CDG Case & Updates

Peymaneh Sarkhail MD.
Pediatric Endocrinologist &
Meatbolic Consultant

The 20th congress of Iranian pediatric endocrinology and metabolism association
January 29-31 2025

Case 1

- Sedna is a 2 years old girl
- She was born from a second cousin parents by C/S without any problem during perinatal and neonatal period
- Her Birth Wt was 3.200 kg and birth Hc was 36 cm
- She suffers from NDD, Hypotonia, ataxia, and seizure
- Her Wt , Ht, and Hc were 11 kg (25%) 87 cm (75%) and 49 cm (75%) respectively.
- Physical examination was normal
- She had strabismus and mild dysmorphism
- Eye exam and retina was normal

Case 1

- In MRI abnormal white matter signal, cerebellar hypoplasia with diffused delayed myelination and atrophy especially in frontotemporal lobes were reported
- EEG showed mild abnormal waves
- VEP also was delayed. Auditory test was normal
- All routine and metabolic screen tests were normal
- She was on B6- Liskantin- Italept and DHA for seizure
- Due to undiagnosed case WES was requested for the patient

Case 1

Re-Analysis Report

CLINICAL INFORMATION

The proband has abnormal MRI imaging (mild bilateral frontotemporal lobes diffuse prolonged atrophy). She has normal EEG and normal MS/MS. The WES has been performed in another center and no relevant variant to the phenotype was identified. Heterozygous pathogenic and likely pathogenic variants in the CYP1B1 (ENST00000610745.5:c.1103G>A), EVC (NM_153717.3:c.2449+1G>A), RHO (NM_000539.3:c.659T>G) and CFTR (NM_000492.4:c.2657+5G>A) genes in the previous center. These variants were also identified in our analysis and they are reported as carrier findings except the one in the CYP1B1 because its ACMG verdict was changed to VUS.

Genes related to the following HPO terms were applied in the analysis:
Brain imaging abnormality, Brain atrophy, Strabismus, Seizures, Neurodevelopmental delay, Generalized hypotonia, Joint laxity, Decreased deep tendon reflexes
Consanguinity: Yes

UNCLEAR RESULT
Variant of uncertain significance (VUS) identified

INTERPRETATION

A homozygous variant of uncertain significance in the **SLC39A8** gene was identified. The genetic diagnosis of autosomal recessive **Congenital disorder of glycosylation, type IIn** is possible.

No copy number variants (CNVs) were detected in the analyzed genes.

***The whole exome sequencing genotyping has been performed in another lab and FASTQ/VCF file provided to us for re-analysis.**

Case 1

- Sanger sequencing to confirm the detected variant in the **SLC39A8** gene as the possible cause of the disease in the proband is recommended. Next, retrospective clinical and biochemical analysis to check the compatibility of the identified variant with the phenotype is suggested.
- Genetic counseling is also recommended.

RESULT SUMMAR

Gene	Variant Coordinates	Zygosity	In Silico Parameters	Allele Frequencies*	Type and Classification**
SLC39A8	Chr4(hg38):g.102344485 ENST00000356736.5: c.178C>T p.(Arg60Cys) Exon 2/9	Hom	MutationTaster: Polymorphism FATHMM_MKL: Neutral SIFT: Damaging	gnomAD: 0.000006576 ExAC: NA Iranome: NA	Missense VUS (Class 3)

*Genome Aggregation Database (gnomAD) Genome version:3.0, Exome Aggregation Consortium (ExAC) version:1.0 and Iranome **Variant classification is based on ACMG recommendations: Class 1: Pathogenic, Class 2: Likely pathogenic, Class 3: Variant of uncertain significance (VUS), Class 4: Likely benign, Class 5: Benign

VARIANT INTERPRETATION

SLC39A8, c.178C>T p.(Arg60Cys)

The SLC39A8 variant c.178C>T p.(Arg60Cys) causes an amino acid change from Arg to Cys at position 60. This is classified **variant of uncertain significance (VUS)** according to **ACMG**. Pathogenic variants in the SLC39A8 gene are associated with autosomal recessive **Congenital disorder of glycosylation, type IIn** (OMIM: 616721).

Autosomal recessive Congenital disorder of glycosylation type IIn (CDG2N) is a severe multisystem developmental disorder characterized by delayed psychomotor development apparent from infancy, hypotonia, and variable additional features, such as short stature, seizures, visual impairment, and cerebellar atrophy.

Boycott *et al.*, (2015) reported 10 patients with a profound multisystem developmental disorder ranging from 6 to 23 years of age. They all had **severe psychomotor retardation** with **delayed head control, severe hypotonia, inability**

Case 1

Molecular Genetic study

for the c.178C>T, c.2449+1G>A, c.659T>G, c.454G>A, c.2657+5G>A and c.595T>C variants in SLC39A8, EVC, RHO, GUSB, CFTR and EXT2 genes, respectively found through WES study

Family Segregation

	Affection Status	Zygosity	Genotype	Nucleic Acid Alteration	Gene	RefSeq
Sedna Ghamari (Proband)	Affected	Hom	T/T	c.178C>T p.(Arg60Cys)	SLC39A8	ENST00000156736.5
Saeed Ghamari (Father)	Healthy	Het	C/T			
Samaneh Miri Panah (Mother)	Healthy	Het	C/T			

Interpretation

Based on this molecular analysis, **Saeed Ghamari** and **Samaeh Miri Panah** are both **heterozygote** for the c.178C>T variant in *SLC39A8* gene, therefore this variant **can be** considered as the cause of the disease in **Sedna Ghamari**. Genetic counseling is highly recommended.

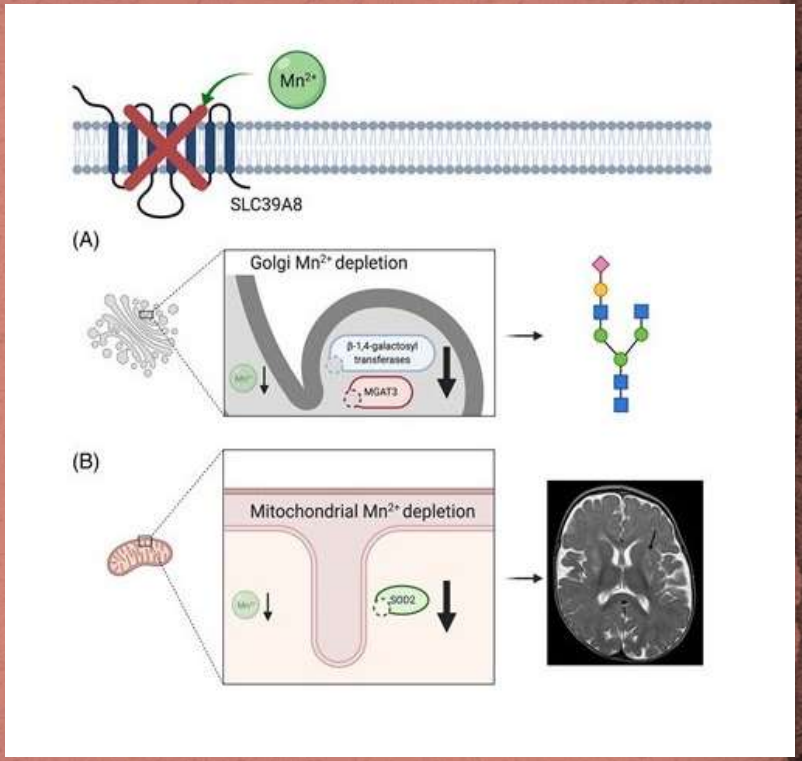
Carrier screening for the incidental finding variants

	Zygosity	Genotype	Nucleic Acid Alteration	Gene	RefSeq
Sedna Ghamari	Het	G/A	c.2449+1G>A	CFTR	NM_153717.3
Samaneh Miri Panah	Normal	G/G			

	Zygosity	Genotype	Nucleic Acid Alteration	Gene	RefSeq
Sedna Ghamari	Het	T/G	c.659T>G (p.Phe220Cys)	RHO	NM_000539.3
Samaneh Miri Panah	Het	T/G			

Case 1

- SLC39A8-CDG is caused by mutations in the gene encoding manganese uptake transporter
- Due to the reliance of several transferases on Mn2+ as a cofactor, mutations in SLC39A8 resulting in intracellular manganese depletion
- This cofactor impairs the function of galactosyltransferases (GalT). cause secondary glycosylation defects corresponding to a type II CDG pattern



ACS Chem Biol. 2022 November 18; 17(11): 2962–2971

Frontiers in Genetics September 2021 | Volume 12 | Article 735348

Case 1

- Clinically, it is characterized by NDD, short stature, severe seizures, cerebellar atrophy, cranial synostoses, as well as visual and auditory impairment
- In addition, Leigh like mitochondrial disease, possibly due to impaired function of manganese-dependent SOD2 (super oxide dismutase 2)
- A dose of 15-20 mg of $MnSO_4$/kg/day was given to two SLC39A8-CDG individuals and showed some improvement in glycosylation and had a considerable impact on clinical parameters by near normalization of EEG patterns and cessation of seizures
- Galactose with dose of up to 3.75 g/kg/day (five equal doses) led to near complete normalization transferrin glycosylation in 2 weeks

ACS Chem Biol. 2022 November 18; 17(11): 2962–2971

Frontiers in Genetics September 2021 | Volume 12 | Article 735348

Case 2

- Narmila was a 1.5 years old girl who was referred to clinic because of NDD , hypotonia and abnormal LFT
- She was born from first cousin parents by C/S
- She was a preterm (29 w) and VLBW (900 g) neonate who admitted for 2 months in NICU
- In her FH her grandfather passed away due to sever liver involvement without any defined Dx
- Her parents also complained she has no tear
- PE:
- Wt= 8.9 kg (5%) Ht= 72 (10%) Hc=45 (25%)
- The liver was in 2 cm below costal margin with firm texture

