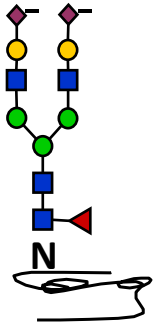
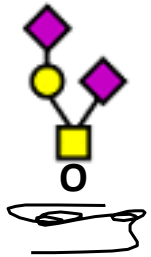
Congenital Disorders of Glycosylation:

Diagnostic steps



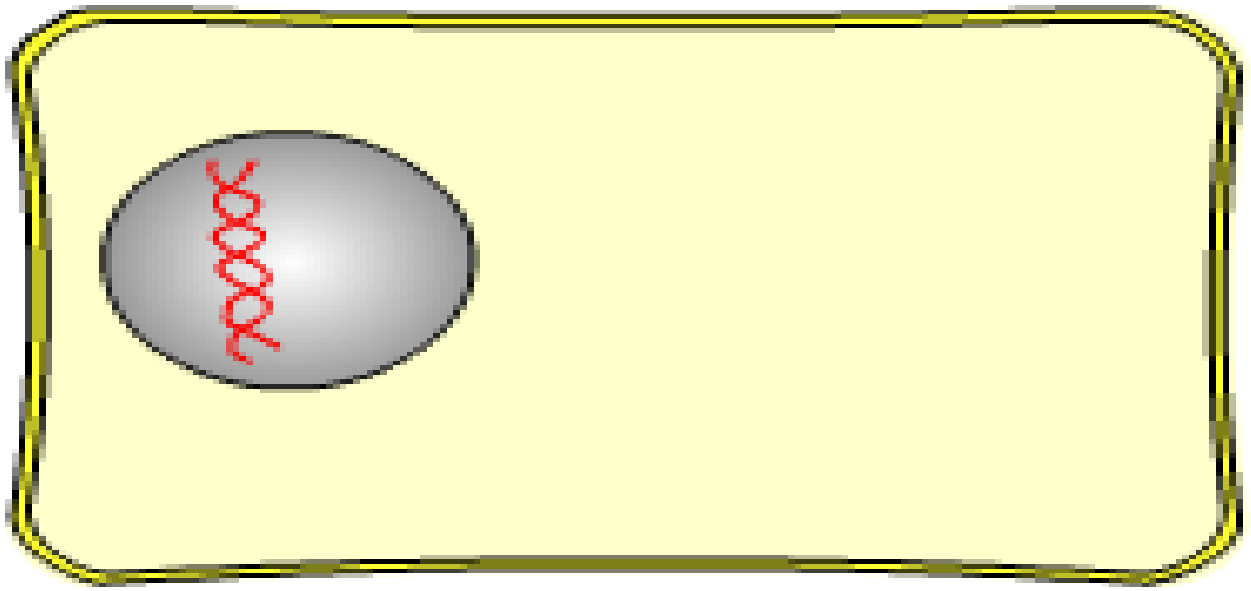


ERNDIM Meeting, Basel 2009

Dirk J. Lefeber (D.Lefeber@neuro.umcn.nl)

