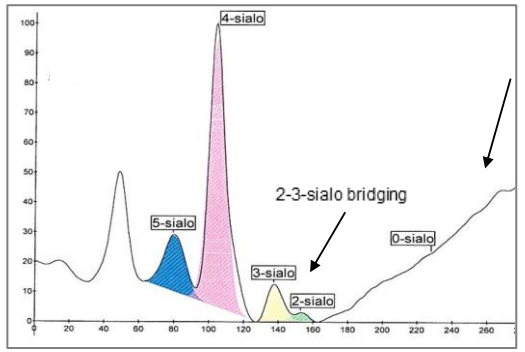




- Transferrin amino acid variants

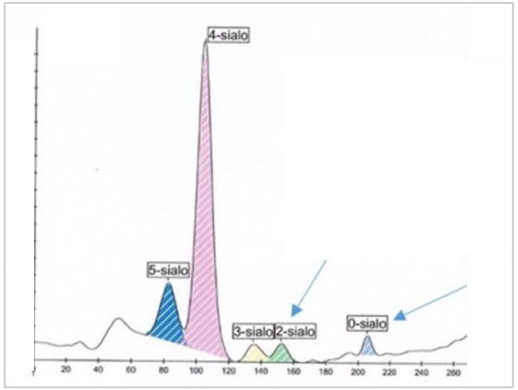
- can modify the electric charge of the protein backbone and produce abnormal results independent of glycosylation status

- how to deal with it: treat sample with sialidase/neuraminidase

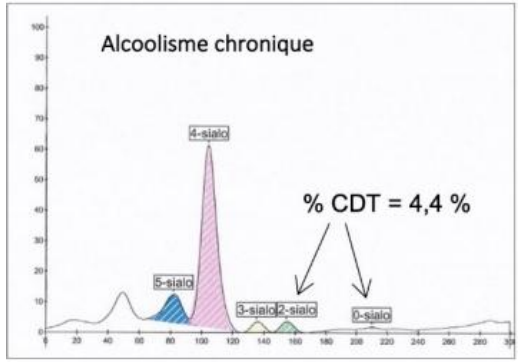


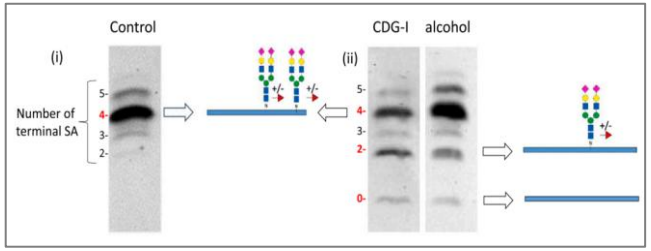
- Hepatopathy / Alcohol abuse

- *may result in abnormal transferrin glycosylation independent of CDG*
- *how to deal with it: tricky; clinical context and genetic testing help interpret results. Also, look for a tri-disialo bridging.*



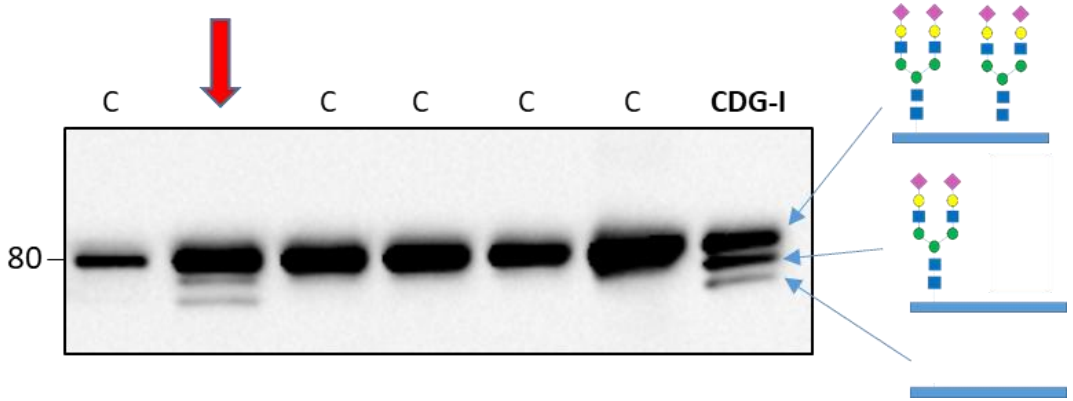
- Galactosemia / Fructosemia



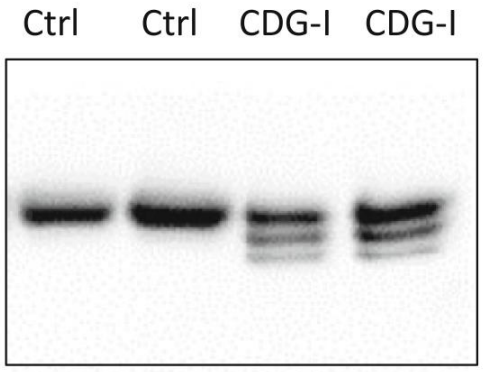


Western Blot

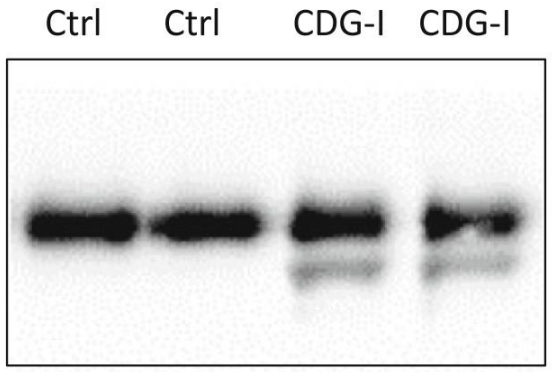
- To confirm a CDG type 1, when we only have filter paper (Guthrie test card), for external quality control when the volume is too low
- Transferrin, α1-antitrypsin, α1-acid glycoprotein, and haptoglobin in CDG-I affected individuals typically display additional bands of decreased molecular weights