Case 2

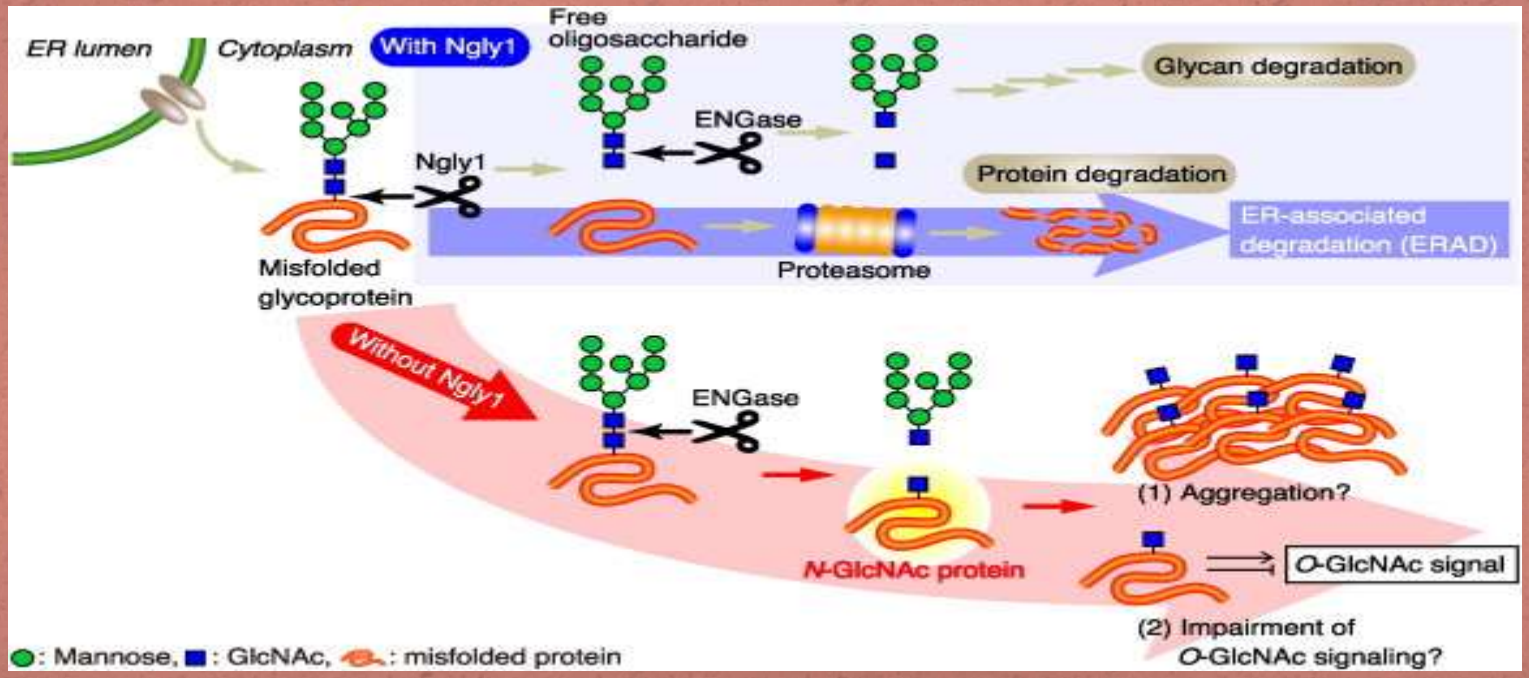
- Lab tests ;
- AST=227 ALT=450 ALP= 723 AFP=2655 GGT=40
- Lactate= 59 NH3=167 Vit D=110
- Other routine lab tests and PT, PTT were in normal range
- All metabolic screen tests were normal
- In sonography the size of liver and spleen reported normal with high echogenicity
- Liver biopsy showed severe steatosis and fibrosis
- She was on levothyroxine therapy since neonatal period and Orso , livergol , vitamin E , DHA , Mitochondrial cocktail started
- WES was requested

Case 2

- In the next examination at 2.5-year-old of age the LFT decreased significantly ; AST=90 ALT=71 ALP=441 AFP=12 PT=14.8 PTT=38
- Ammonia and lactate became normal
- In WES two homozygote mutations were found ;
- A VUS mutation for MTRR gene (Methionine synthase reductase) and a pathogenic mutation for NLGY1 gene (CDDG)
- NLGY1 mutation corresponded with her symptoms; liver involvement, NDD, hypotonia, and alacrimia

Case 2

- In NGLY1 deficiency, the mutations in the NGLY1 gene result in reduced or absent N-glycanase 1 activity.
- This leads to the accumulation of abnormal glycoproteins within the ER, disrupting the normal protein quality control mechanisms.
- As a result, the misfolded proteins can not be properly degraded and cleared from the cells.



Journal of Rare Diseases (2023) 2:20 doi.org/10.1007/s44162-023-00024-9

Case 2

- This ultra-rare AR disorder with approximately 100 patients identified worldwide to date.
- The disorder is characterized by five core features:
- (1) global developmental delay and/or intellectual disability
- (2) a primarily hyperkinetic movement disorder
- (3) transient elevation of liver transaminases
- (4) hypo- or alacrima
- (5) peripheral neuropathy
- Additionally, acquired microcephaly, hypotonia, EEG abnormalities with or without overt seizures, brain imaging abnormalities, GI disturbances, and a history of IUGR

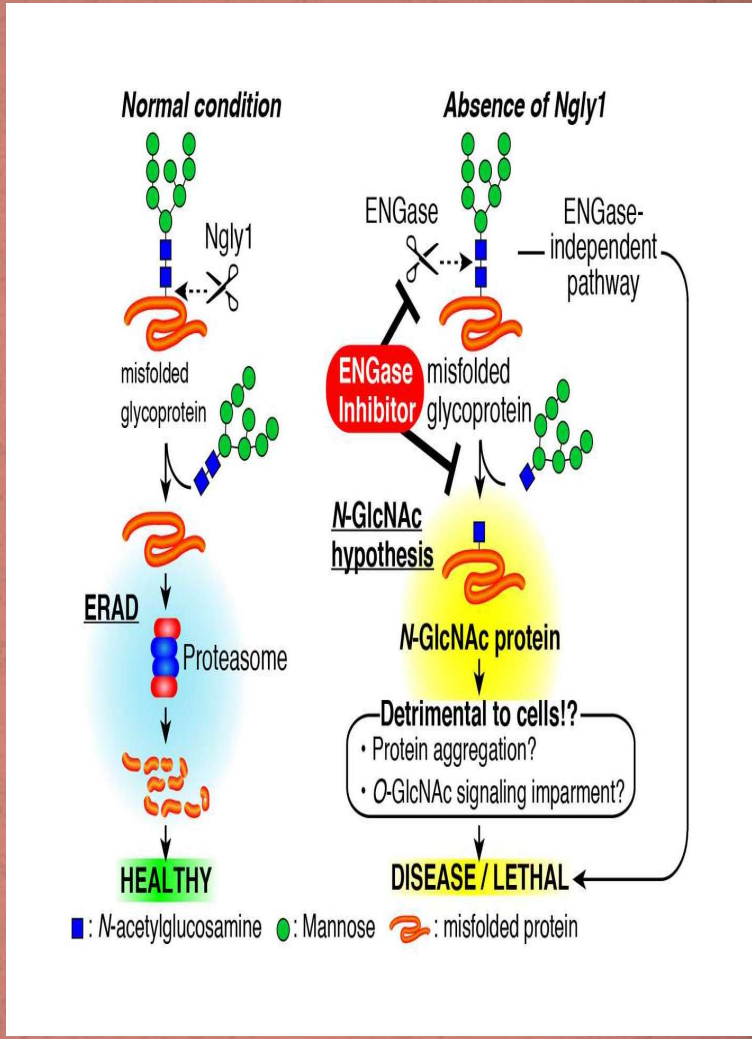
Case 2

- At present, the condition is diagnosed via genetic testing.
- However, screening for the recently described NGLY1 Deficiency biomarker, GlcNAc-Asn, may help identify patients and increase the rate of diagnosis
- Treatment options are presently limited to supportive care.
- However, a gene therapy is currently under development.
- Thus, it is increasingly important to identify affected families to participate in patient registries

Journal of Rare Diseases (2023) 2:20 doi.org/10.1007/s44162-023-00024-9

Case 2

- ***1) Enzyme replacement therapy***: A potential treatment option that is currently in the pre-clinical stages
- ***2)ENGase inhibitors:*** A potential treatment option in the pre-clinical stages
- Endo-β-*N*-acetylglucosaminidase (ENGase) is a key enzyme involved in the processing of free oligosaccharides in the cytosol.
- ENGase inhibitors are molecules that stop the activity ENGASE enzyme.
- ***3)Gene therapy:*** A promising treatment option that involves replacing the NGLY1 gene (GS-100 gene therapy)



JCI Insight. 2024;9(19):e183189.

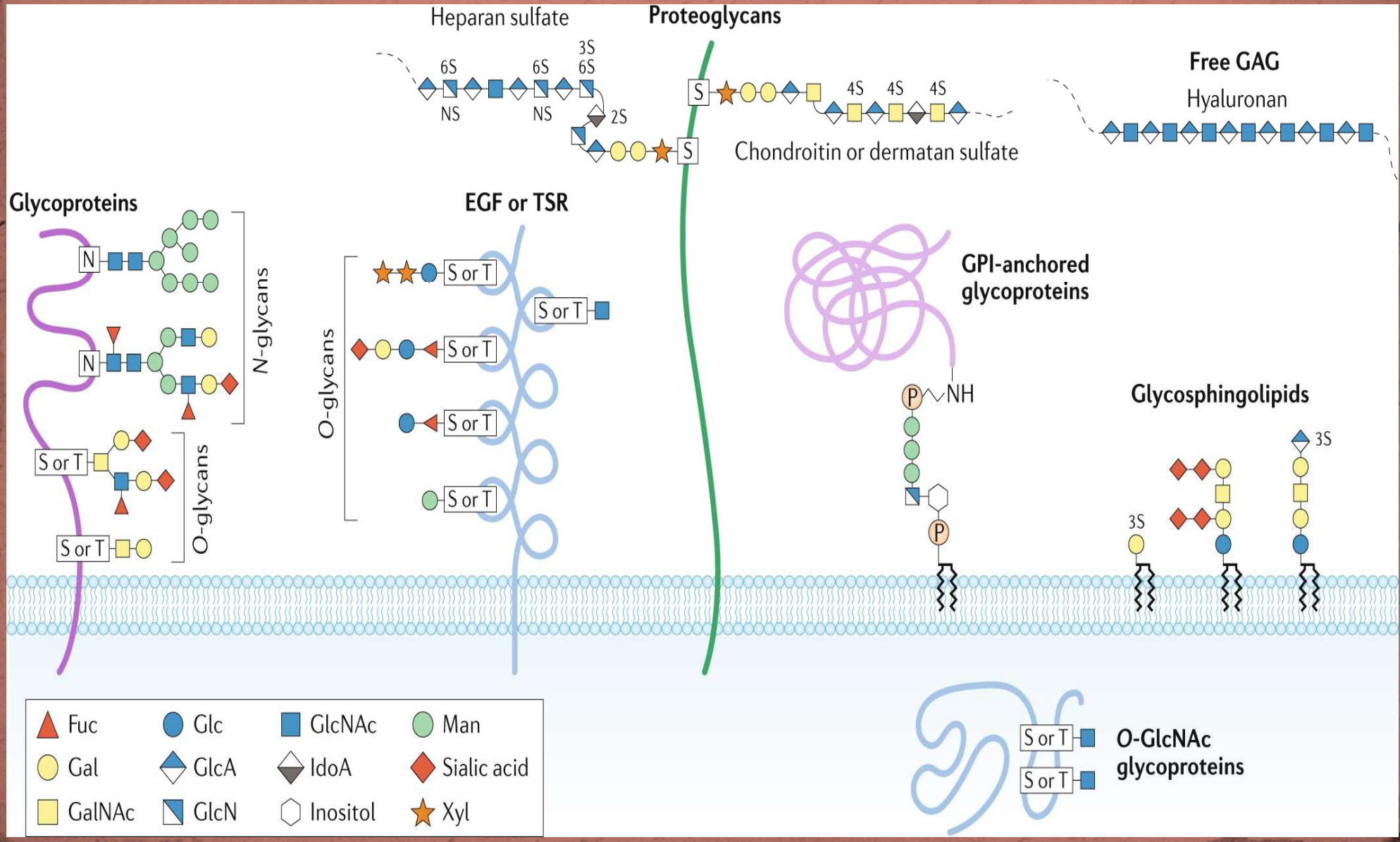
Introduction

- Congenital disorders of glycosylation (CDG) are a varied group of rare genetic diseases characterized by protein and lipid hypoglycosylation
- CDGs are categorized into groups based on their defects
- 1) N-glycosylation defects
- 2) O-glycosylation defects
- 3) lipid and GPI (glycosylphosphatidylinositol) glycosylation defects
- 4) multiple glycosylation pathways
- 5)Additionally, deglycosylation defects (CDDG) like NGLY1-CDG have also been identified