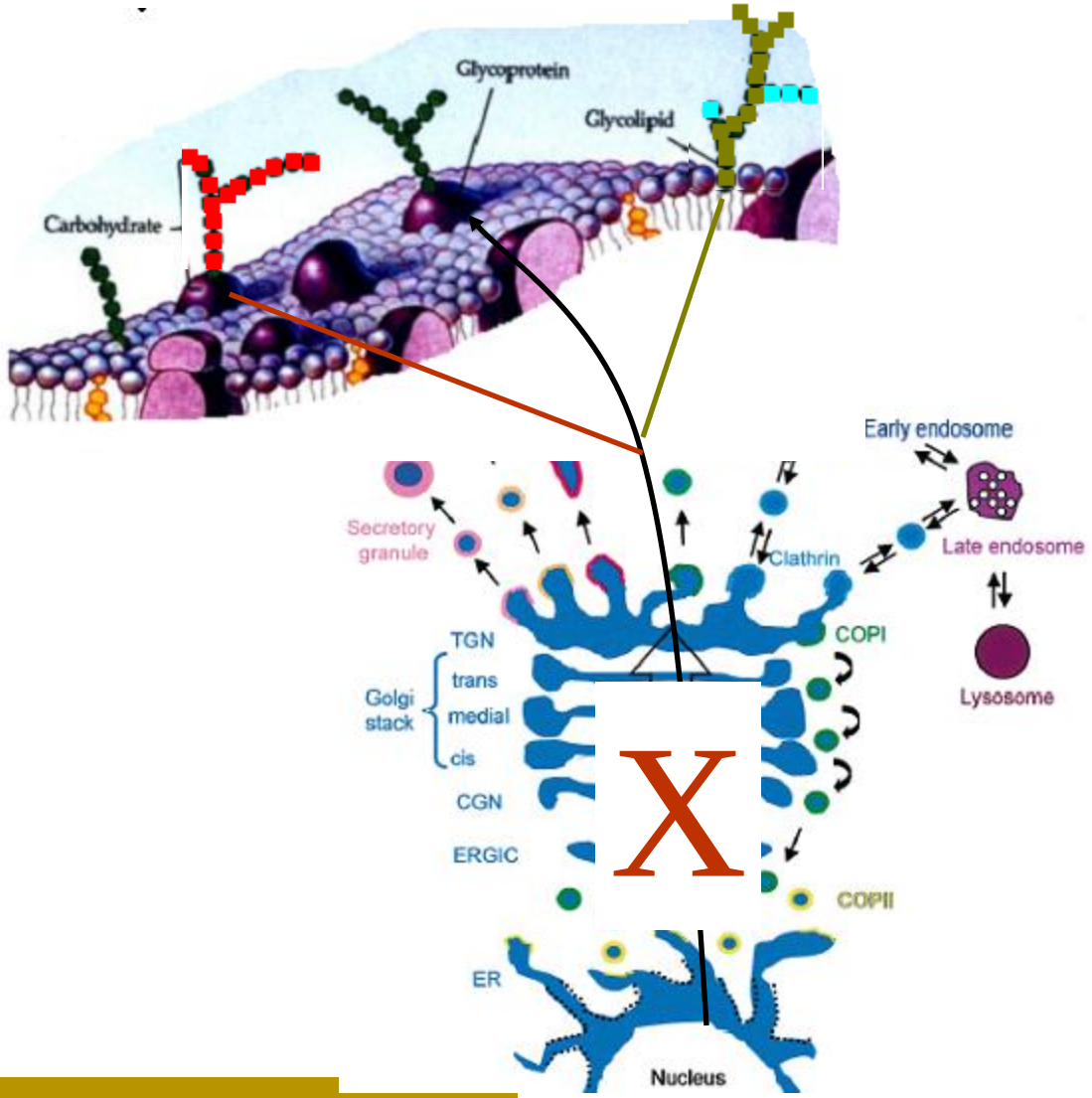
Nijmegen, The Netherlands

Dynamic glycosylation pathway

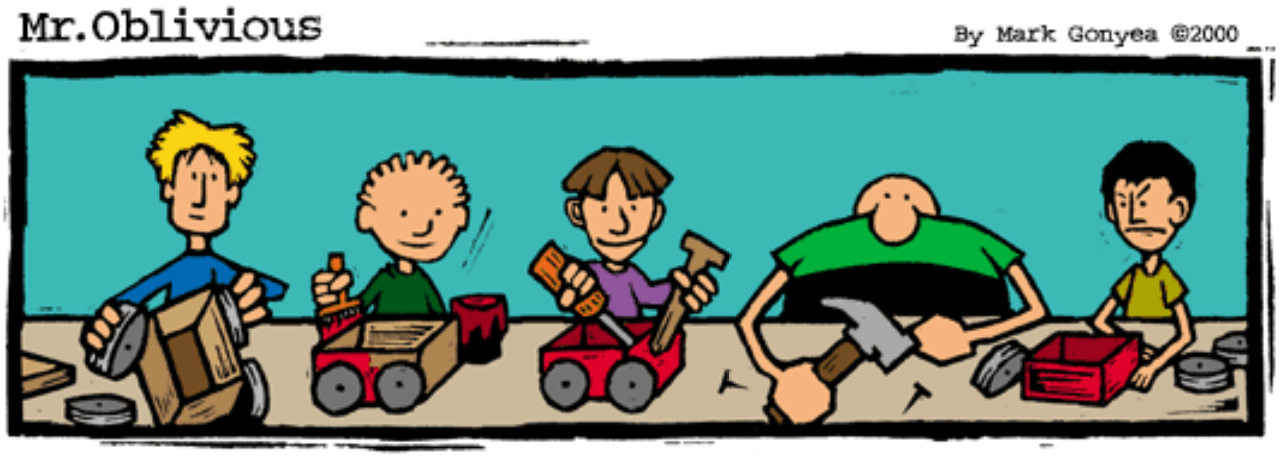


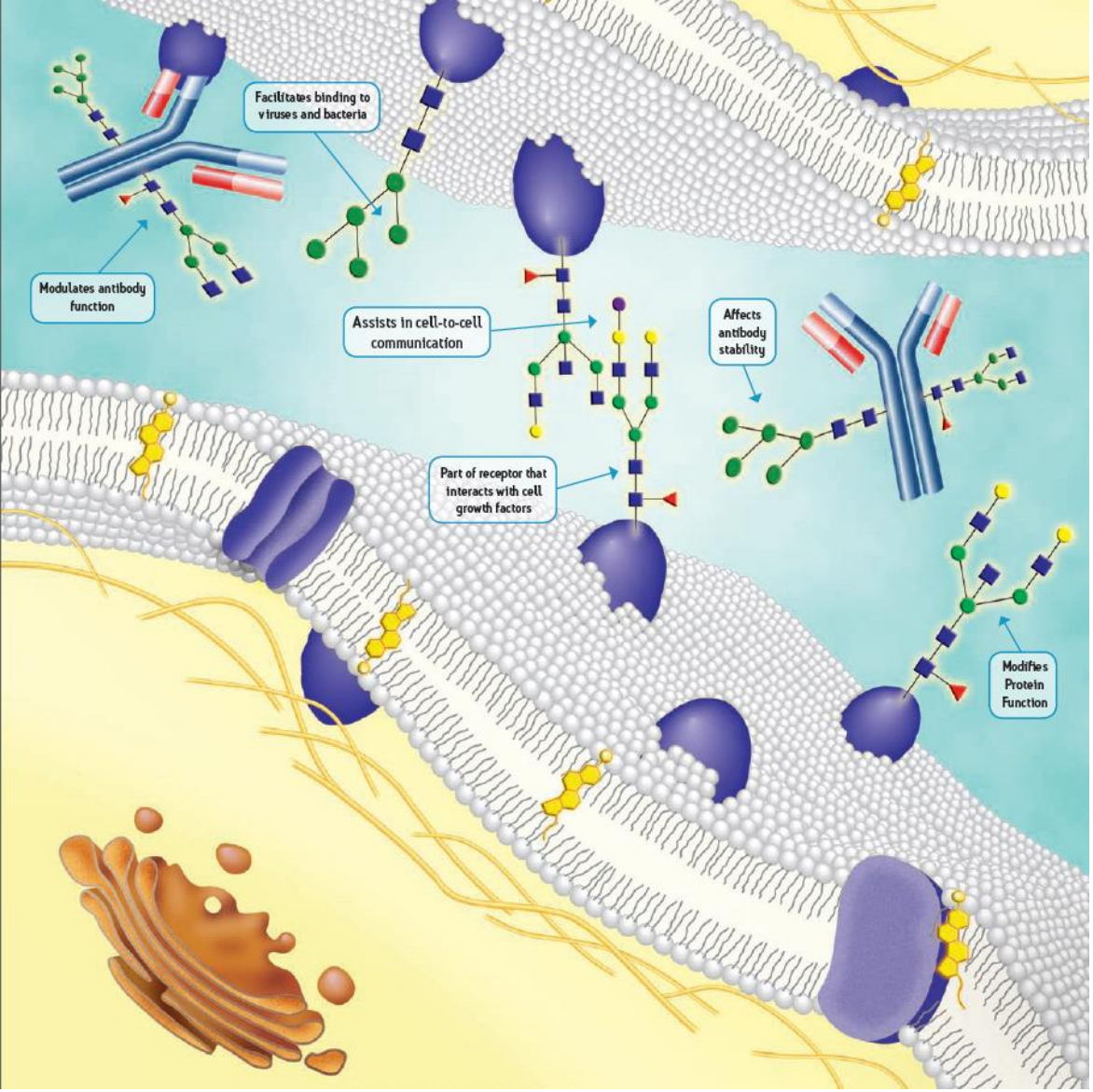
Courtesy of Dr. T. Hennet (Zurich)

General principle of Congenital Disorders of Glycosylation



Congenital Disorders of Glycosylation: Errors in the assembly line





Glycoprotein Function

- Protein stability, solubility and structure
- Protection against proteases
- Cell – cell interactions
- Target – receptor interaction
- Localization of proteins
- Signal transduction
- Bacterial adhesion
- …

> 50 % of human proteins are glycosylated
> 1 % of our genes is involved in glycosylation

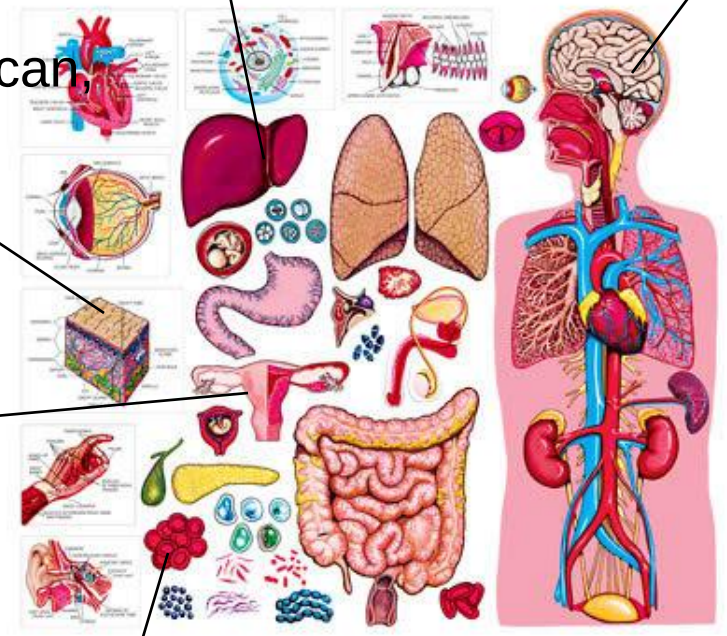
N-glycosylation: multisystemic

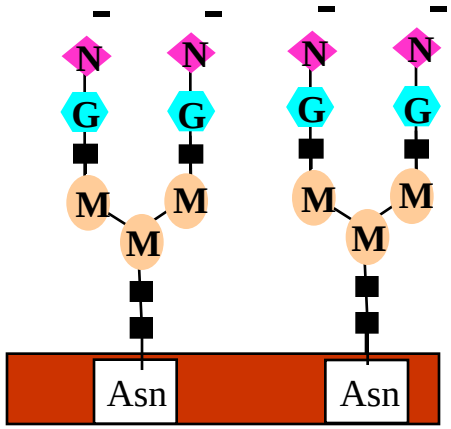
Brain (MAG, P0, neurexin)

Liver (transferrin)

Muscle (Dystroglycan, agrin)