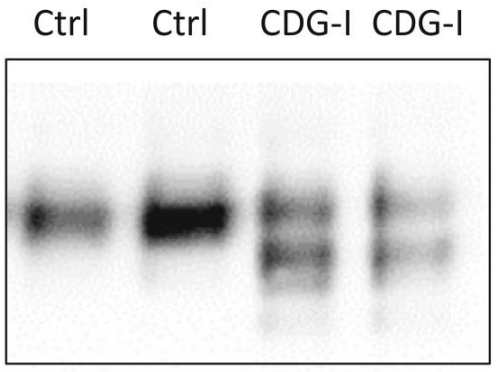
transferrin



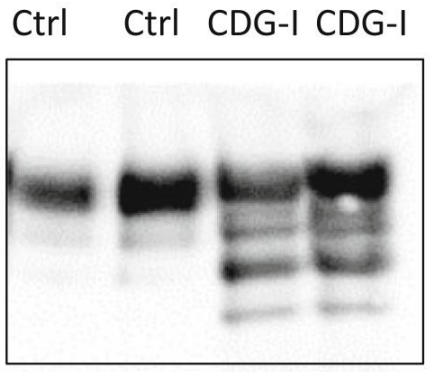
α1-antitrypsin



α1-acid glycoprotein



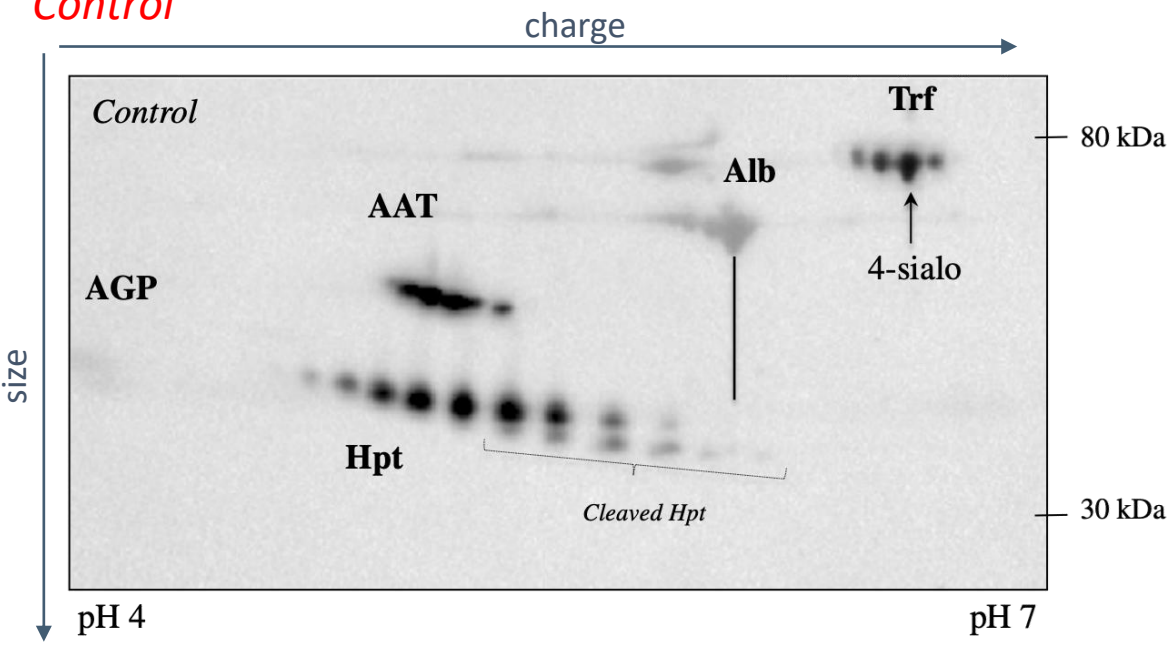
haptoglobin

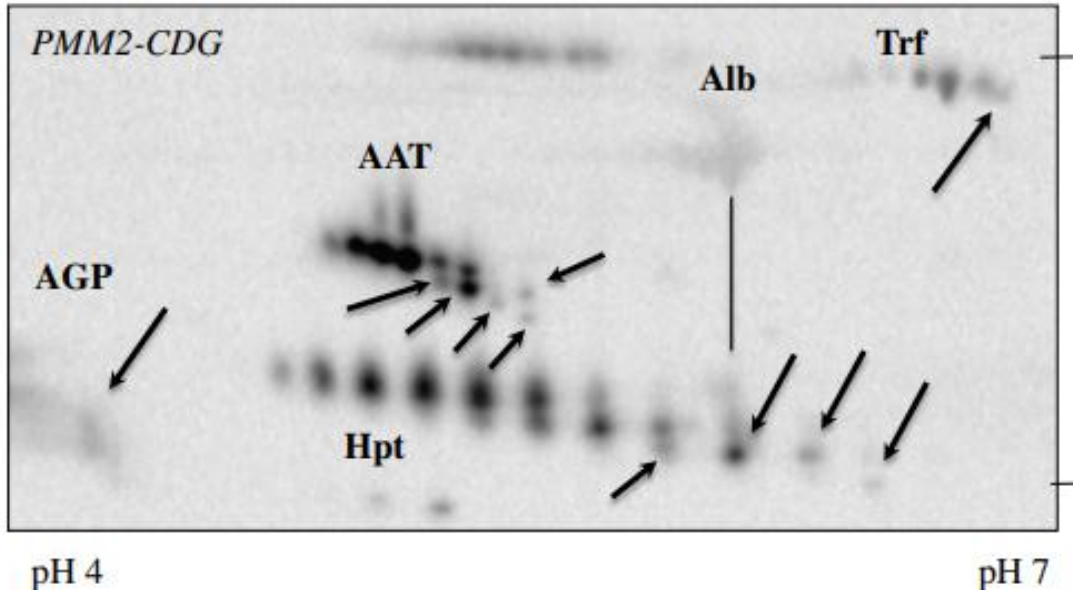


2D Electrophoresis

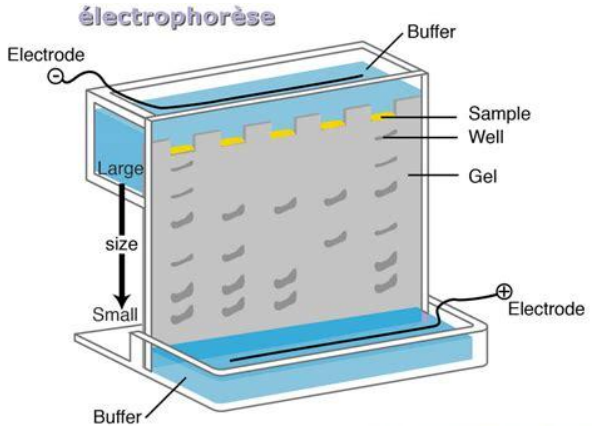
- Complementary biochemical method, such as two-dimensional gel electrophoresis of N-glycoproteins, allows the distinction between type I and type II CDG