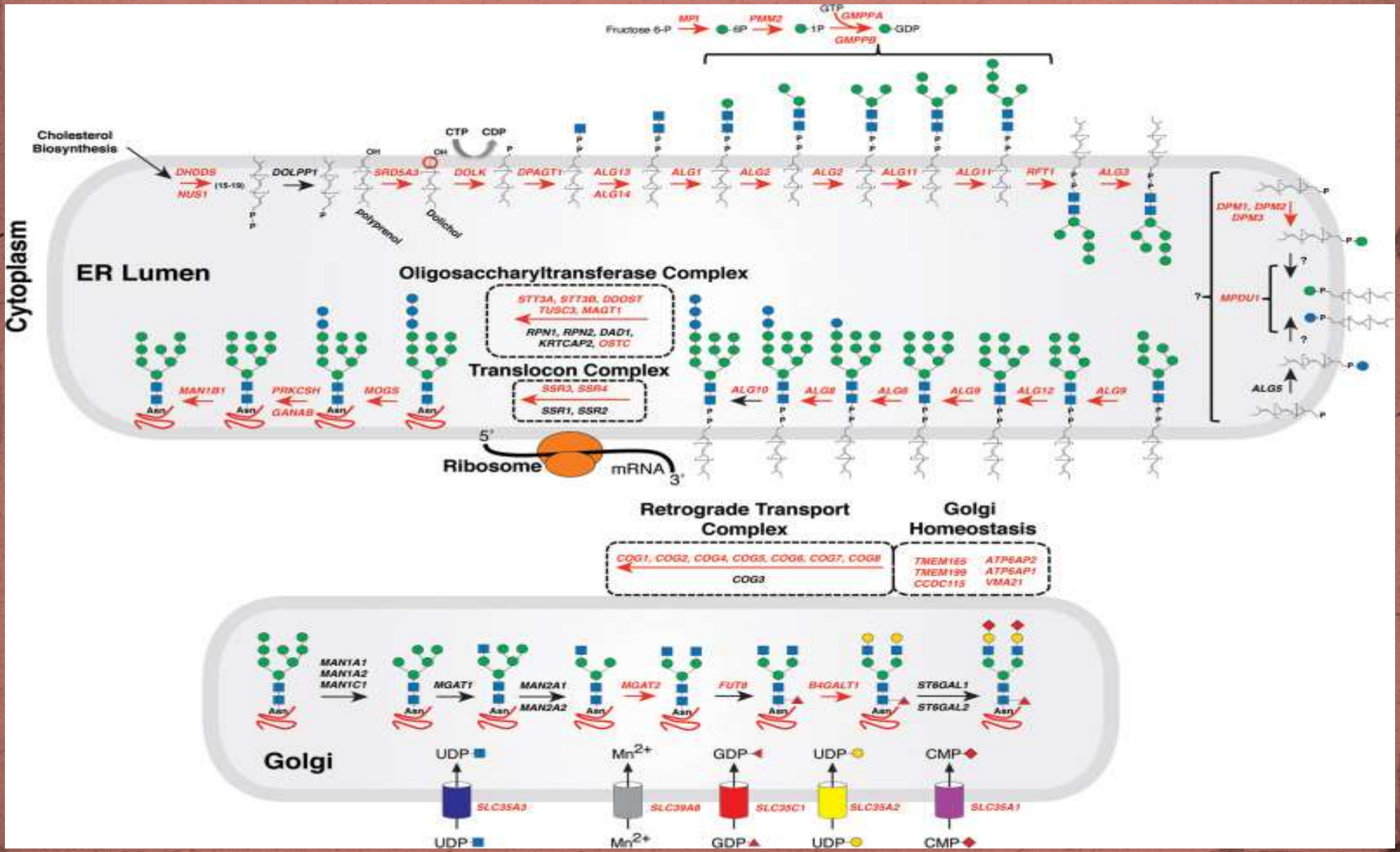
Orphanet Journal of Rare Diseases (2023) 18:329

Journal of Child 2023;23(3):31-40

Introduction



Pathway


Fructose-6-P
MPI
6P
PMM2
1P
GTP
GMPPA
GMPPB
GDP
Cytoplasm
Cholesterol Biosynthesis
DHDDS
NUS1
(15-19)
DOLPP1
CTP
CDP
SRD5A3
DOLK
DPAGT1
ALG13
ALG14
ALG1
ALG2
ALG2
ALG11
ALG11
RFT1
ALG3
polyprenol
Dolichol
DPM1, DPM2
DPM3
MPDU1
ALGS
ER Lumen
Oligosaccharyltransferase Complex
STT3A, STT3B, DDOST
TUSC3, MAGT1
RPN1, RPN2, DAD1,
KRTCAP2, OSTC
Translocon Complex
SSR3, SSR4
SSR1, SSR2
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
TMEM165
TMEM199
CCDC115
ATP6AP2
ATP6AP1
VMA21
MAN1A1
MAN1A2
MAN1C1
MGAT1
MAN2A1
MAN2A2
MGAT2
FUT8
B4GALT1
ST6GAL1
ST6GAL2
Golgi
UDP
Mn2+
GDP
UDP
CMP
SLC35A3
SLC39A6
SLC35C1
SLC35A2
SLC35A1

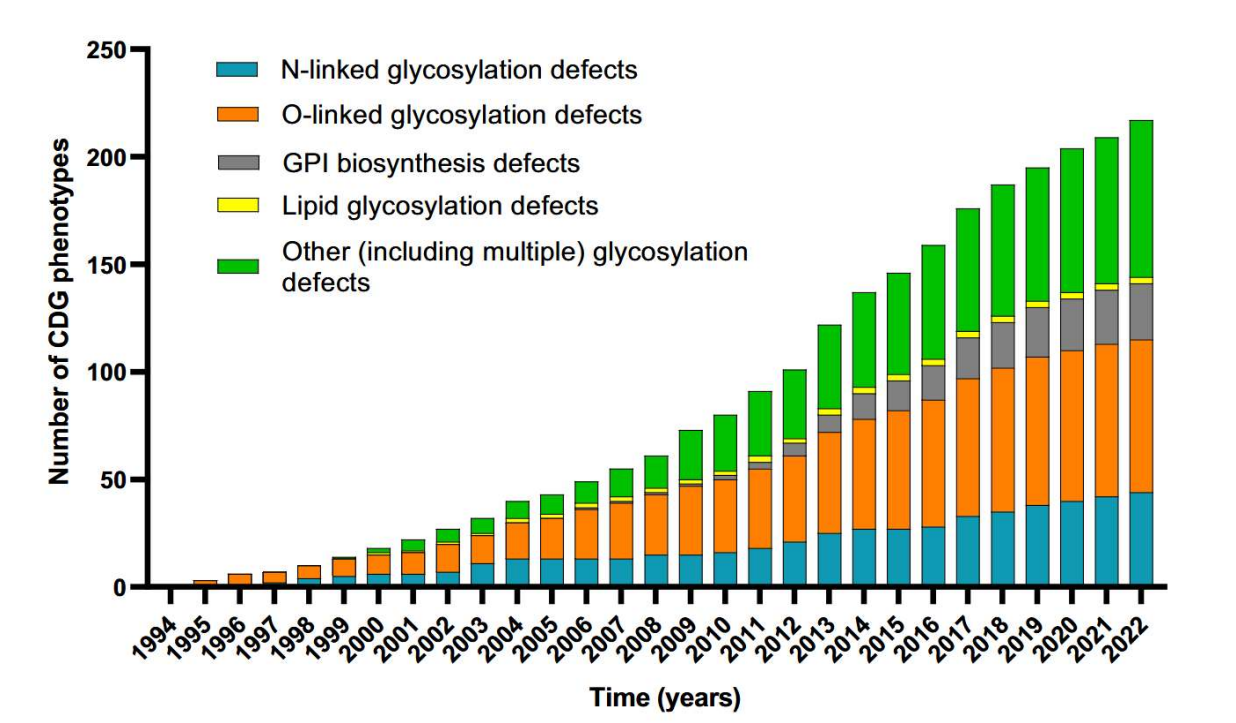
Epidemiology

- The total incidence and prevalence for all CDG types is not known;
- however, cases have been documented worldwide, encompassing many ethnic backgrounds with both sexes affected.
- The prevalence of CDG in European populations has been estimated to range from 1/10,000 (for PMM2-CDG; the most common type) to 0.1–0.5/100,000
- With the current estimated prevalence ranging from 1/20,000 in Dutch populations to 1/77,000 in Estonia (as per isolated reports) to 1/286,726 in Turkey

J Genet Couns. 2024;00:1–7.
Frontiers in Pediatrics · September 2021 Volume 9 | Article 715151

Epidemiology

- Over 190 known CDG encompass 220 different phenotypes or diseases
- Most of these disorders have been discovered in the past two decades ago especially after in recent years by WES

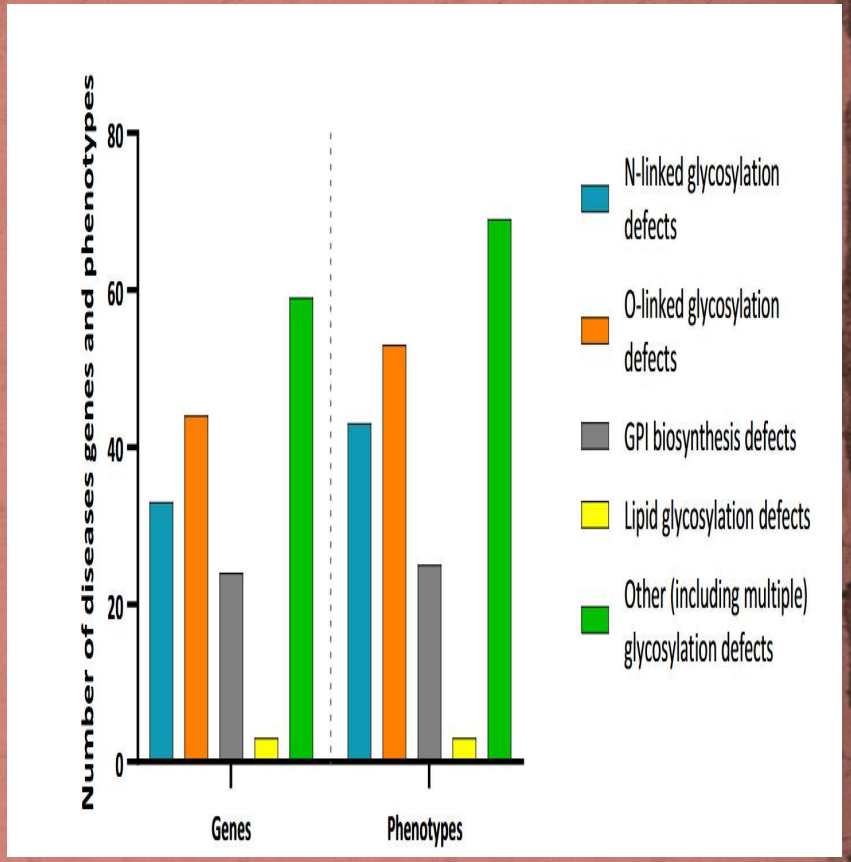


Orphanet Journal of Rare Diseases (2023) 18:329

Frontiers in Pediatrics · September 2021 Volume 9 | Article 715151

Epidemiology

- N-linked glycosylation (n = 43) are caused by variants in 33 genes;
- the 53 O-linked glycosylation are caused by defects in 44 genes;
- GPI biosynthesis defects (n = 25) are due to variants affecting 24 genes,
- while variants in 3 genes cause the 3 lipid glycosylation defects.
- The 69 disorders affecting other (including multiple) glycosylation pathways described are caused by defects in 59 genes



Orphanet Journal of Rare Diseases (2023) 18:329

Frontiers in Pediatrics · September 2021 Volume 9 | Article 715151

Epidemiology

- The majority (80%) of CDG have an AR inheritance pattern (n = 161)
- AD patterns have been described in N-linked (n = 6), O-linked (n = 5), and other (including multiple) glycosylation pathway defects (n = 4). (n=15)
- X-linked defects (n = 12), both of dominant (XLD) and recessive (XLR) inheritance, have been described in all glycosylation pathways except for lipid glycosylation defects.
- 7% of the clinical presentations have an autosomal dominant (AD) transmission, and 6% are X-linked (XL).