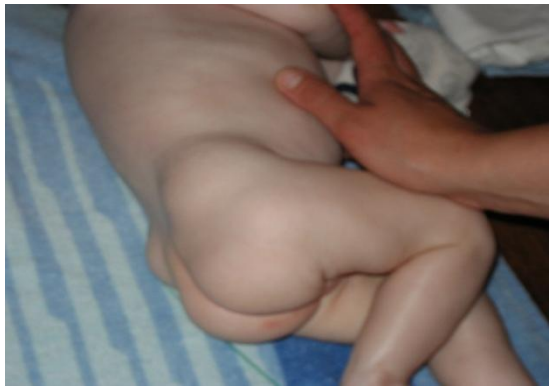
Glycoprotein hormones (LH/FSH/TSH)





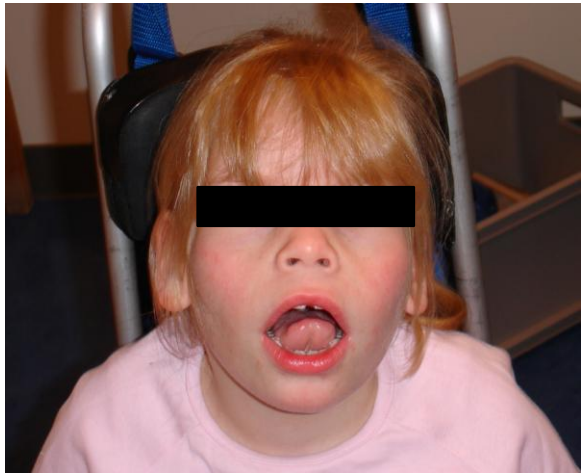
Coagulation (factor VII, IX, ATIII)

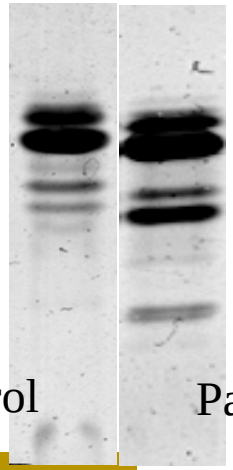
Clinical aspects of Congenital Disorders of Glycosylation



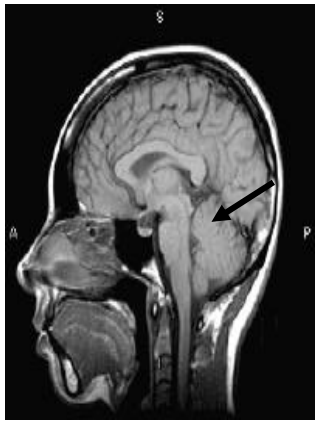
Classical picture of CDG:

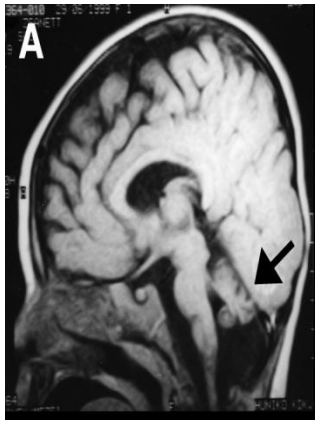
- hypotonia/epilepsy/cerebellar atrophy, inverted nipples fat pads, strabismus, feeding problems, ataxia, hypogonadism, mental retardation