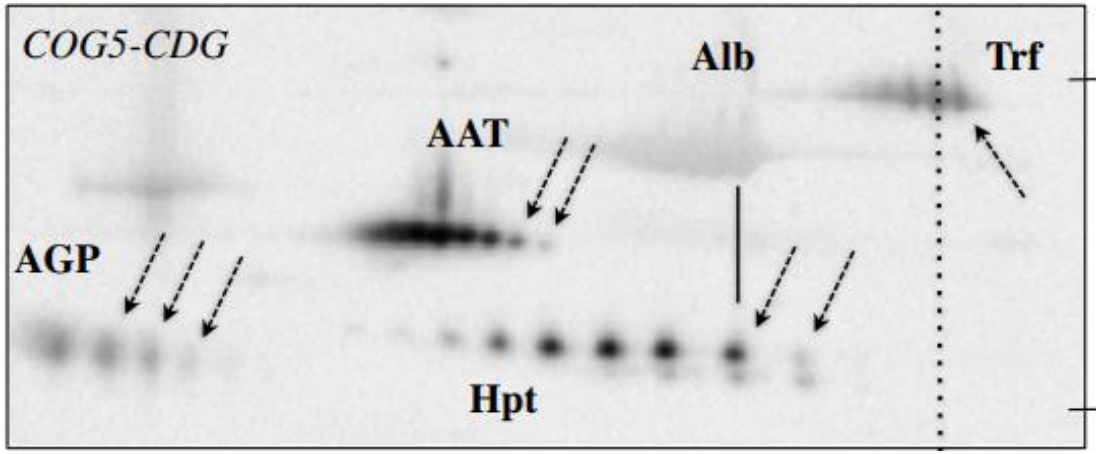
Control





Type 1 CDG profile

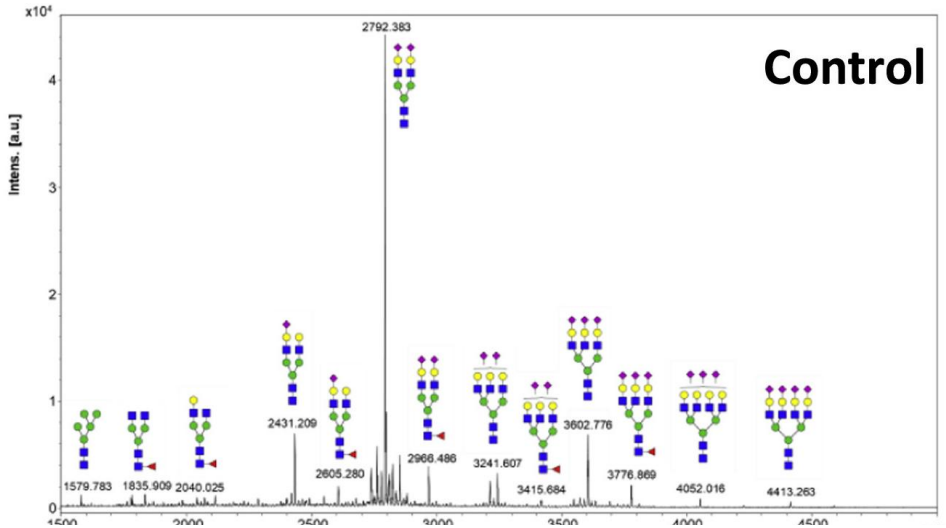


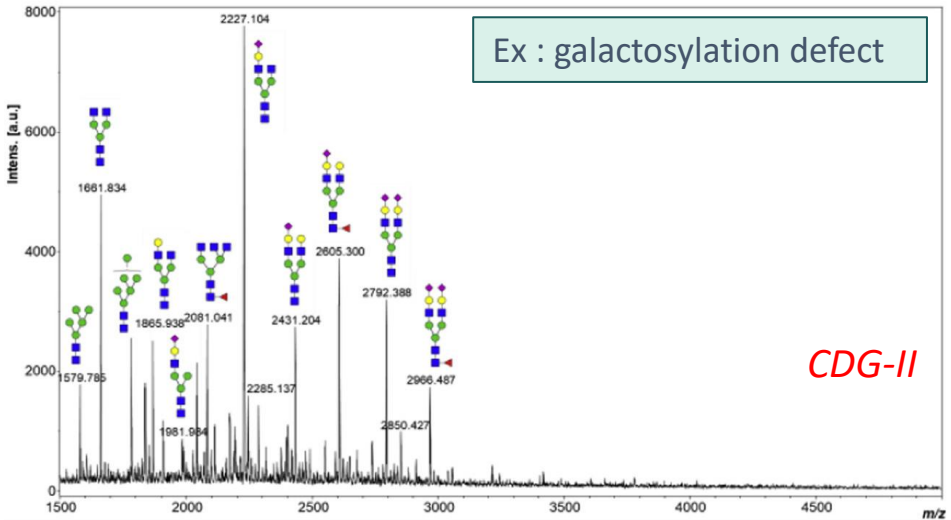


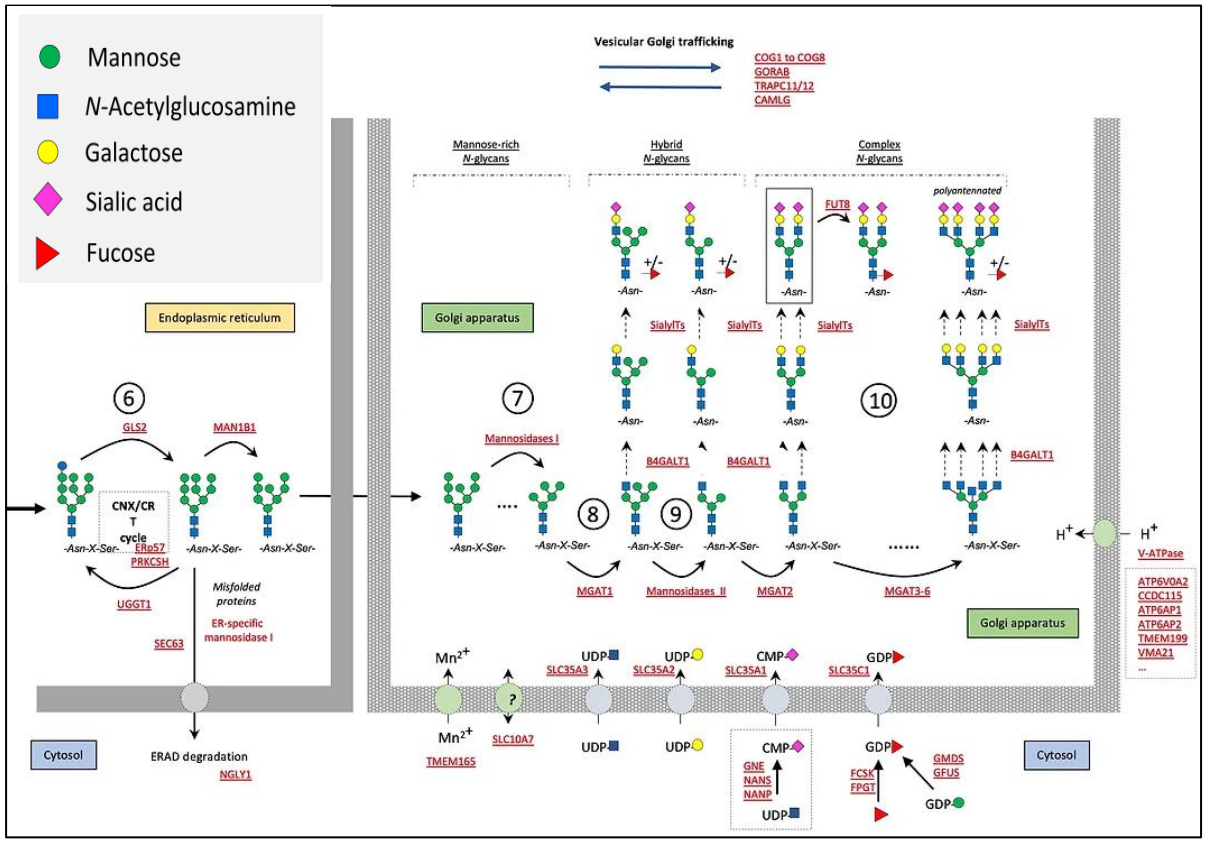
Type 2 CDG profile

Mass spectrometry (1)

- Mass spectrometry of intact serum transferrin allows precise characterization of glycosylation abnormalities in CDG-II