Glycosylation defects	Inheritance patterns					
	AR	AD	XL	XLR	XLD	NA
N-linked	30	6	1	3		3
O-linked	45	5	0	2		1
GPI	24	0	0	1		0
Lipid	2	0	0	0		1
Other (including multiple)	60	4		4	1	

Orphanet Journal of Rare Diseases (2023) 18:329

Clinical manifestations

- Overall, CDGs are usually multi-systematic and often present with NDD, FTT, hypotonia, neurologic abnormalities, as well as varying abnormalities of the endocrinologic, hepatic, ophthalmologic, dermatologic, gastrologic, immunologic, skeletal, and coagulation systems.
- The severity of clinical expression extends from perinatal death (and probably even miscarriage) to mild adult involvement
- The heterogeneity can result in multiple clinical presentations depending on the involved variant.
- For this reason, CDGs should routinely be on the differential, especially for individuals with a multi-systemic disease of an unknown etiology with neurologic abnormalities or developmental delay

Orphanet Journal of Rare Diseases (2023) 18:329

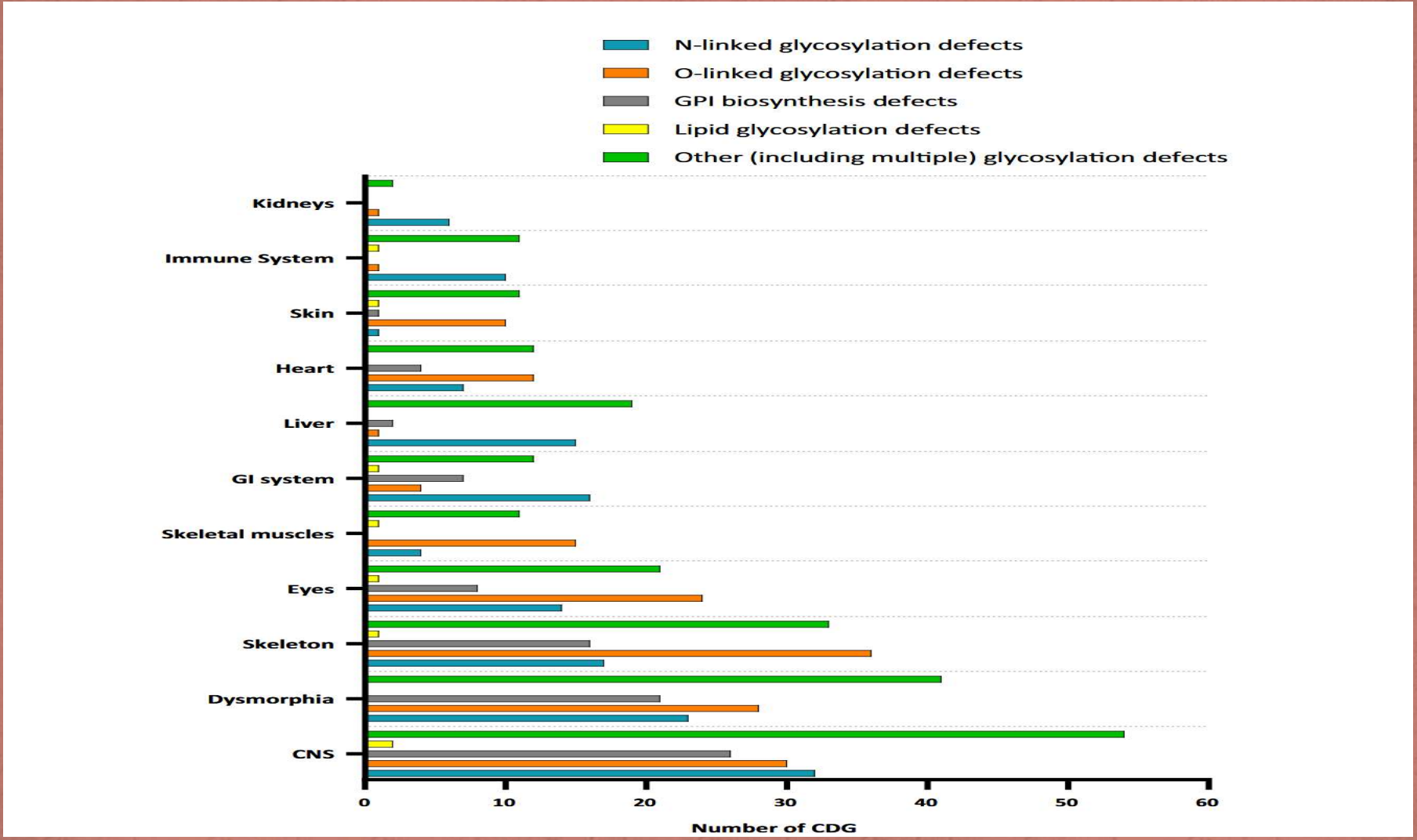
J Genet Couns. 2024;00:1–7.

Clinical manifestations

- The most affected system across the majority of CDG is the central nervous system
- After the CNS, the skeleton is the most commonly affected organ in all CDG groups, except for lipid glycosylation defects.
- Among the other glycosylation pathway defects, the eyes, GI system, and the liver are the most affected systems.
- The skeletal muscle and the eyes are commonly affected organs among O-linked glycosylation defects.
- A few mono-organ or pauci-organ CDG have been reported, such as ;
- DHDDS-CDG (MIM: 613861), with one phenotype only associated with a form of familial retinitis pigmentosa
- GNE-CDG (MIM: 605820) that manifests as a progressive myopathy
- GANAB-CDG presenting as a polycystic kidney or liver diseases (MIM: 600666).

Orphanet Journal of Rare Diseases (2023) 18:329

Clinical manifestations



Orphanet Journal of Rare Diseases (2023) 18:329

Diagnostic methods

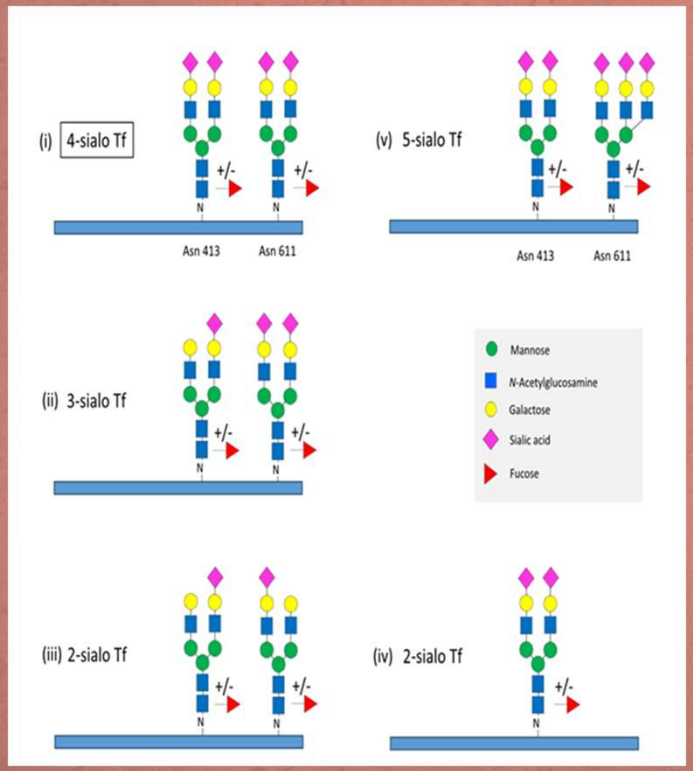
- Contrary to other IMD, CDGs are due to defects occurring in several cell organelles, mainly the cytosol, the endoplasmic reticulum (ER), the Golgi, and the sarcolemmal membrane which causes difficult diagnosis
- Glycosylation of serum glycoproteins, such as transferrin (TF) or apolipoprotein C-III, can be used as a diagnostic biomarkers for N and O glycosylation defects respectively.
- Several other laboratory techniques have been used for the separation and quantification of serum Tf isoforms, including high-performance liquid chromatography (HPLC), capillary zone electrophoresis (CZE), and mass spectrometry (MS)
- Every diagnostic method has its own limitations.

Orphanet Journal of Rare Diseases (2023) 18:329

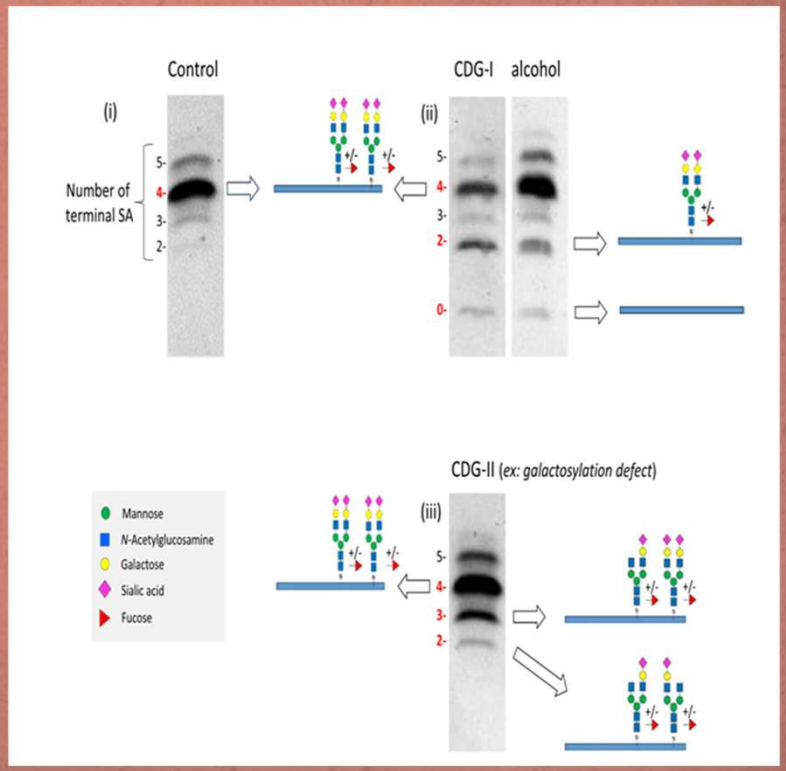
ACS Chem Biol. 2022 November 18; 17(11): 2962–2971

Diagnostic methods

- ***1-Tf-IEF:***
- Normal human serum contains tetra-sialo-TRF, (usually 75–80% of total TRF) with two bisialylated biantennary glycans.
- The penta-sialo, hexasialo and tri-sialo glycoforms can also be found in healthy subjects