Control Patient

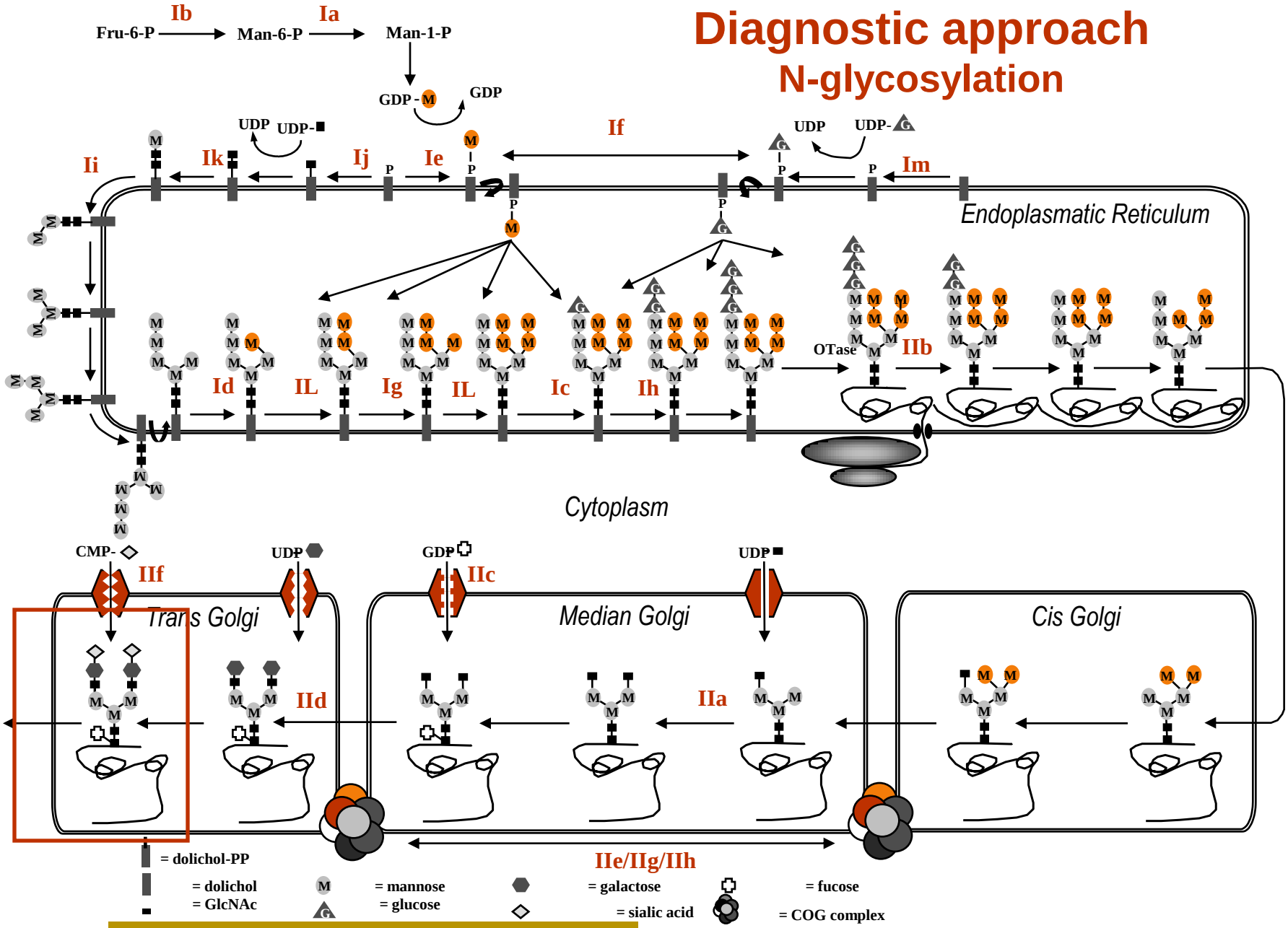




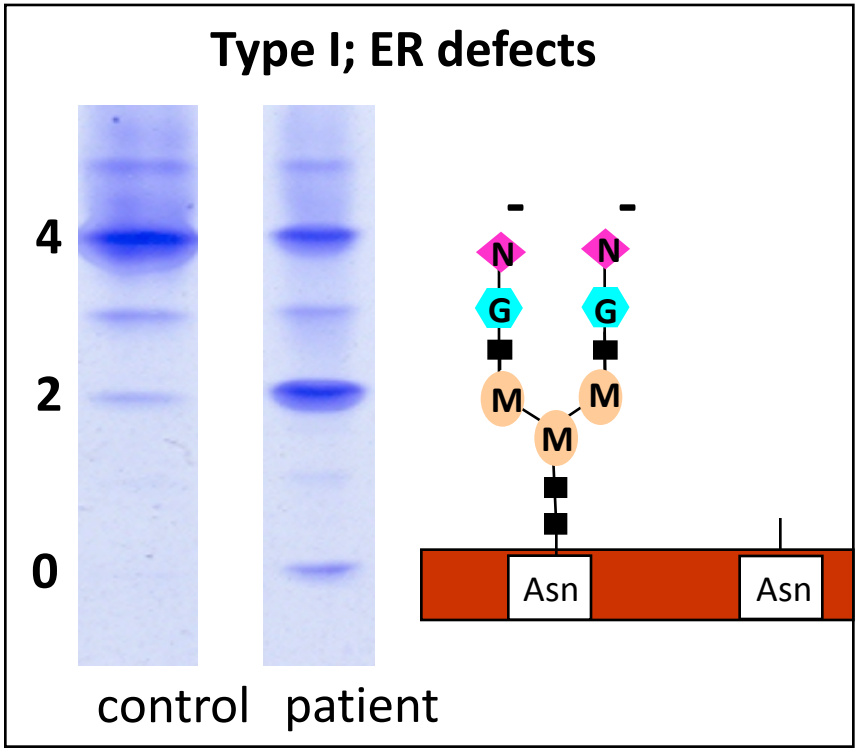
When to perform transferrin isofocusing?

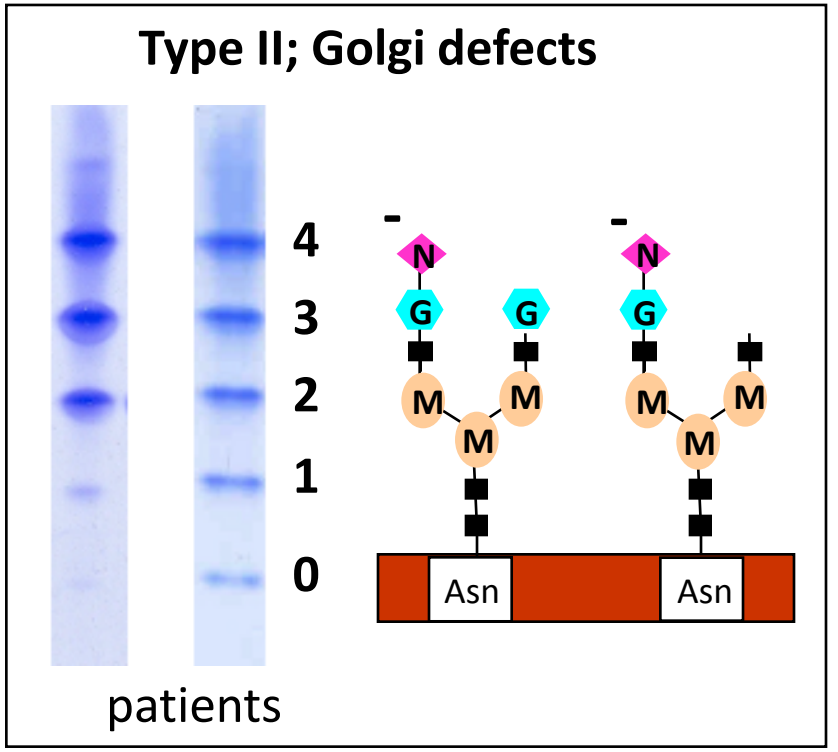
- All patients with a suspicion of a metabolic disorder
- Reason:
  - CDG: wide spectrum, mild isolated to severe multisystem
  - CDG-Ib/h, fructosemia
  - CDG-Ix with isolated myopathy, optic nerve atrophy, DCM, ichthyosis
  - New phenotypes in CDG-II: adducted thumbs/microcephaly; cutis laxa; complex vertebral malformations
  - CDG-II with liver pathology as main feature

Diagnostic approach
N-glycosylation



CDG patients with different profiles





86 CDG-I; solved
8 CDG-Ix; unsolved

17 CDG-II; solved
29 CDG-IIx; unsolved

Nomenclature changes in CDG

- 1999: CDGS-I to VI to CDG-I(a-o) and CDG-II(a-h)
- 2009: from CDG-I//II to PMM2-CDG

PMM2-CDG

MGAT2-CDG

ALG6-CDG

B4GALT1-CDG

PMI-CDG

POMT1-CDG

CDG-I

CDG-II

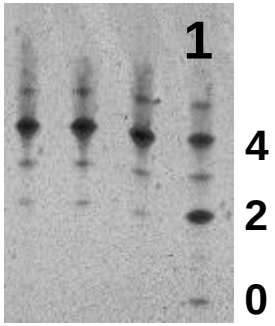
Walker Warburg Syndrome (WWS)

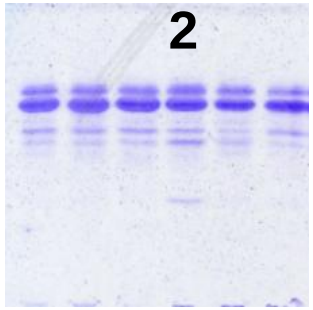
Keep CDG-I/II with gene name?? CDG-I(ALG3)

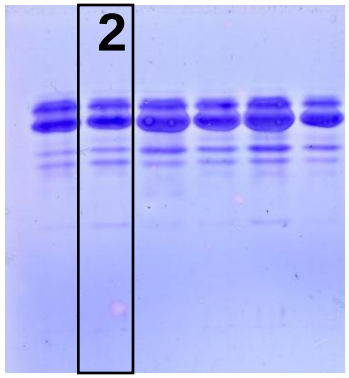
Stage 1: Secondary causes & Type I/II determination

Type I:

1. Galactosemia
2. Fructosemia
3. Alcohol abuse

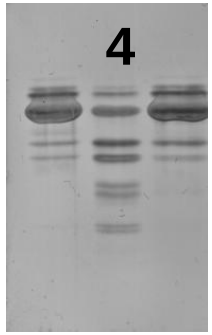


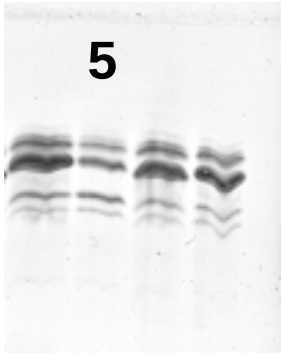


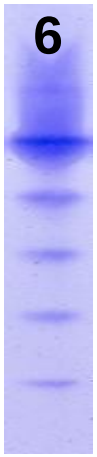


Type II:

4. Haemolytic Uremic Syndrome (HUS)
5. Severe liver disease



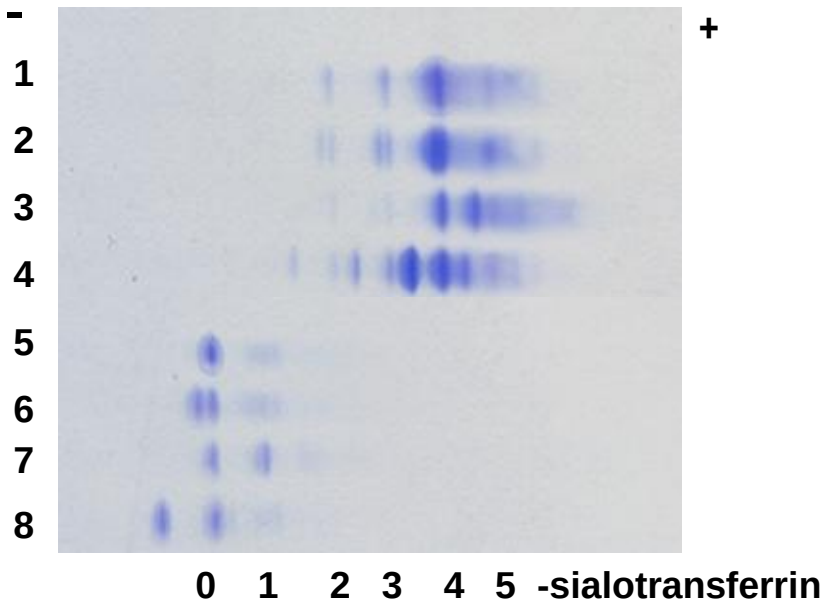




6. Young age (<1-2 months)
7. Transferrin protein polymorphism

Transferrin protein polymorphism

Lanes 1 - 4: No neuraminidase treatment
Lanes 5 - 8: Neuraminidase treatment



1/5: normal

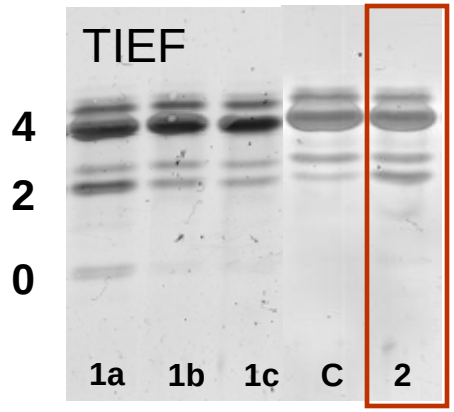
2/6: The frequently occurring C_1/C_3 variant