- Also used to « take a photography » of all serum N-glycans (i.e., N-glycome), collected after cleaving from glycoproteins with PNGase F (useful in type 2 CDG)



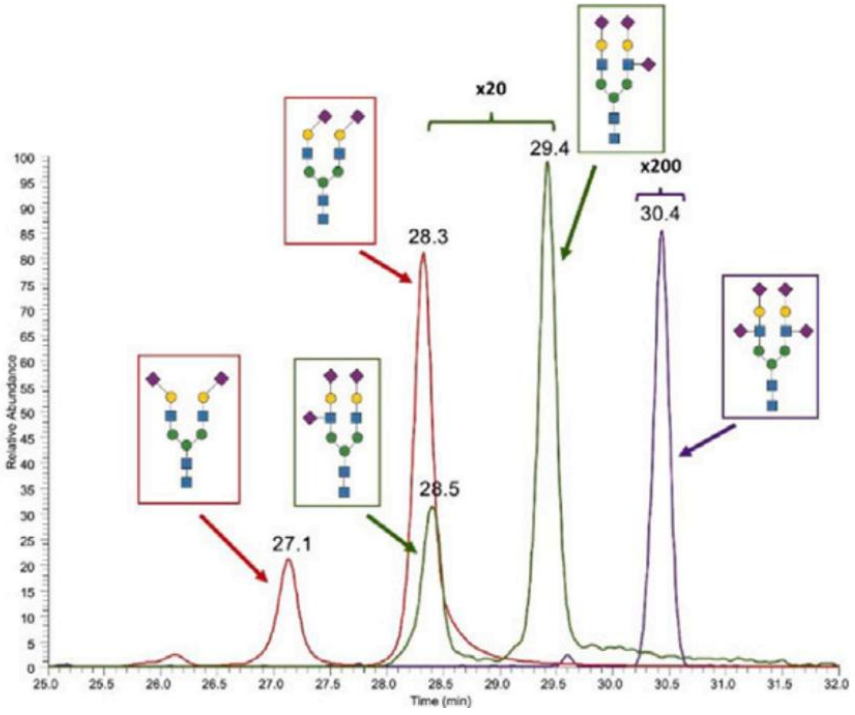


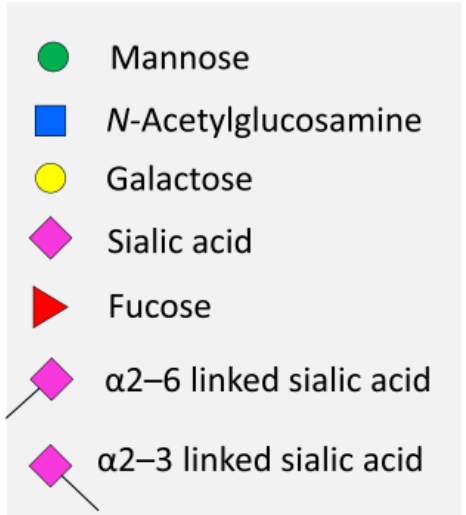


NEW

Mass spectrometry (2)