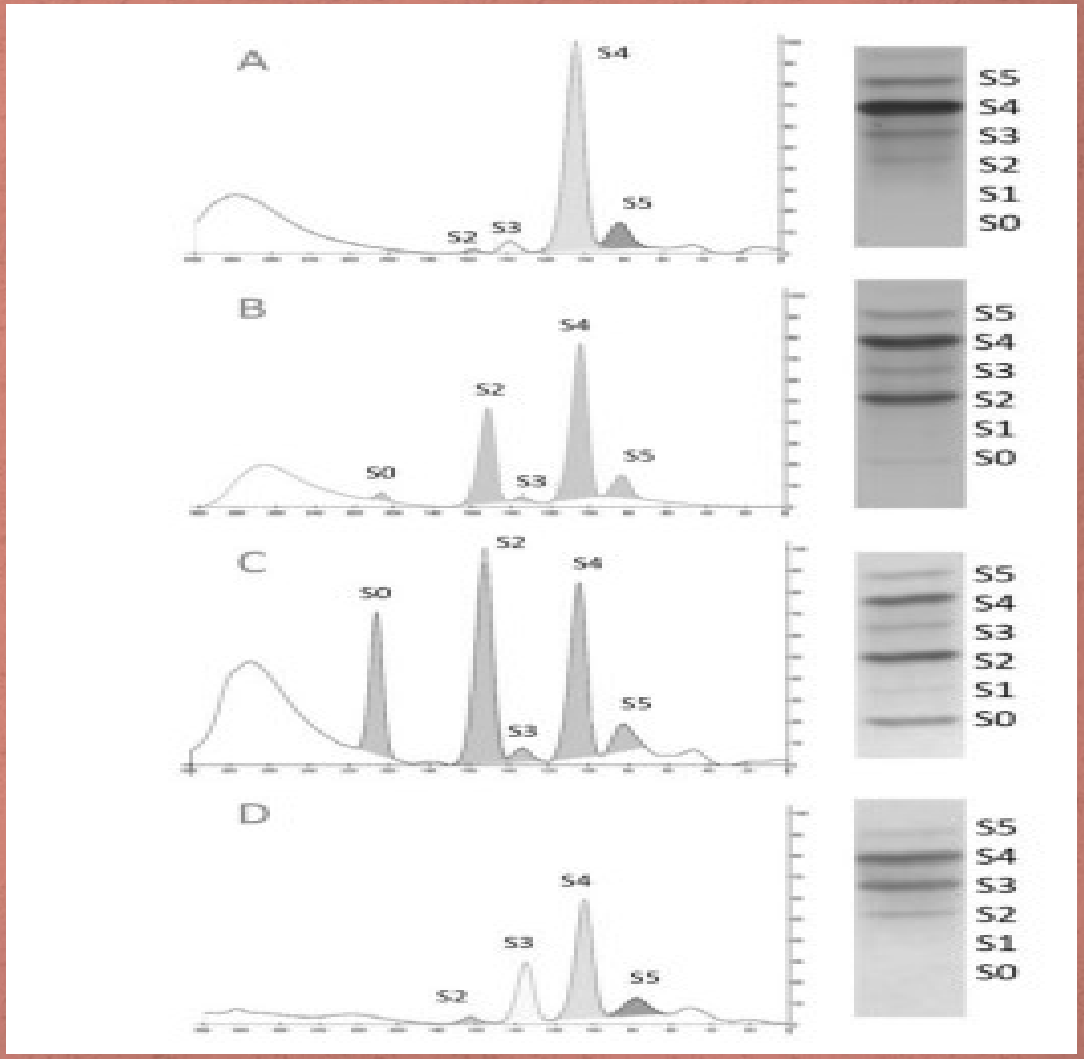
Advances in Clinical Chemistry
https://doi.org/10.1016/bs.acc.2024.03.001



Frontiers in Genetics September 2021 | Volume 12 | Article 735348

Diagnostic methods

- *2-CZE*
- The normal pattern of IEF and CZE (A)
- CDG type 1 pattern (B , C)
- CDG type II pattern (D)



Frontiers in Genetics September 2021 | Volume 12 | Article 735348

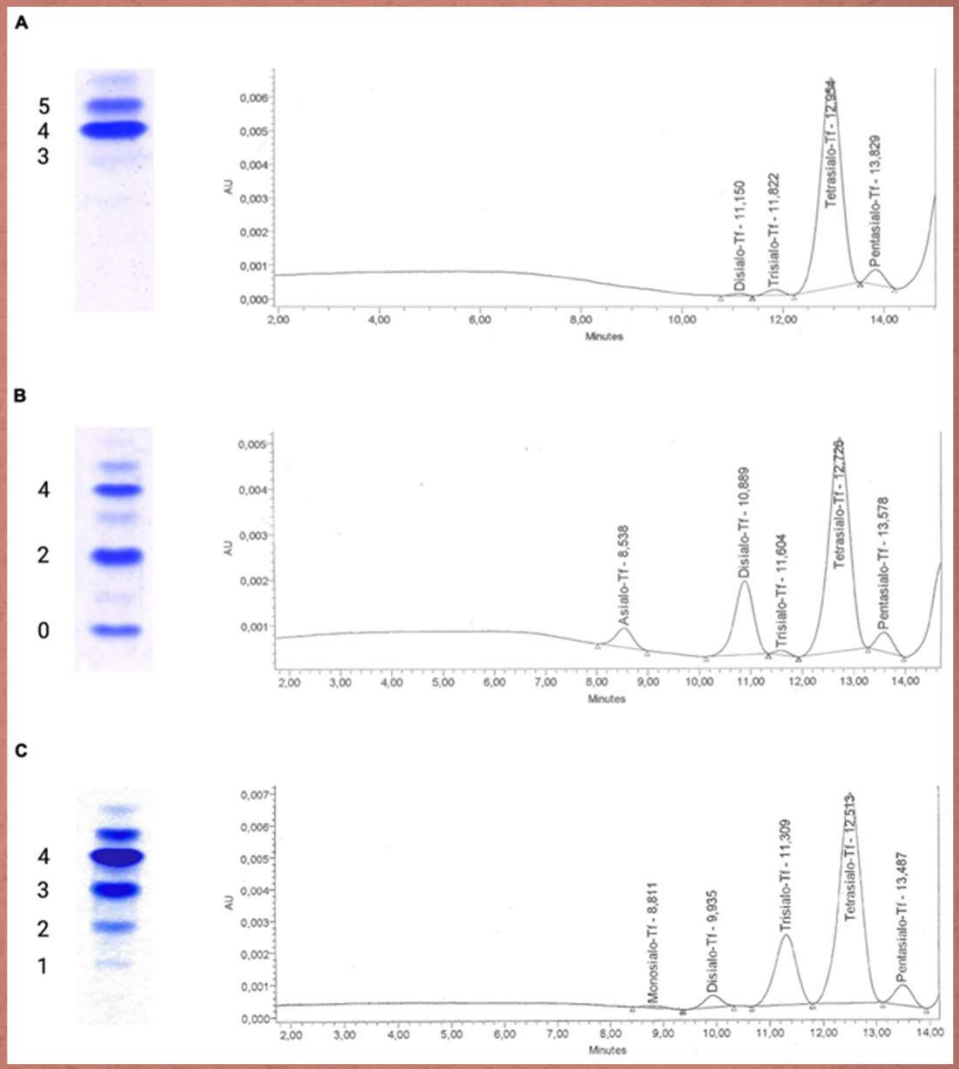
Diagnostic methods

3-HPLC

(A) normal glycosylation profile (pentasialo-transferrin 4.97%, tetrasialo-transferrin 92.44%, trisialo-transferrin 1.94%, and disialo-transferrin 0.65%).

B) Impaired transferrin glycosylation in a PMM2-CDG patient, type I CDG pattern.

(C) In a COG6-CDG patient, type II pattern.



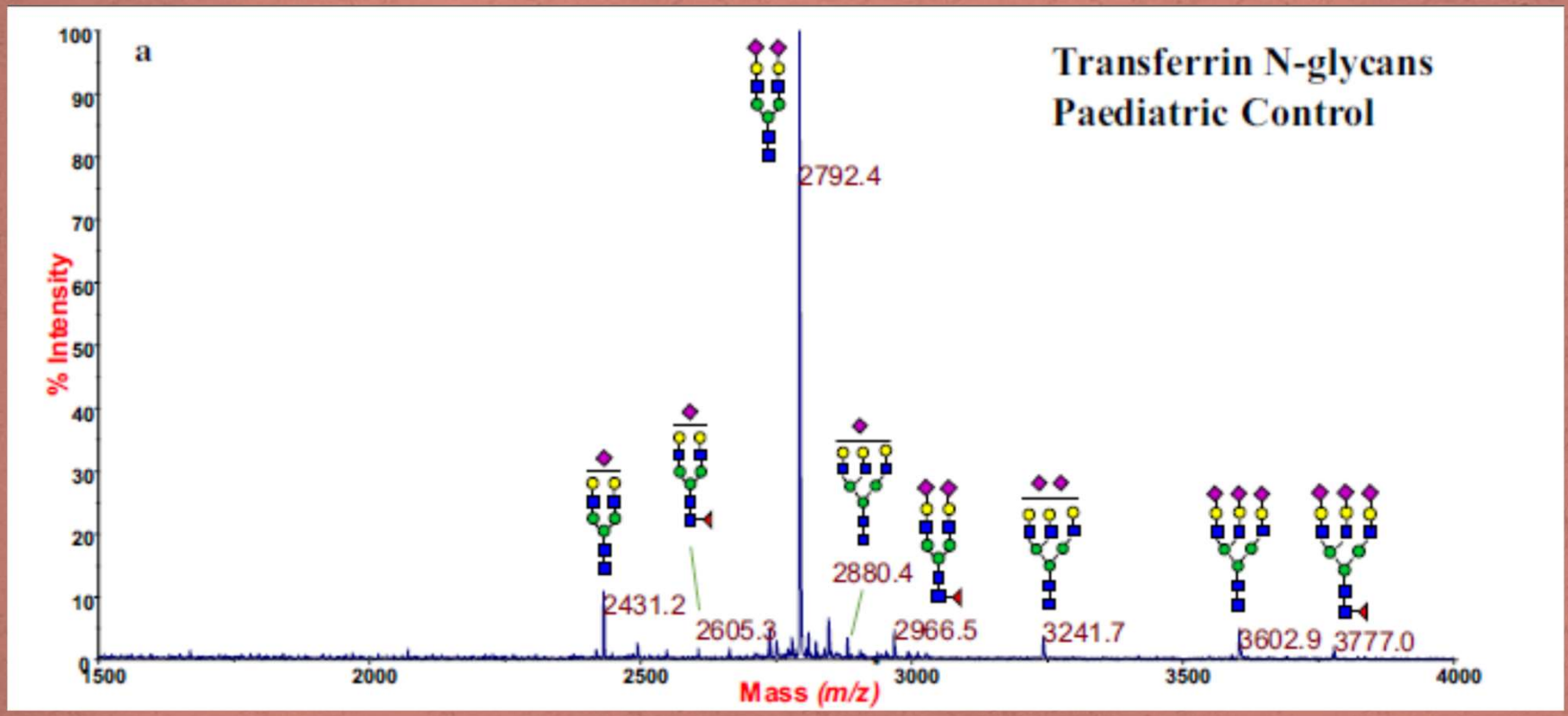
Diagnostic approaches

- In addition, a more sensitive and specific method was developed using flow injection-electrospray ionization-quadrupole time-of-flight mass spectrometry (ESI-QTOF-MS/MS) for serum N-glycan profiling allowing the identification of novel characteristics of polymannose changes in CDG
- Whole Exome Sequencing and Whole Genome Sequencing techniques are the only and powerful tools to diagnose CDG without known biomarkers