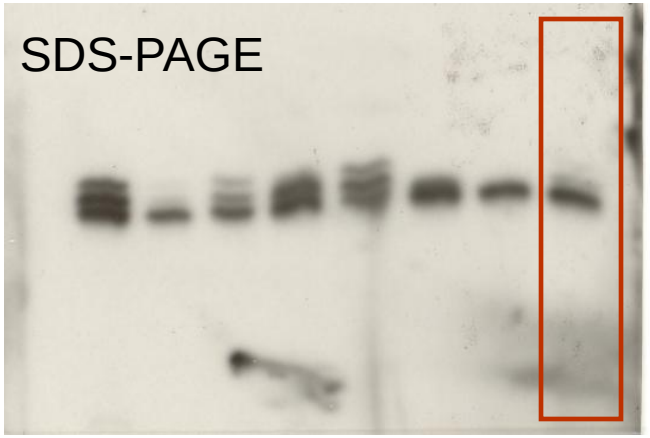
3/7: protein polymorphism shifting towards anode (= B variants)

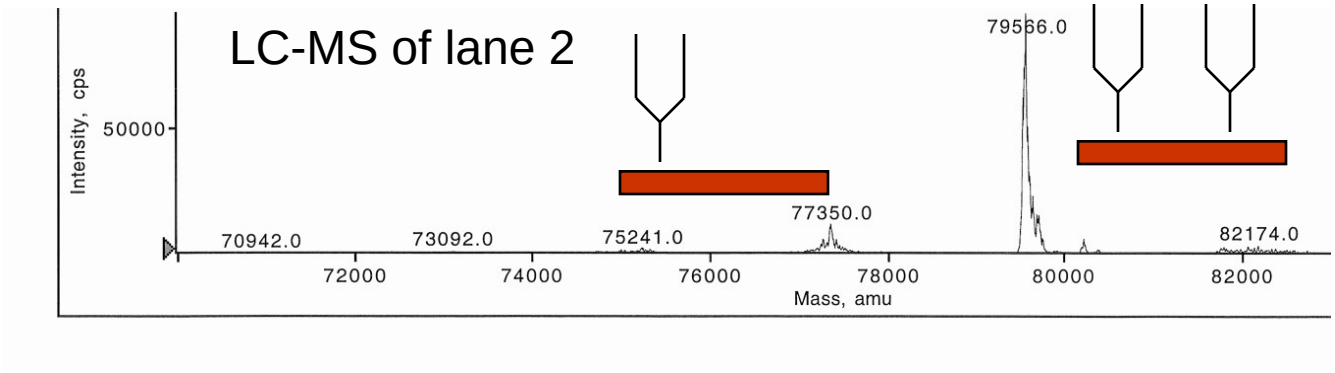
4/8: protein polymorphism shifting towards cathode (D variants)

Type I/II classification

- In some cases, assignment of type I or type II is difficult
- SDS-PAGE of transferrin might help

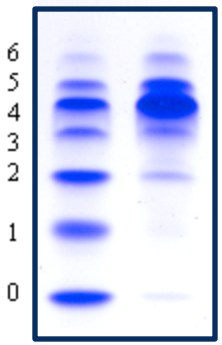


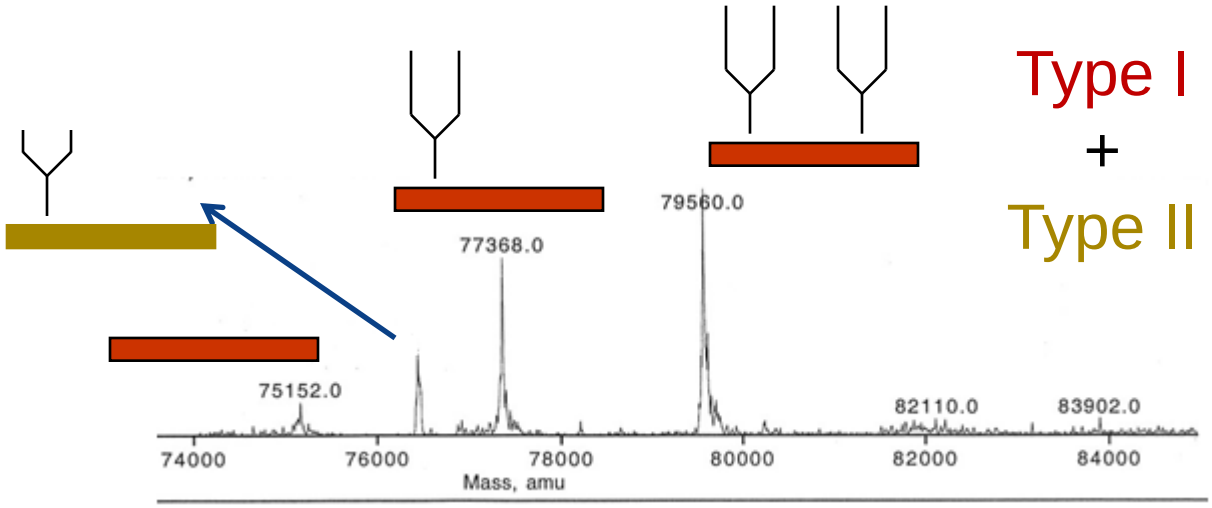




Escape of the CDG-I vs CDG-II classification?