- Emergent MS techniques: isomeric mass spectrometry, glycoproteomics (provides protein-specific and site-specific informations)

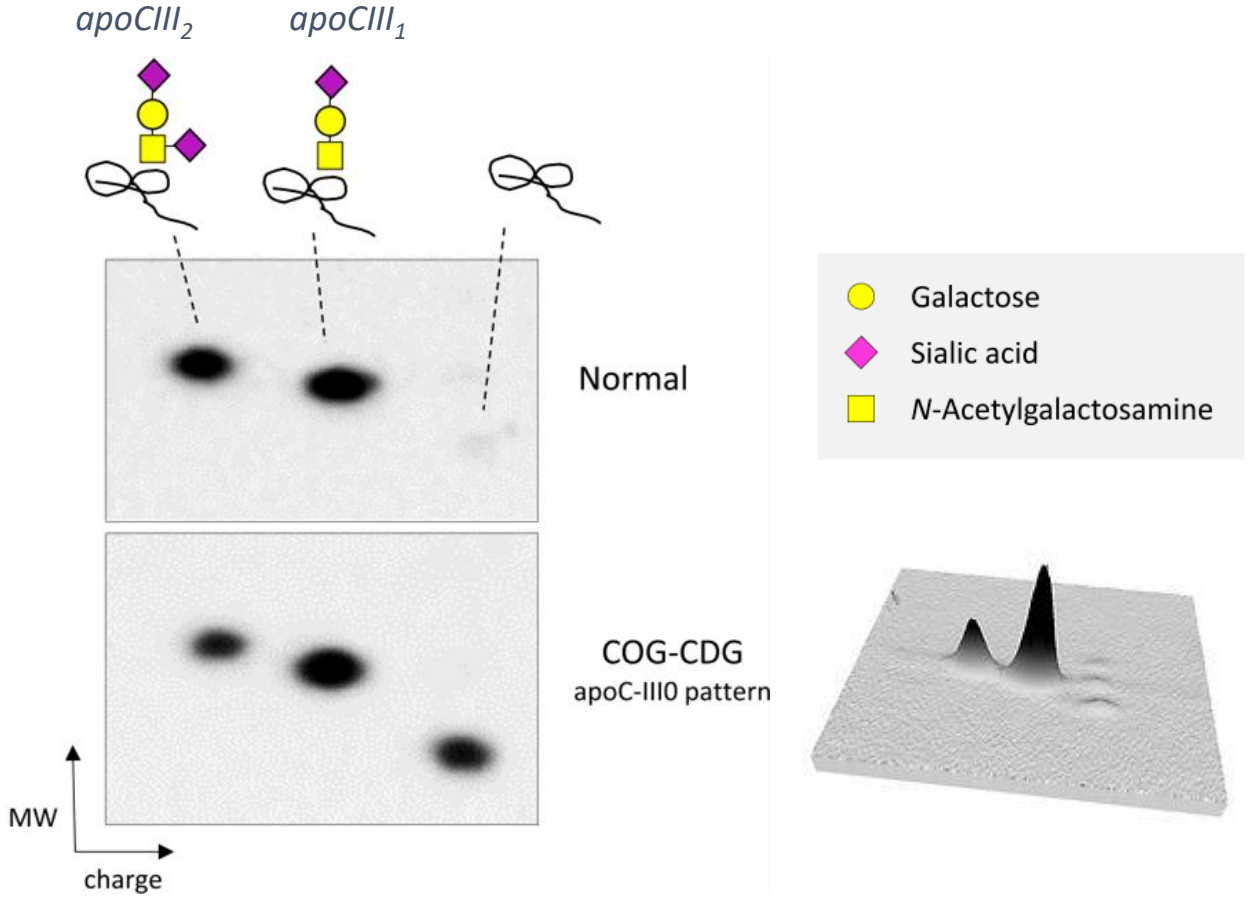


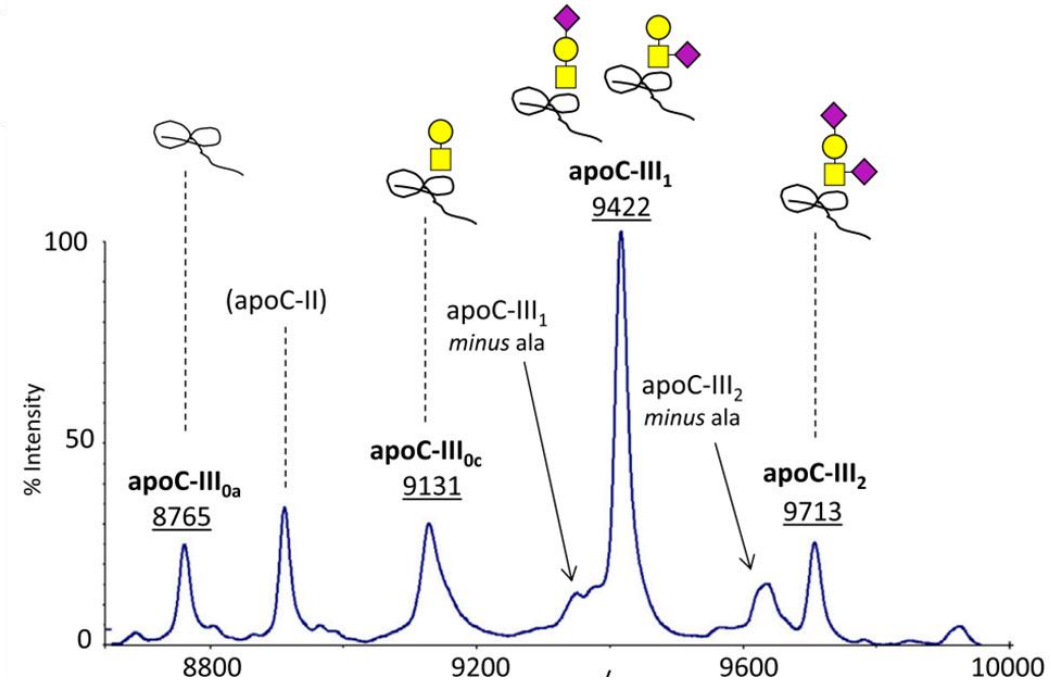


From Sturiale L, et al. doi: 10.1016/j.isci.2021.102323.