Orphanet Journal of Rare Diseases (2023) 18:329

Diagnostic methods

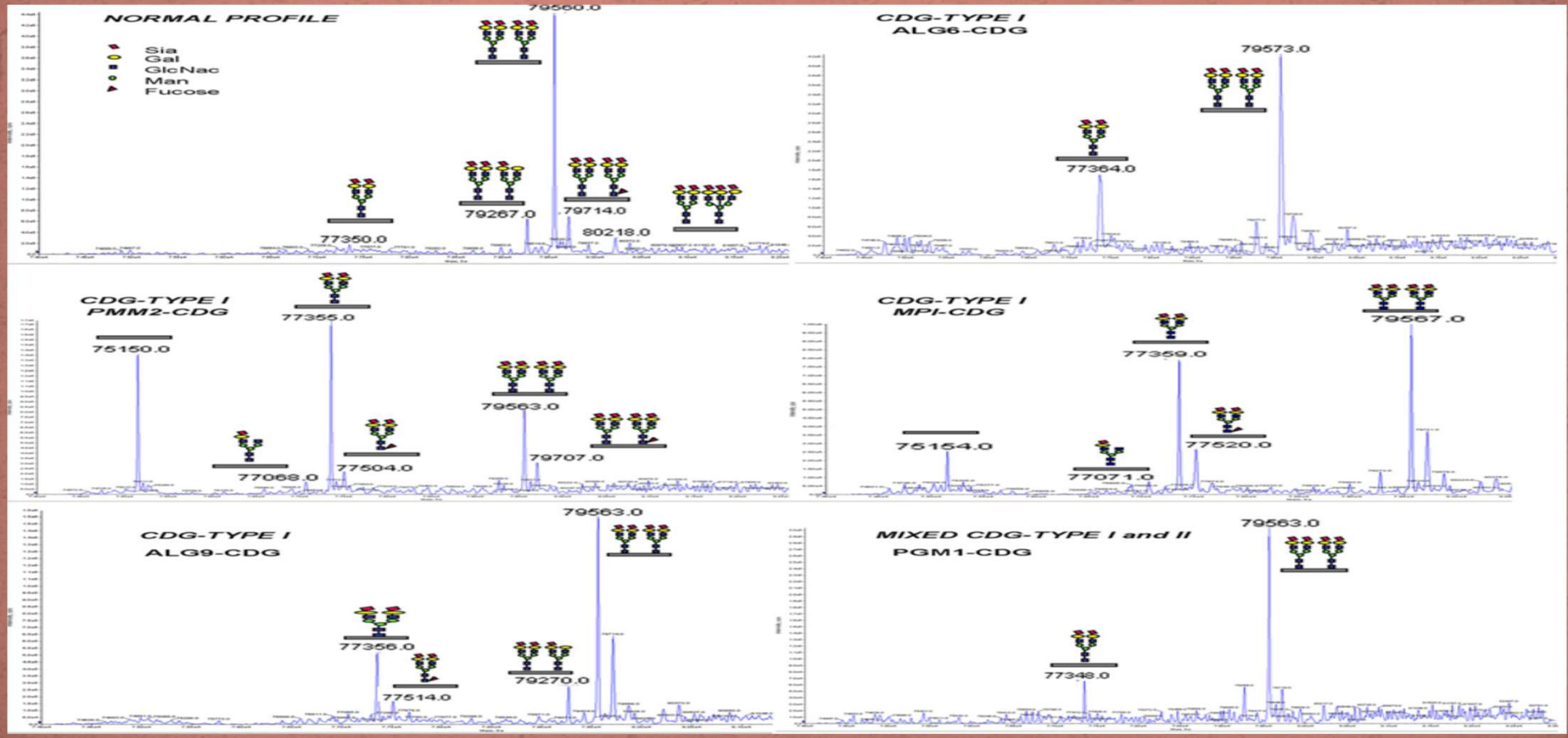
Normal TRF-isoform profile



Orphanet J Rare Dis (2021) 16:307

Diagnostic methods

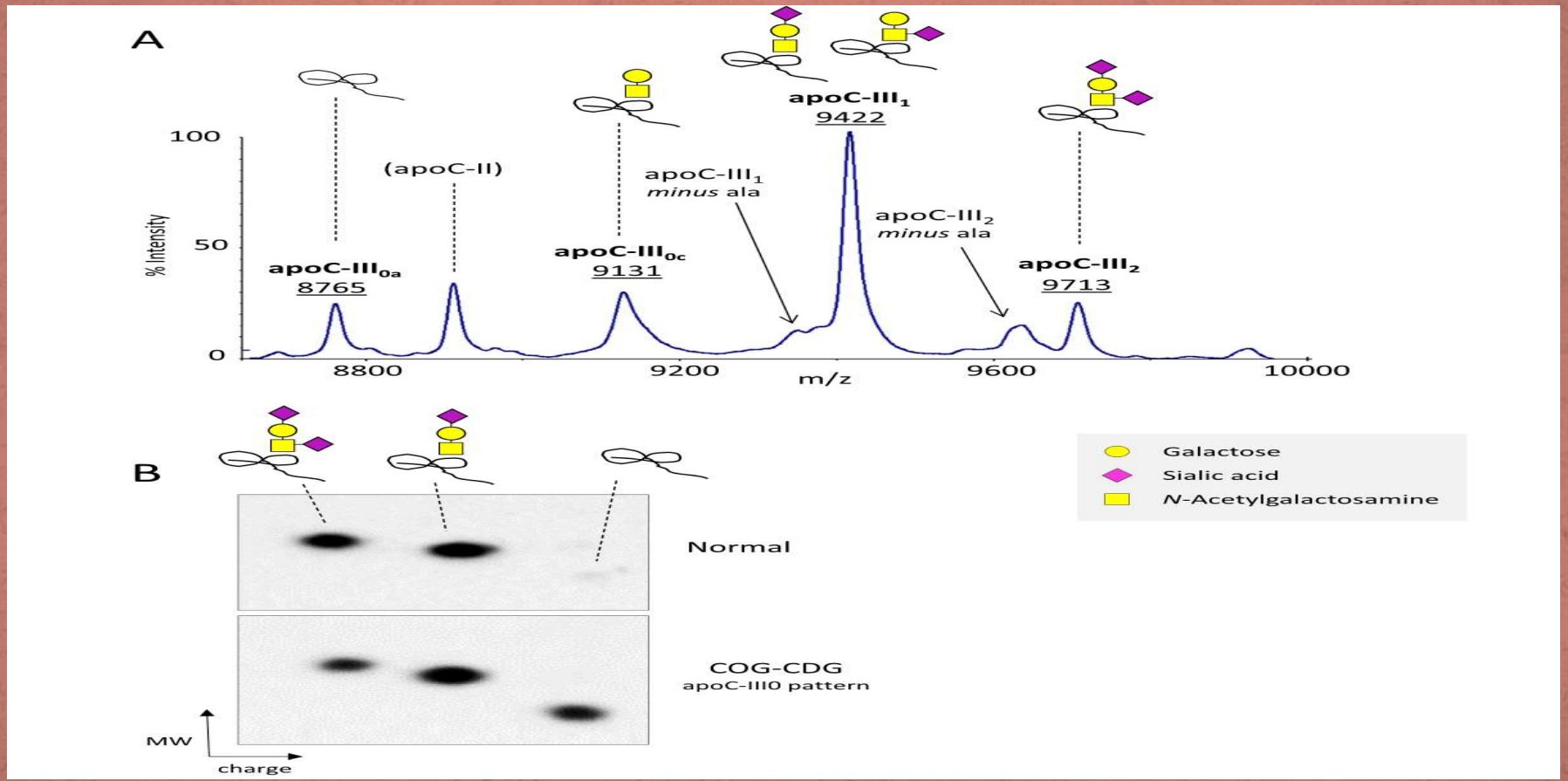
TRF-isoform profiles for different patients



Clin Chem Lab Med 2021; 59(1): 165–171

Diagnostic methods

- MALDI-TOF and two-dimensional electrophoresis of apolipoprotein C-III glycoforms



Advances in Clinical Chemistry
https://doi.org/10.1016/bs.acc.2024.03.001

Diagnostic methods

- ***New Biomarkers***
- 1-Apolipoprotein C-III (ApoC-III); used as a marker for mucin type 1 O-glycan biosynthesis defects, for Golgi-apparatus impairment (e.g.,COG-CDG, ATP6VOA2-CDG, CCDC115-CDG and GALNT2-CDG)
- it was analyzed in a group of glycogen storage diseases (GSD; types 0, Ia, non-Ia, III and IX), Significantly reduced was found in GSD types III and IX
- 2-Haptoglobulin ;Two-dimensional electrophoresis of haptoglobin β glycoforms was found to be a good additional biomarker for combined CDG (CDG-I and CDG-II)
- 3-Bikunin; is a plasma proteinase inhibitor primarily known for its role as a key inhibitor of the trypsin family of serine proteases.
- Bikunin analysis by western blot enabled distinction of some linkeropathies (O-xylose glycosaminoglycan defects in bikunin for GAGs biosynthesis defects, namely the mutations in B4GALT7, B3GALT6 and B3GAT3)

BBA - General Subjects 1865 (2021) 129751

Orphanet Journal of Rare Diseases (2024) 19:407

The therapeutic approaches

- Although the number of known CDG is rapidly growing, an effective treatment is known only for a small part of them.
- Effective therapy for 12 out of the 190 CDG (7 by monosaccharide substitution and 5 by other treatment modalities)
- Organ transplantation and other treatment modalities are known to ameliorate certain clinical aspects of 13 other CDG.
- In vitro experiments are testing other options.

BBA - General Subjects 1865 (2021) 129751

The therapeutic approaches

- ***I-Substrate (Precursor) Supplementation***
- Supplement substrates of the affected enzymes with the aim to shift the reaction equilibrium toward the favoured product.
- In cases where the direct substrate is either not available, the enzymatic defect was bypassed, utilizing alternative pathways
- ***II-Cofactor Supplementation***
- In several CDG subtypes, supplementation of the affected enzyme with essential cofactors has been employed as a means to improve glycosylation
- ***III-Pharmacological Chaperones***
- While frameshift and non-sense mutations frequently lead to a total loss of protein function, missense mutations can result in impaired protein folding.
- Pharmacological chaperones are small molecules bind to the altered structure of mutated proteins and facilitating correct folding

The therapeutic approaches

Effective therapies in CDG.