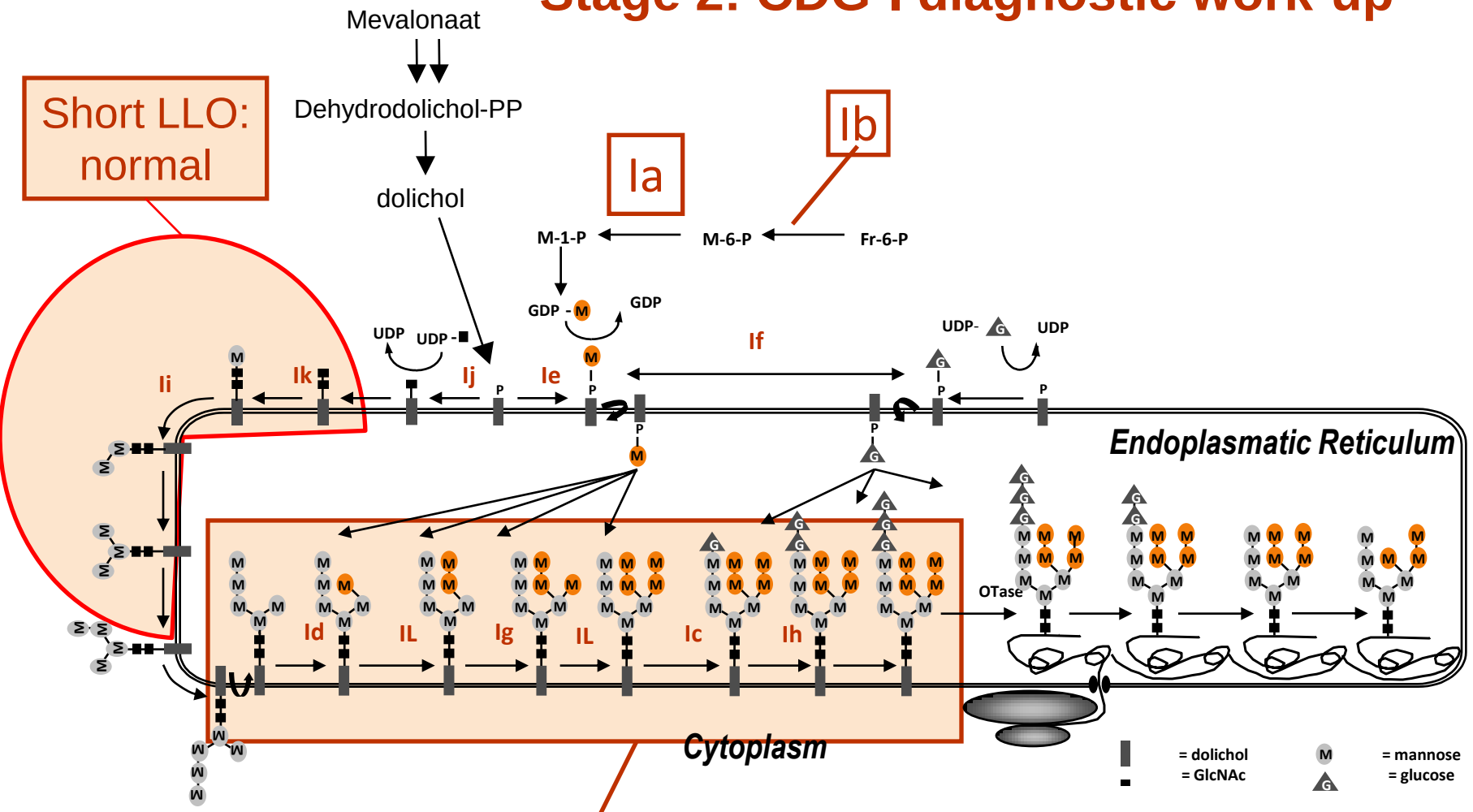
9 yr girl:
cleft palate, dilated cardiomyopathy and chronic hepatitis