ApoCIII

- Apolipoprotein C-III (apoC-III) is a biomarker of CDG affecting mucin core 1 type O-glycosylation
- Particular interest in the diagnosis of type 2 CDG with impaired Golgi homeostasis
- Classical technique: isoelectric focusing
- Refinement: 2-DE (allows discrimination of aglycosylated apoC-III from asialylated apoC-III)

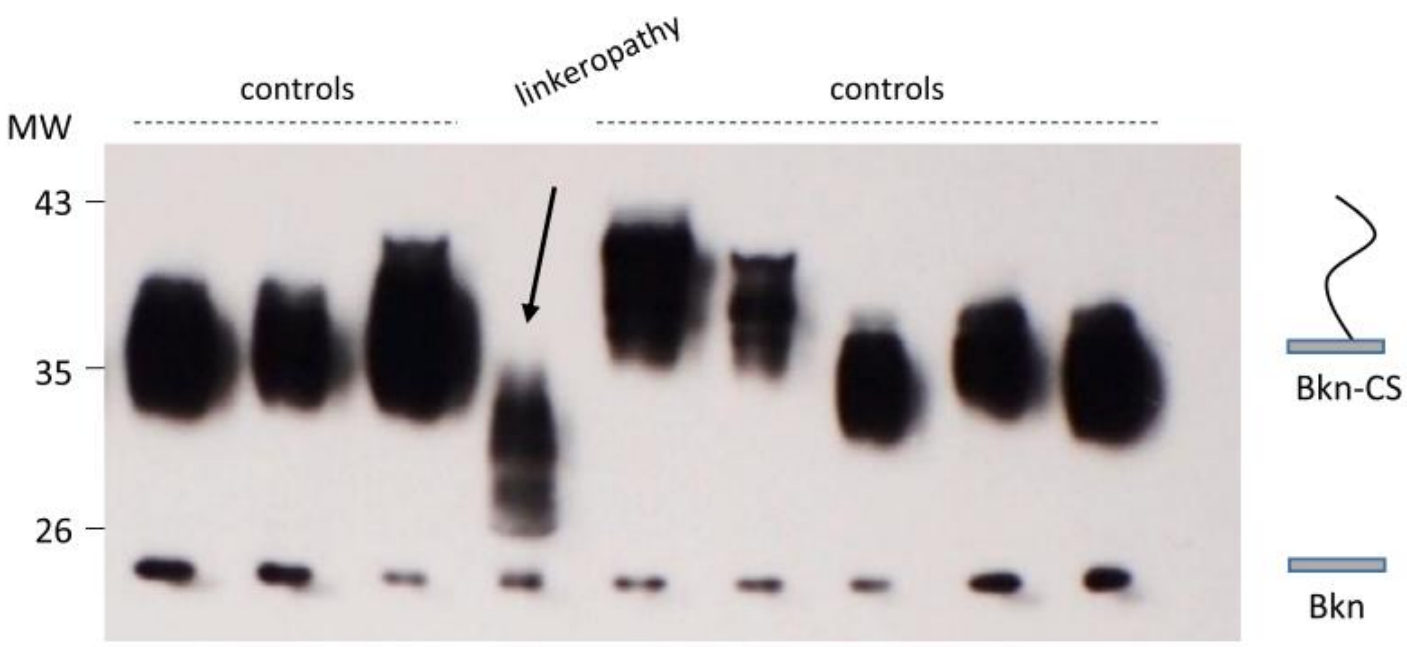


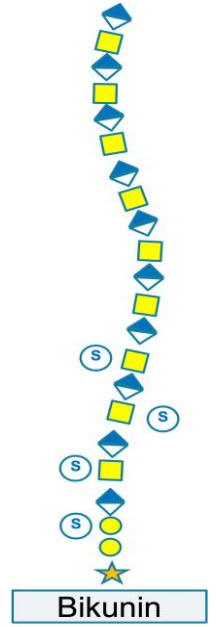


From Yen-Nicolay S. et al. doi: 10.1002/prca.201400187.

Western Blot of Bikunin

- Glycosaminoglycans: bikunin is a serum protein bearing a chondroitin sulfate (CS) chain
- Abundance of bikunin with shortened CS chain in the sera of patients with linkeropathies

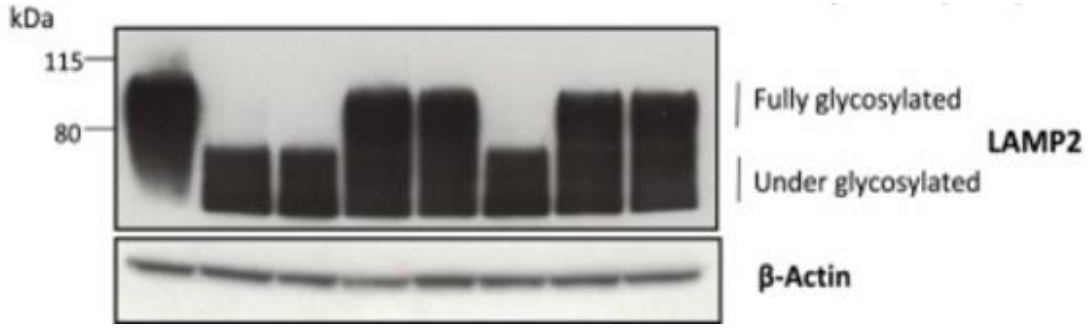




Cellular CDG biomarkers (LAMP2, TGN46)

- Other techniques can be performed in cells if serum analyses are not informative (e.g., some CDG have little/no hepatic expression and therefore do not alter the majority of serum glycoproteins)

e.g.: SDS-PAGE/Western-blot of LAMP2 and TGN46 in fibroblasts (study of N- and O-glycosylation, respectively)



From Durin Z, et al. doi: 10.1016/j.trsl.2023.11.005.