CDG	Treatment	Recommended dose	Number of treated/reported patients	Clinical effects	Side effects
MPI-CDG	Mannose	600–1200 mg/kg BW/ day in 4–5 oral doses	20/35	Improvement of digestive symptoms (13/14), coagulopathy (12/12) and hypoglycaemia (9/9), no long-term effect on liver symptoms	Abdominal pain and diarrhoea (5/10; responding to dose adjustment)
PGM1-CDG	Galactose	500–2500 mg/kg BW/ day in 3–6 oral doses	23/57	Improvement of hepatopathy (15/18), muscle symptoms (14/14), coagulopathy (10/12), hypoglycaemia (10/13) and pubertal delay (2/2), minimal effect on growth (1/8), ID (0/2) and cardiologic symptoms (0/2)	Not reported
SLC35A2-CDG	Galactose	500–1500 mg/kg BW/ day in 5 oral doses	11/62	Improvement of psychomotor development (5/10), growth (n.s.), gastrointestinal symptoms (n.s.) and seizures (3/5)	Not reported
SLC35C1-CDG	Fucose	500–1000 mg/kg BW/day in 4–5 oral doses	5/13	Normalization of neutrophil count (5/5), decreased incidence of severe infections (3/3), improvement of psychomotor development (1/5), no effect on adult form of the disease (ID, ataxia, epilepsy, autism)	Autoimmune neutropenia and hemolysis (responding to dose adjustment)
CAD-CDG	Uridine	100 mg/kg/day in 4 oral doses	2/6	Cessation of pharmacoresistant seizures (3/3), significant developmental progress (3/3), resolution of anaemia (2/2) and anisopoikilocytosis (3/3)	Not reported
SLC39A8-CDG	Manganese	15–20 mg $MnSO4*H_2O$ /kg BW/day in 5 oral doses	3/12	Improvement of glycosylation (2/2), motor abilities (2/2), epilepsy (1/1), ataxia (1/1) and hearing (1/1)	Not reported, risk of manganism
	Galactose	500–3750 mg/kg BW/day in 5 oral doses	2/12	Improvement of glycosylation (2/2), no clinical improvement reported	Not reported
TMEM165-CDG	Galactose	1000 mg/kg/day orally	2/10	Improvement of glycosylation (2/2), hepatopathy (2/2), coagulopathy (2/2) and IGF1 levels (2/2) (clinical improvement not mentioned)	Not reported
GFPT1-CDG	Pyridostigmine (cholinesterase inhibitors)	2.5–15 mg/kg/day in 3 oral doses	56/62	Improvement of muscle weakness (52/56)	Muscle twitching, depression and anxiety (2/56)
ALG2-CDG	Pyridostigmine (cholinesterase inhibitors)	Oral, dose n.s.	1/11	Improvement of muscle weakness (1/1)	Not reported
ALG14-CDG	Pyridostigmine (cholinesterase inhibitors)	5–8 mg/kg/day in 2–6 oral doses	4/7	Improvement of muscle weakness (4/4, in 1 patient the effect was only temporary)	Not reported
PIGM-CDG	Sodium butyrate	60–90 mg/kg/day in 3 oral doses	4/7	Effect on seizures (3/3), developmental delay (2/2) and increase in the expression of GPI-linked blood cell surface markers (2/4)	Not reported
PMM2-CDG	Acetazolamide	7–17 mg/kg/day in 2 oral doses	24/ > 900	Effect on cerebellar symptoms (20/23), speech (20/23), anxiety (20/23), some coagulation parameters (20/23), stereotypic movements (5/5) and SLE (1/1). No improvement of ID and non-neurologic symptoms.	Acidosis with significant decrease of serum bicarbonate (9/23), asthenia (4/23) and paresthesia (2/23) responding to dose adjustments; risk of urolithiasis, osteopenia

BBA - General Subjects 1865 (2021) 129751

New treatments

- ***Enzyme replacement therapy***
- Although enzyme therapy is theoretically possible in PMM2-CDG, several difficulties limit the application of enzyme replacement as a feasible treatment in CDG.
- Of these, cell compartmental targeting, low levels of replaced enzyme, cellular uptake of the supplemented enzyme, and delivery across the BBB remain most problematic.
- Even if an enzyme crosses the blood–brain barrier, and gets into the right cells, it would only affect current glycosylation-dependent cell functions and may or may not affect developmental problems

Genet Med. 2020 February ; 22(2): 268–279

New treatments

- ***Gene therapy***
- Gene therapy restore the wild-type sequence of the mutated gene through transgene introduction
- Non-viral transgene delivery methods include zinc-finger nucleases, TALENs, and CRISPR/Cas9 technology.
- Adeno-associated viral vector serotype 9 (AAV9) therapy; AAV9 gene encoding N-glycanase 1 (NGLY1) is the first human open-label gene therapy for CDG (GS-100 gene therapy) by intracerebroventricular infusion of GS-100 in patients aged 2 to 18 years old.
- Gene therapy is being considered for several types of CDG, including: PMM2-CDG, GNE-CDG, and PIGA-CDG

JCI Insight. 2024;9(19):e183189.

Genet Med. 2020 February ; 22(2): 268–279

New treatments

- AI has boosted both diagnosis and classification as therapeutic developments in rare diseases .
- Over 6,000 rare diseases have been identified, affecting 8–10% of the world's population
- According to the Orphan Drug Act, a rare disease is a disease or condition that impacts fewer than 200,000 people in the US
- European Union considers as rare a disease affecting fewer than 5 people in 10,000
- Recently in CDG, AI has been used to elucidate basic disease mechanisms and facilitate diagnosis, classification, and new modalities for effective treament.

Orphanet Journal of Rare Diseases (2023) 18:247
Orphanet Journal of Rare Diseases (2022) 17:303

New treatments

Patent id/Title/ earliest publication	Compound(s)	Tested	Defect/CDG/protein	Category/rationale
DE19758059A1 WO9933474A1 The use of mannose for combating protein loss enteropathy (1999)	Mannose or mannose-containing sugar which releases mannose in the gastro-intestinal tract	Mannose supplementation was successfully tested on CDGIb patient' fibroblasts (2 [^{3} H]mannose incorporation into glycoconjugates was measured and normal glycosylation of glycoproteins was recorded). Mannose was given orally to the patient too. The clinical symptoms disappeared and transferrin glycoprofile normalized. [35, 36, 37]	Protein N-glycosylation: • MPI-CDG Phosphomannose isomerase	Sugar supplementation therapy Mannose provided by the diet becomes the only source of mannose 6-phosphate
EP1521761A2 WO03104247A2 Treatment of congenital disorders of glycosylation (CDG) using mannose (2003)	Hydrophobically masked derivatives of mannose-1P	Enzymatic tests have been conducted to demonstrate that protecting groups were cleaved to restore the original mannose-1P. [48]	Protein N-glycosylation: • PMM2-CDG Phosphomannomutase 2	Substrate replacement therapy The hydrophobically masked mannose 1-phosphate derivatives having an increased lipophilicity can cross the cell membrane. Once inside the cell, endogenous nonspecific enzymes should ensure the release of the free monophosphate sugar
FR2897779A1 FR2897779B1 US2009054353A1 Drug composition, useful as cellular sources of mannose-1-phosphate against carbohydrate deficient glycoprotein type I syndrome, comprises excipient and active ingredient e.g. mono(alpha-D-mannopyranosyl-1) phosphate (2007)	Mono-(mannopyranosyl-1), di(mannopyranosyl-1) and tri(mannopyranosyl-1) phosphates	Lymphoblast cells derived from a control subject and a CDG-Ia patient were used. Inhibition of 2 [^{3} H]mannose incorporation into glycoconjugates was recorded. The toxicity of prodrugs has also been assessed. [49]	Protein N-glycosylation: • PMM2-CDG Phosphomannomutase 2	Substrate replacement therapy
WO2011116355A2 WO2011116355A3 Benzoisothiazolones as inhibitors of phosphomannose isomerase (2011)	Benzoisothiazolones	The potency and selectivity of the compounds were assessed running enzymatic assays on human PMI and PMM2. Fresh plasma or liver microsomes (mouse) were used for stability assays. Cellular assay was conducted on HeLa cells (2 [^{3} H]mannose incorporation into glycoconjugates was measured). C57BL/6 mice were used for in vivo pharmacokinetics. [50]	Protein N-glycosylation: • PMM2-CDG Phosphomannomutase 2	Phosphomannose isomerase (PMI) inhibitors The inhibition of PMI can push the flux of mannose-6P towards the production of mannose-1P

Orphanet Journal of Rare Diseases (2023) 18:247

New treatments

Patent id/Title/ earliest publication	Compound(s)	Tested	Defect/CDG/protein	Category/rationale
EP3275863A1 Compounds for treating congenital disorders of glycosylation (2018)	Amide and urea derivatives (e.g.: 3-(3-Chloro-phenyl)-1,1-di-pyridin-2-yl-urea and 1,3-Bis-(3-chlorophenyl)-urea)	Pure, homodimeric WT-PMM2 and mutants (p.Val44Ala, p.Asp65Tyr, p.Arg123Gln, p.Arg141His, p.Arg162Trp, p.Thr237Met, p.Cys241Ser) were analysed by Differential Scanning Fluorimetry. Patient-derived fibroblasts (p.Arg141His/p.Asp65Tyr), (p.Pro113Leu/p.Pro113Leu), (p.Arg141His/p.Arg162Trp) and (c.640-9T > G/p.Thr237Met) were transduced with their own folding or oligomerization variant, that is p.Asp65Tyr, p.Pro113Leu, p.Arg162Trp, and p.Thr237Met respectively. These cellular models were used to evaluate PMM2 activity after the treatment with selected drugs. [56]	Protein N-glycosylation: • PMM2-CDG Phosphomannomutase 2	Pharmacological chaperone therapy The drug binds to and stabilize wt and mutated PMM2s. When the variant does not affect the enzymatic activity but only causes a loss of stability, a PC prevents the premature degradation of the mutated protein
WO2020040831A1 Methods for treating congenital disorders of glycosylation (2020)	α-cyano-4-hydroxycinnamic acid, epalrestat, rhetsinine, theasinensin, suramin	Drug screen/test was conducted in: -yeast models of PMM2-CDG (pACT1-F126L – p.Phe126Leu, pSEC53-V238M – p.Val238Met, and pSEC53-F126L -p.Phe126Leu haploids and pACT1-F126L/pACT1-R148H – p.Phe126Leu/p.Arg148His, pSEC53-V238M/pSEC53-R148H – p.Val238Met/p.Arg148His, and pSEC53-F126L/pSEC53-R148H – p.Phe126Leu/p.Arg148His heterozygous diploids). - a p.Phe119Leu variant strain, orthologous p.Phe125Leu in worms - WT and PMM2 (GM20942-p.Arg141His/p.Phe119Leu) compound heterozygous fibroblasts. [58, 59]	Protein N-glycosylation: • PMM2-CDG Phosphomannomutase 2	Aldose reductase inhibitors Aldose reductase inhibitors may reduce the flux of glucose through the polyol pathway, which can lead to inhibition of tissue accumulation of sorbitol and fructose and prevention of reduction of redox potentials
US2022017535A1 Inhibitors of aldose reductase (2020)	A novel family of aldose reductase inhibitors	In vitro studies measuring aldose reductase enzymatic inhibition were conducted. Ex vivo studies on an isovolumic isolated rat heart preparation (male Wistar rats) were also conducted.	Protein N-glycosylation • PMM2-CDG Phosphomannomutase 2	Aldose reductase inhibitors
CA3153108A1 WO2021071965A1 Aldose reductase inhibitors for treatment of phosphomannomutase 2 deficiency (2021)	Zopolrestat, ponalistat, epalrestat, sobinil or sorbinol, mirlistat, AND-138, CT-112 (Risarestat), zopostat, denastat, BAL-AR18, AD-5467, M-79,175, torilista, alconil, statil, berberine or SPR-210	PMM2 enzymatic assay was conducted on four protein extracts from PMM2-CDG patient-derived fibroblasts (p.Phe138Ser/p.Arg141His, p.Arg141His/p.Pro113Leu, p.Arg141His/p.Phe119Leu p.Arg141His/p.Asn216Ile) treated with the drugs.	Protein N-glycosylation: • PMM2-CDG Phosphomannomutase 2	Aldose reductase inhibitors Single or combined therapy (with a second aldose reductase inhibitor, an antioxidant, or both)