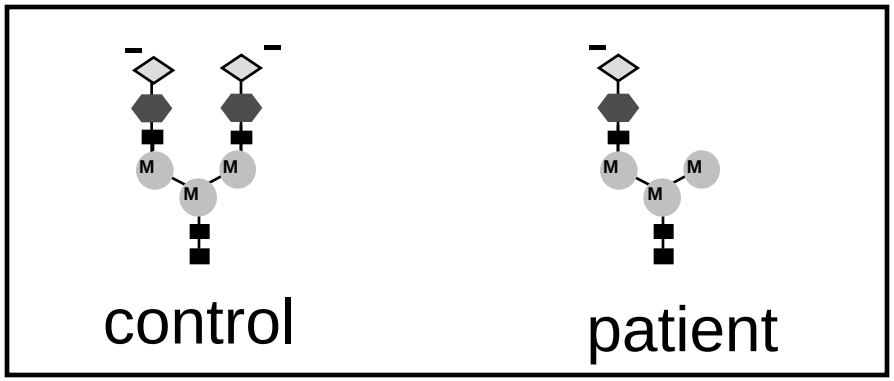
Type I
+
Type II

Stage 2: CDG-I diagnostic work-up

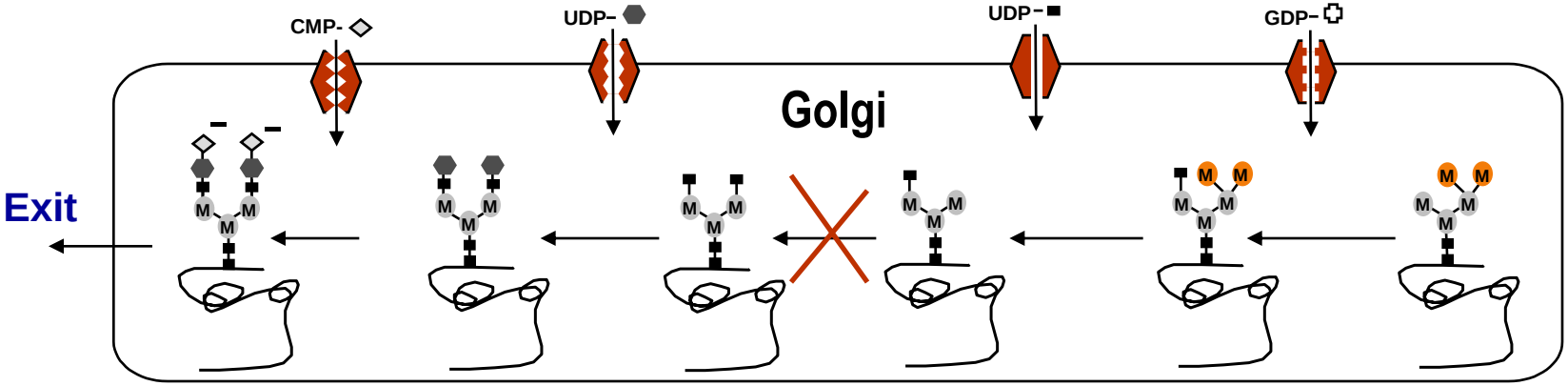


Lipid-linked Oligosaccharide (LLO) analysis

Stage 2: CDG-II diagnostic work-up



N-Glycan structural analysis

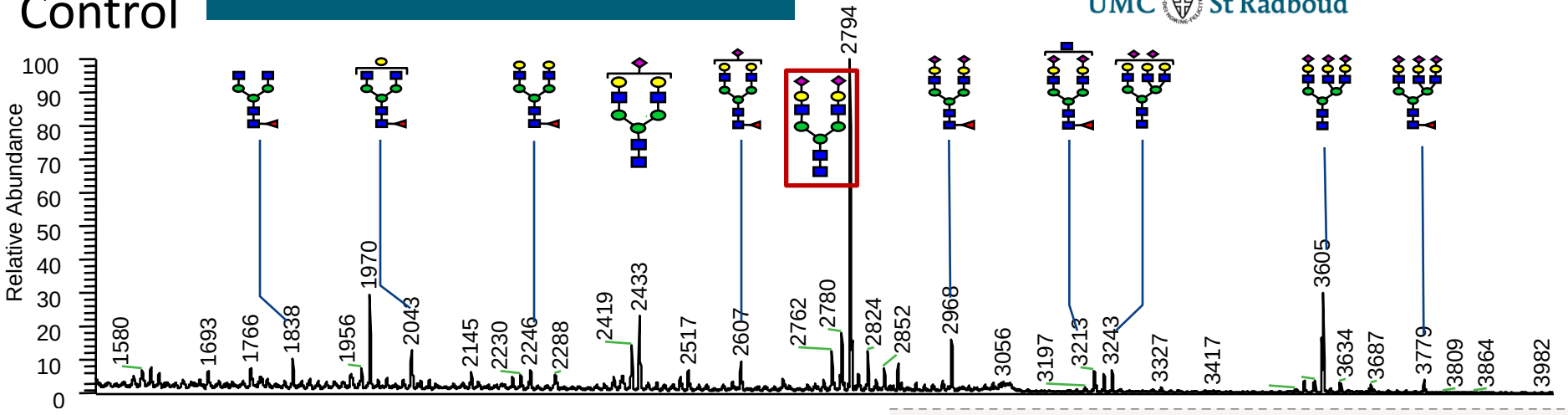


MGAT2

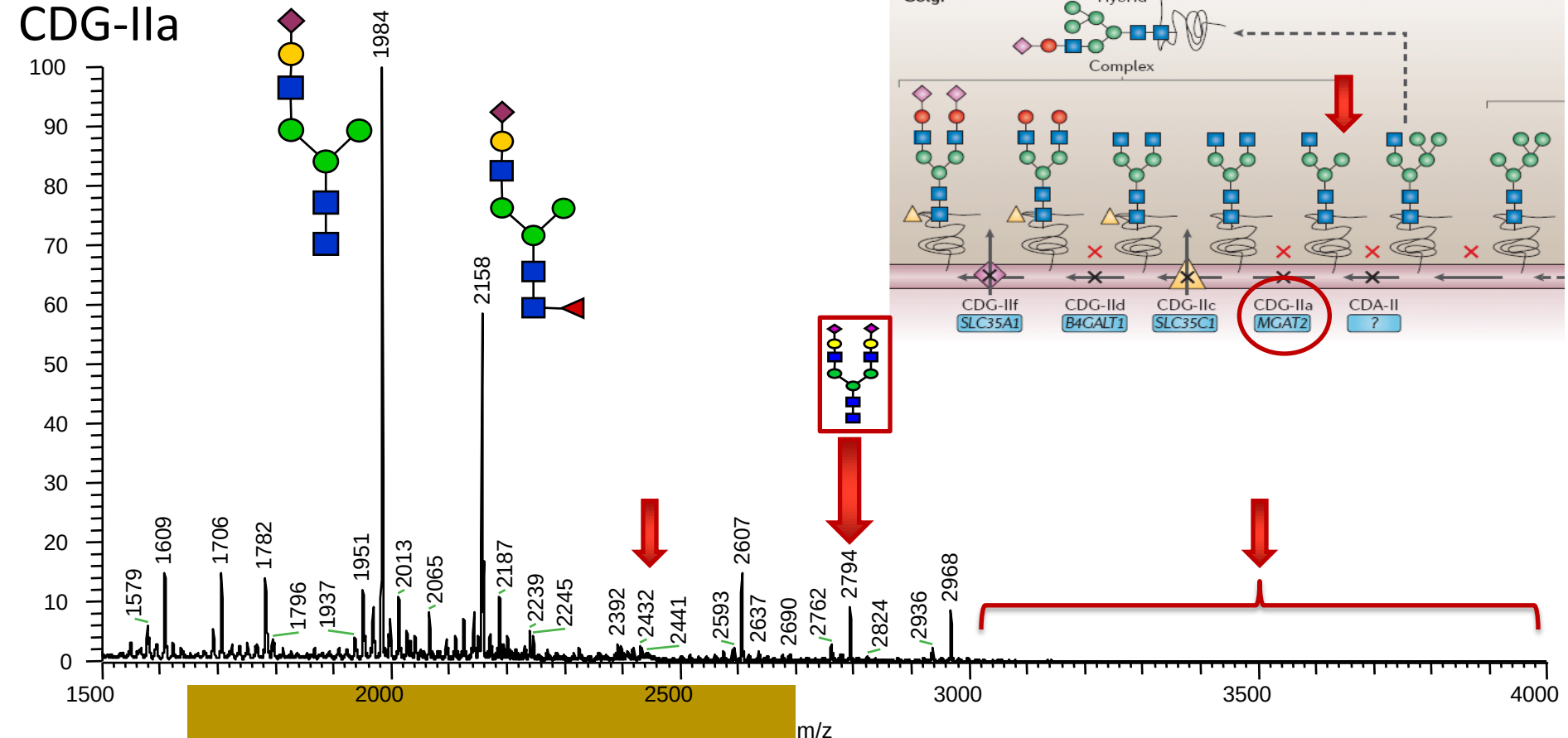
CDG-IIa

UMC St Radboud

Control