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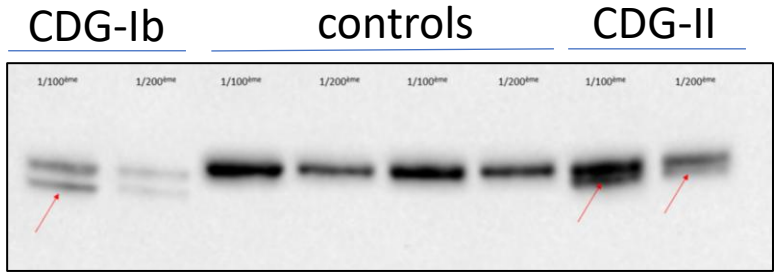
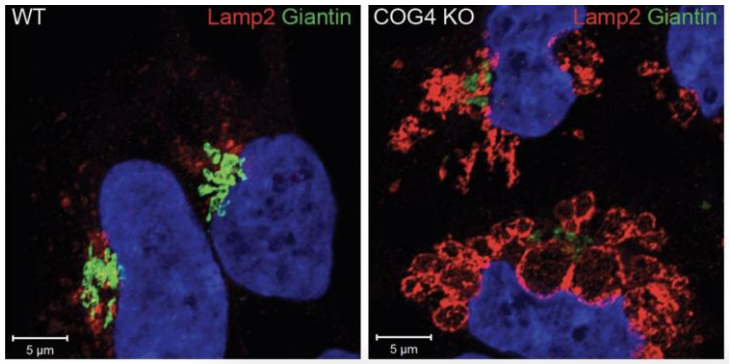
- Promising emerging biomarkers: Antithrombin III



- Complementary techniques can highlight defective pathways/processes:

e.g.: Lysotracker®, pHluorin, brefeldin A treatment to study Golgi trafficking…

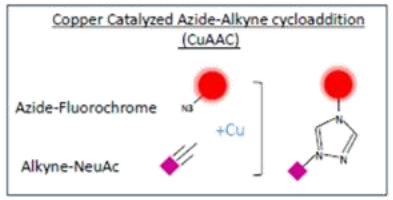


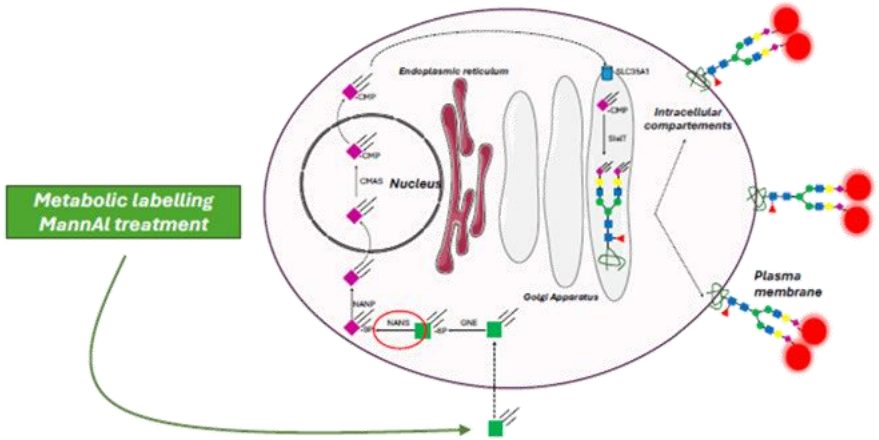
Lupashin et al.

NEW

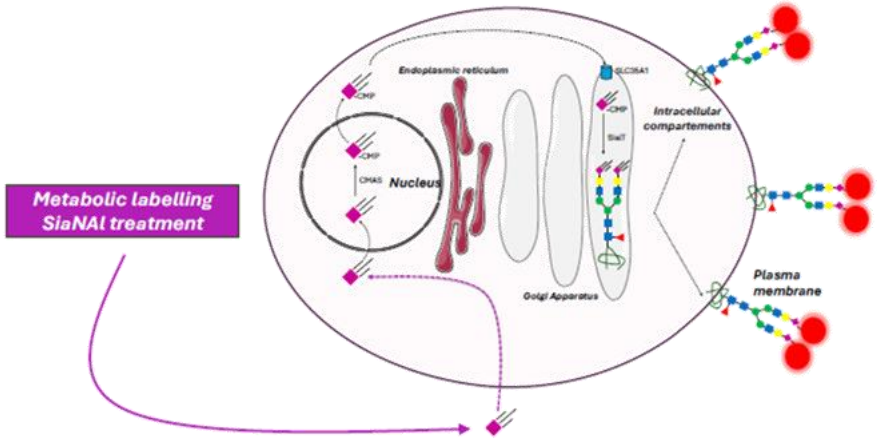
Click-chemistry

Click Reaction





Besoin de l'enzyme NANS pour faire de l'acide sialique et donc des glycanes sialylés