Orphanet Journal of Rare Diseases (2023) 18:247

New treatments

Patent id/Title/ earliest publication	Compound(s)	Tested	Defect/CDG/protein	Category/rationale
GB2597315A Use of cannabidiol in the treatment of seizures associated with rare epilepsy syndromes related to genetic abnormalities (2022)	Cannabidiol	CBD was able to significantly reduce the number of seizures in one ALG11-CDG patient.	Protein N-glycosylation: • ALG11-CDG ALG11 Alpha-1,2-Mannosyltransferase	Treatment of seizures associated with rare epilepsy syndromes Single or combined therapy with one or more other antiepileptic drugs. The combined administration can be done sequentially or simultaneously
EP2905621A1 Means and methods for diagnosing and treating cdg caused by a deficiency of PGM1 (2015)	Galactose and uridine	Galactose was added to culture medium of fibroblasts from patients. An enhancement of the glycosylation was recorded on ICAM-1 and Glyc-ER-GFP. The effect on Glyc-ER-GFP was further enhanced by the addition of uridine to the culture medium. Six patients received dietary supplementation with lactose or galactose. Transferrin IEF profiles showed a substantial improvement of glycosylation after dietary intake of galactose. Other clinical signs have been monitored too. [79]	Disorder of multiple glycosylation pathways: • PGM1-CDG Phosphoglucomutase 1	Sugar supplementation therapy
WO2016028894A1 Treatment of glycosylation deficiency diseases (2016)	Uridine prodrug (e.g.: uridine triacetate) and sugars	A patient diagnosed with a CDG by detection of a variation affecting glycosylation by reducing availability of a UDP-sugar was treated with uridine triacetate. One GNE-myopathy patient, one PGM1-CDG patient, one DGAPT1-CDG patient were treated with uridine triacetate in combination with N-acetylmannosamine, D-galactose, N-acetylglucosamine respectively. The combined therapies produced a clinical improvement better than the sugars alone.	Disorder of multiple glycosylation pathways: • PGM1-CDG Phosphoglucomutase 1 • GNE-CDG UDP-GlcNAc 2-epimerase/ManNAc kinase Protein N-glycosylation: • DPAGT1-CDG UDP-GlcNAc: dolichylphosphate N-Acetylglucosaminephosphotransferase 1	Combined therapy (uridine prodrug and sugars). The administration of uridine prodrug increases the intracellular UTP. The coadministration of the specific sugar or its precursor, causes increased intracellular concentrations of the UDP-sugar
EP3806866A1 EP3806866A4 EP3806866B1 Methods and materials for treating glycosylation disorders (2019)	UDP-galactose, UDP-glucose and derivatives, in the presence or absence of D-gal	The effect of D-gal has been studied in vivo on nine PGM1-CDG patients, but also in vitro on patient skin fibroblasts. Many chemical, biochemical, clinical parameters have been checked to assess the validity of the treatment. The efficacy of the administration of UDP-Gal in the presence of glucose or a derivative thereof (e.g., UDP-glucose) to efficiently correct the PGM1-CDG phenotype, has been assessed using PGM1-CDG patient skin fibroblasts. [64]	Disorder of multiple glycosylation pathways: • PGM1-CDG Phosphoglucomutase 1 Protein N-glycosylation: • PMM2-CDG Phosphomannomutase 2 • MPI-CDG Phosphomannose isomerase	Combined sugar supplementation therapy (UDP-glycans and D-galactose)

Orphanet Journal of Rare Diseases (2023) 18:247

New treatments

Patent id/Title/ earliest publication	Compound(s)	Tested	Defect/CDG/protein	Category/rationale
WO2022272056A2 Compositions and methods for treating PGM1 deficiency (2022)	AAV9 hPGM1 gene	AAV9-hPGM1 gene replacement prevents cardiac dysfunction in Pgm2 cKO mice. [68]	Disorder of multiple glycosylation pathways: • PGM1-CDG Phosphoglucomutase 1	Gene replacement therapy Recombinant adeno-associated virus (AAV) gene therapy
EP3175859A1 EP3175859B1 N-acetyl mannosamine for the treatment of a kidney-disease (2008)	N-acetyl mannosamine and derivatives	$Gne^{p.Met712Thr/p.Met712Thr}$, $Gne^{p.Met712Thr/+}$ and $Gne^{+/+}$ mice were used to test the drugs. N-acetyl mannosamine and derivatives were useful for treating myopathies, muscular atrophy and/or muscular dystrophy and kidney conditions and diseases (e.g., those involving proteinuria and hematuria). [65]	Disorder of multiple glycosylation pathways: • GNE-CDG (UDP-N-Acetyl)-2-epimerase/N-Acetylmannosamine kinase	Substrate replacement therapy Administration of N-acetyl mannosamine and/or its derivatives promotes formation of sialic acid
CN104271125A WO2013109906A2 Method and formulation for treating sialic acid deficiencies (2013)	Sialic acid, oral administration in different formulations	27 patients were enrolled for a phase 1 study that was successful. Tests on animals (dogs) were conducted too. [66]	Disorder of multiple glycosylation pathways: • GNE-CDG (UDP-N-Acetyl)-2-epimerase/N-Acetylmannosamine kinase	Substrate replacement therapy Single or combined therapy was applied with other compounds (for example ManNAc) or derivatives of the sialic acid biosynthetic pathway. Sustained release formulation was also practiced Variations in the gene *GNE* cause a decrease in activity in either the isomerase or kinase domains, resulting in less formation of ManNAc-6-P and ultimately less Neu5AcA.
WO2019118486A1 Monosaccharide phosphoramidate prodrugs (2019)	Phosphoramidate prodrug of N-acetyl-D-mannosamine (ManNAc) 6-phosphate	Tests have been conducted to demonstrate that protecting groups were cleaved to restore the monophosphate sugar upon exposure to carboxypeptidase. Lec3 mutant CHO cells and GNE myopathy patient-derived myoblasts were treated with phosphoramidate prodrug of ManNAc 6-phosphate and the increase of free and total sialic acid recorded was higher than that obtained when ManNAc 6-phosphate has been was administered. [67]	Disorder of multiple glycosylation pathways: • GNE-CDG UDP-GlcNAc 2-epimerase/ManNAc kinase	Substrate replacement therapy Phosphoramidate derivative of sugars (N-acetyl-D-mannosamine 6-phosphate, mannose, etc.) enables the intracellular delivery of the monosaccharide

Orphanet Journal of Rare Diseases (2023) 18:247

New treatments

Patent id/Title/ earliest publication	Compound(s)	Tested	Defect/CDG/protein	Category/rationale
US2006276376A1 Increasing functional glycosylation of alpha-dystroglycan in the treatment of muscle degeneration (2006)	Glycosyltransferase (LARGE or LARGE2, or glycosyltransferases other than LARGE).	Direct injection of glycosyltransferase or exogenous construct harboring an expressible cDNA construct. FCMD myoblasts MEB fibroblasts WWS cells MCK-DG null mice expressing DG deletion mutant proteins.	Disorder of O-mannosylation: • LGMD2I limb-girdle muscular dystrophy linked to the FKRP gene MDC1D but also of FCMD, MEB, WWS, LGMD2I and other glycosyltransferase-deficient muscular dystrophies	LARGE expression can prevent muscle degeneration in various types of muscular dystrophy Increasing glycosyltransferase activity in the muscle of the subject would increase functional glycosylation of [alpha]-dystroglycan
JP2020510416A/ US2020317761A1 Multispecific binding molecules having specificity to dystroglycan and laminin-2 (2018)	A bispecific binding molecule comprising a first binding domain that binds an extracellular portion of α-dystroglycan and a second binding domain that binds laminin-2	In vivo studies on LARGEmyd-3 J/GrsJ mice. [71]	Disorder of O-mannosylation: α-dystroglycanopathy	Bispecific antibody which binds an extracellular portion of α-dystroglycan and laminin-2 Hypoglycosylation of alpha-dystroglycan results in a loss of binding of its ligands (such as laminin-2). This antibody promotes the binding between α-dystroglycan and laminin-2
US10456367B2 Compositions and methods for treating muscular dystrophy and other disorders (2018)	ribitol	p.Phe448Leu mice (containing the p.Phe448Leu variant in the fukutin-related protein gene) demonstrate a dystrophic phenotype similar to that of LGMD2I. Oral administration of ribitol increases levels of ribitol-5-phosphate and CDP-ribitol and restores therapeutic levels of F-α-DG in skeletal and cardiac muscles. [70]	Disorder of O-mannosylation: • FKRP-CDG α-dystroglycanopathy	Ribitol supplementation aims to circumvent ribitol shortage and ultimately the recovery of alpha-DG glycosylation
US10221168B1 Small-compound enhancers for functional O-mannosylation of alpha-dystroglycan, and uses thereof (2019)	Enhancer of O-mannosylation of alpha-dystroglycan	The enhancement of the functional O-mannosyl glycans of a-DG was tested on various cells: Chinese hamster ovary (CHO), mouse myoblasts (C2C12), normal human myoblasts (HSMB), human FKRP deficient myoblasts (FKRPD). [69]	Congenital muscular dystrophy characterized by reduced of O-glycosylation in the mucin-like domain of α-dystroglycan α -dystroglycanopathy	Enhancer of the functional O-mannosyl glycan (FOG) of α-DG on the cell surfaces
US2017368199A1 Methods and compositions for treating dystroglycanopathy disorders (2016)	Synthetic (optimized or not naturally occurring) polynucleotides encoding fukutin related protein (FKRP)	In vivo test in FKRP mutant mouse models. [73]	Disorder of O-mannosylation: • FKRP-CDG Fukutin-related protein	Gene replacement therapy Recombinant adeno-associated virus (AAV) gene therapy

New treatments

Patent id/Title/ earliest publication	Compound(s)	Tested	Defect/CDG/protein	Category/rationale
CN110944656A WO2019008157A1 Novel polynucleotides encoding a human FKRP protein (2019)	Polynucleotides encoding human fukutin-related protein (FKRP) and containing variants that avoid complementary transcripts generated from the frameshift start codon	Gene transfer efficiency was evaluated in C57B16 mice. In vivo functional tests were conducted on HAS-FKRPdel mice. Optimization of human FKRP transgene improved FKRP expression and as a consequence ameliorated the efficacy of the treatment. [72]	Disorder of O-mannosylation: • FKRP-CDG Fukutin-related protein	Gene replacement therapy Recombinant adeno-associated virus (AAV9) gene therapy. FKRP-based gene replacement therapy
WO2021053124A1 Gene therapy expression system alleviating cardiac toxicity of FKRP (2021)	Expression system which ensures the production of a therapeutically effective amount of the protein in the target tissues (mainly the skeletal tissues) and a toxically acceptable amount of the protein in the heart.	The invention aims at alleviating or curing the devastating pathologies linked to a fukutin-related protein (FKRP) deficiency such as Limb-Girdle Muscular Dystrophy type 21 (LGMD2I).	Disorder of O-mannosylation: • LGMD2I Limb-girdle muscular dystrophy linked to the FKRP gene	Gene replacement therapy Recombinant adeno-associated virus (AAV) gene therapy. In order to alleviate toxicity, the construct may contain a target sequence of an miRNA expressed in the heart or a promoter sequence presenting a promoter activity at a toxically acceptable level or even no activity in the heart
WO2022147490A1 Optimized fukutin-related proteins (FKRT) and methods of use (2022)	Synthetic polynucleotide encoding a human FKRP	The evaluation of therapeutic effects of different vectors and different FKRP constructs was conducted on p.Glu310stop /p.Leu276Ile mice.	Disorder of O-mannosylation: • FKRP-CDG Fukutin-related protein	Gene replacement therapy Recombinant adeno-associated virus (AAV9) gene therapy
WO2022076556A2 Therapeutic adeno-associated virus delivery of fukutin related protein (FKRP) for treating dystroglycanopathy disorders including Limb Girdle 2i (LGMD2I) (2022)	AAV9 FKRP vector; the sequence has been codon optimized; viral vectors comprise nucleic acid encoding FKRP polypeptide operatively linked to a muscle-specific promoter	The AAV9 FKRP vector has been tested in the mouse model of LGMD2I. The strength of synthetic specific promoters was tested in H9C2 (a rat BDIX heart myoblast cell line). Determination of FKRP activity using LGMD2I patient- derived cell line.	Disorder of O-mannosylation: • LGMD2I Limb-girdle muscular dystrophy linked to the FKRP gene	Gene replacement therapy AAV gene therapy product candidate containing FKRP

Orphanet Journal of Rare Diseases (2023) 18:247


Thank you