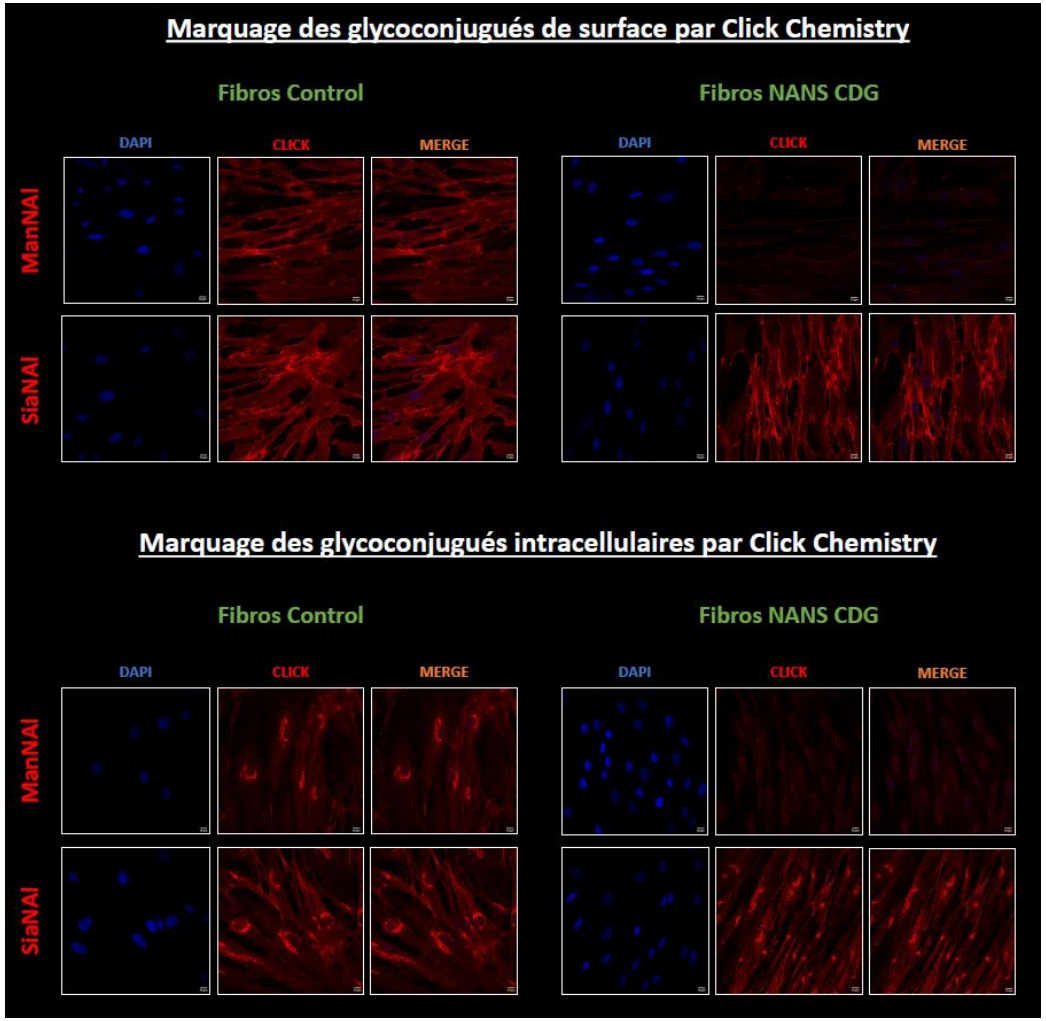
Plus besoin de l'enzyme NANS : L'acide sialique rentre par endocytose et peut-être activé en CMP-Sia pour être utilisé par les sialyltransférases

Oral sialic acid supplementation in NANS-CDG: Results of a single center, open-label, observational pilot study

Bibiche den Hollander [1 2 3], Marion M Brands [1 2 3], Lonneke de Boer [2 4], Charlotte A Haaxma [4 5], Anna Lengyel [6], Peter van Essen [4], Gera Peters [7], Hanneke J T Kwast [8], Willemijn M Klein [9], Karlien L M Coene [8 10], Dirk J Lefeber [2 5 8], Clara D M van Karnebeek [1 2 3 11]

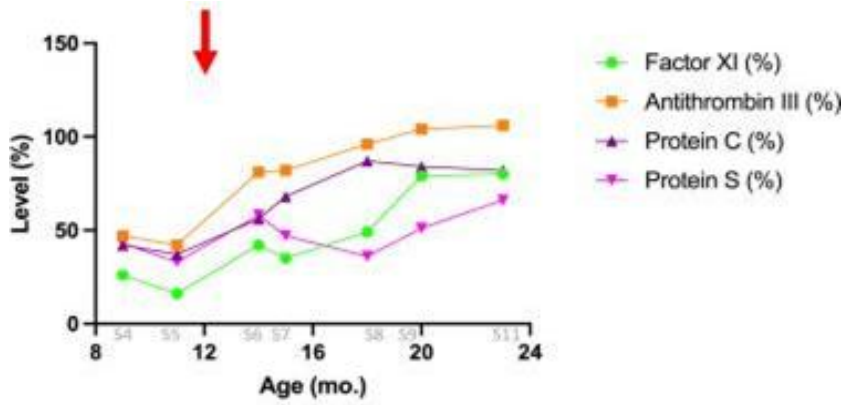
Affiliations + expand

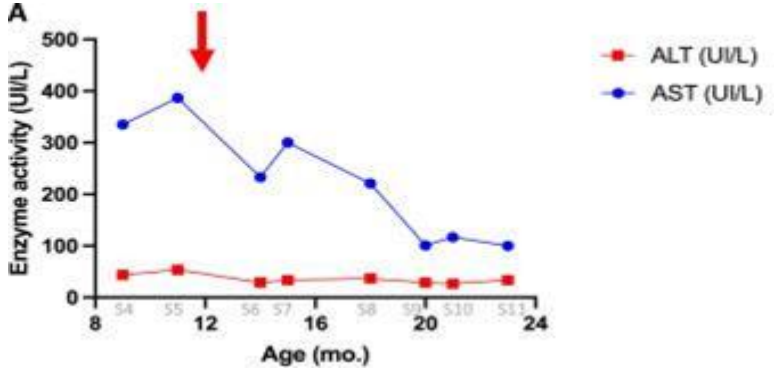
PMID: 37340906 DOI: 10.1002/jimd.12643



Other non-specific laboratory tests

- In routine clinical laboratory analyses: decrease in the concentrations of many plasma/serum glycoproteins are typically observed in CDG
- e.g., decreased pro/anti-coagulant factor XI, antithrombin III, protein C, protein S, factor IX…





- Not specific of CDG, but should make clinicians/laboratorians suspicious of CDG!
- Serum O-glycome: yet to be developed!

Elements on genetic diagnosis

- Historically, genetic testing mostly followed the observation of abnormal glycosylation
- Sequencing of specific genes (e.g., *ATP6V0A2* if *cutis laxa* and type 2 transferrin profile) or of a CDG panel of genes
- Nowadays, with the increasing use of **whole exome** and **whole genome** sequencing, variants in genes involved in glycosylation can be detected before CDG is suspected
- Biochemical analyses provide phenotypic results to support genetic results, and are of particular interest to confirm the pathogenicity of variants of unknown significance
- Metabolomic ?

38èmes JNBC

16-18 Octobre 2025
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THANK YOU !

Elodie.lebredonchel@aphp.fr

LinkedIn : Elodie Lebredonchel

www.cdg-bichat.com

IFCC International Federation of Clinical Chemistry and Laboratory Medicine

SFBC Société Française de Biologie Clinique

Hôpital Bichat Claude-Bernard AP-HP