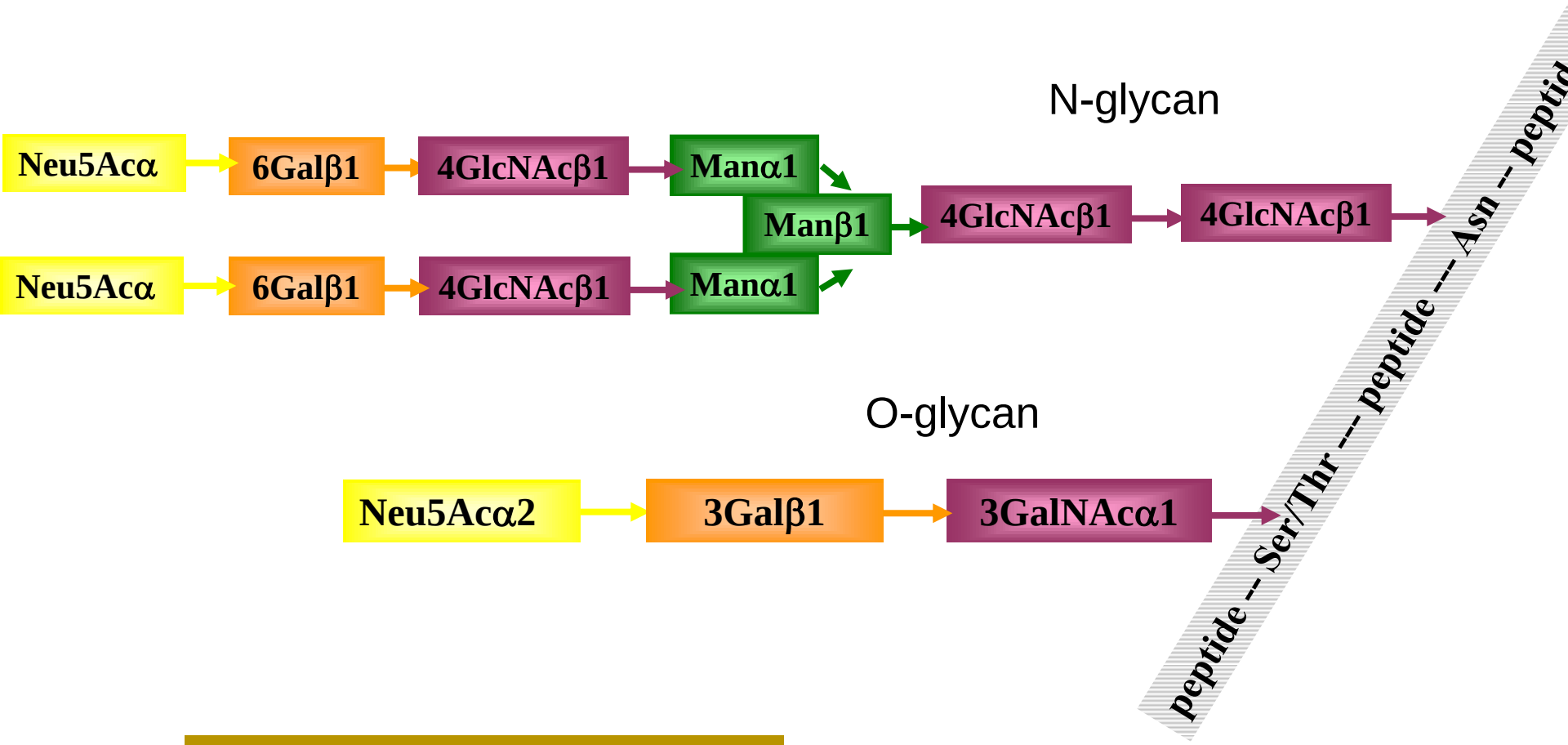


CDG-IIa

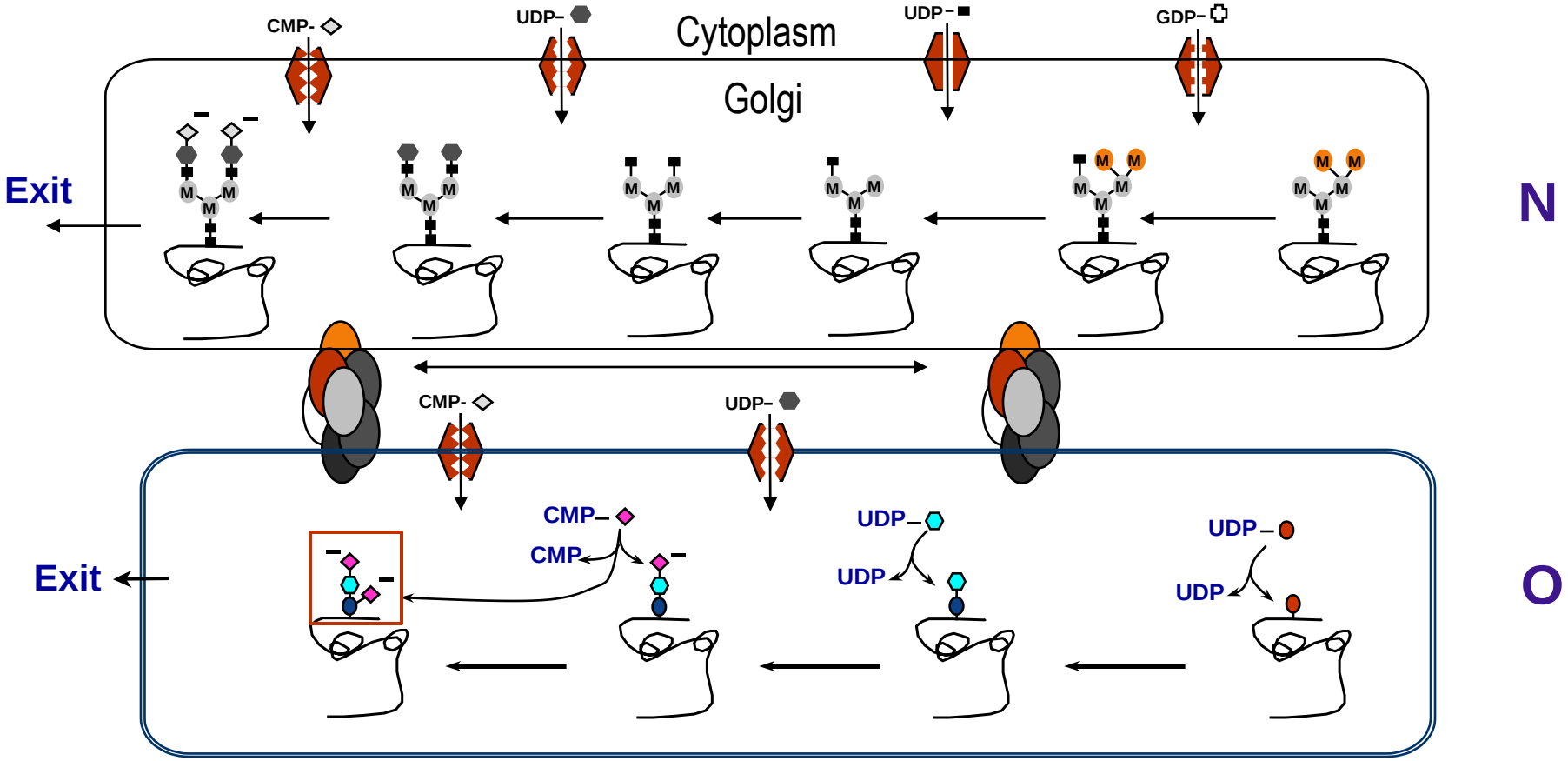


Glycan types

- N-glycosylation: amide (NH2) binding with Asparagine (ASN)
- O-glycosylation: hydroxy (OH) binding with Serine (Ser) or Threonine (Thr)

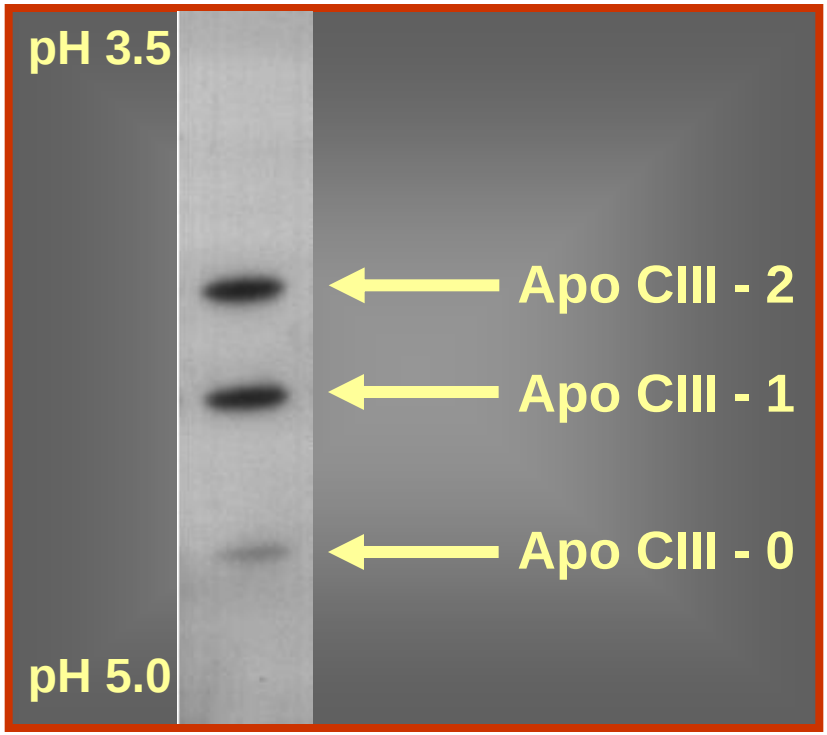


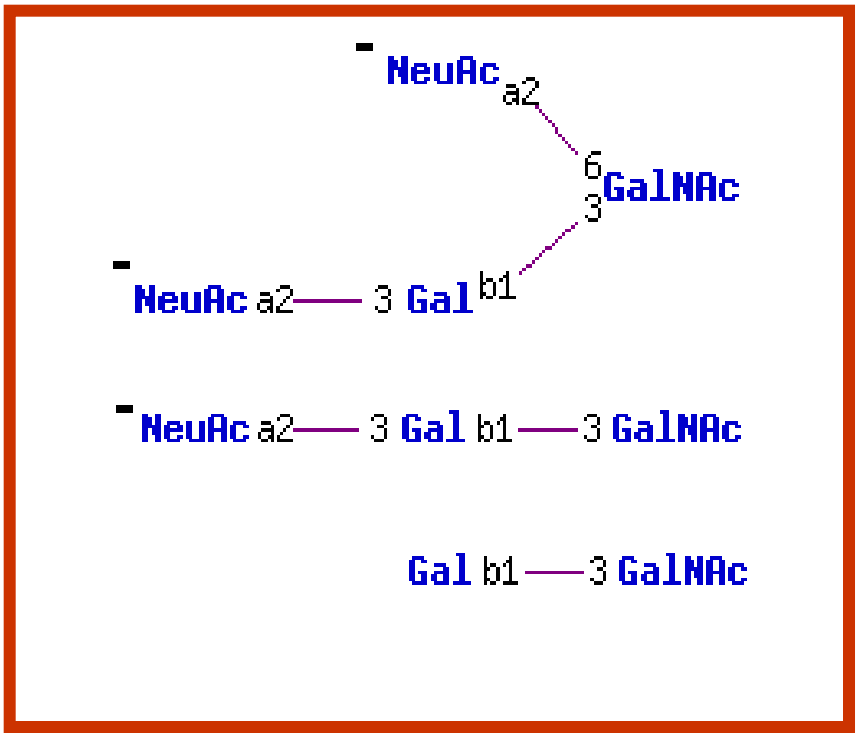
More options in the Golgi



Isoelectric focusing of serum apolipoproteinC-III

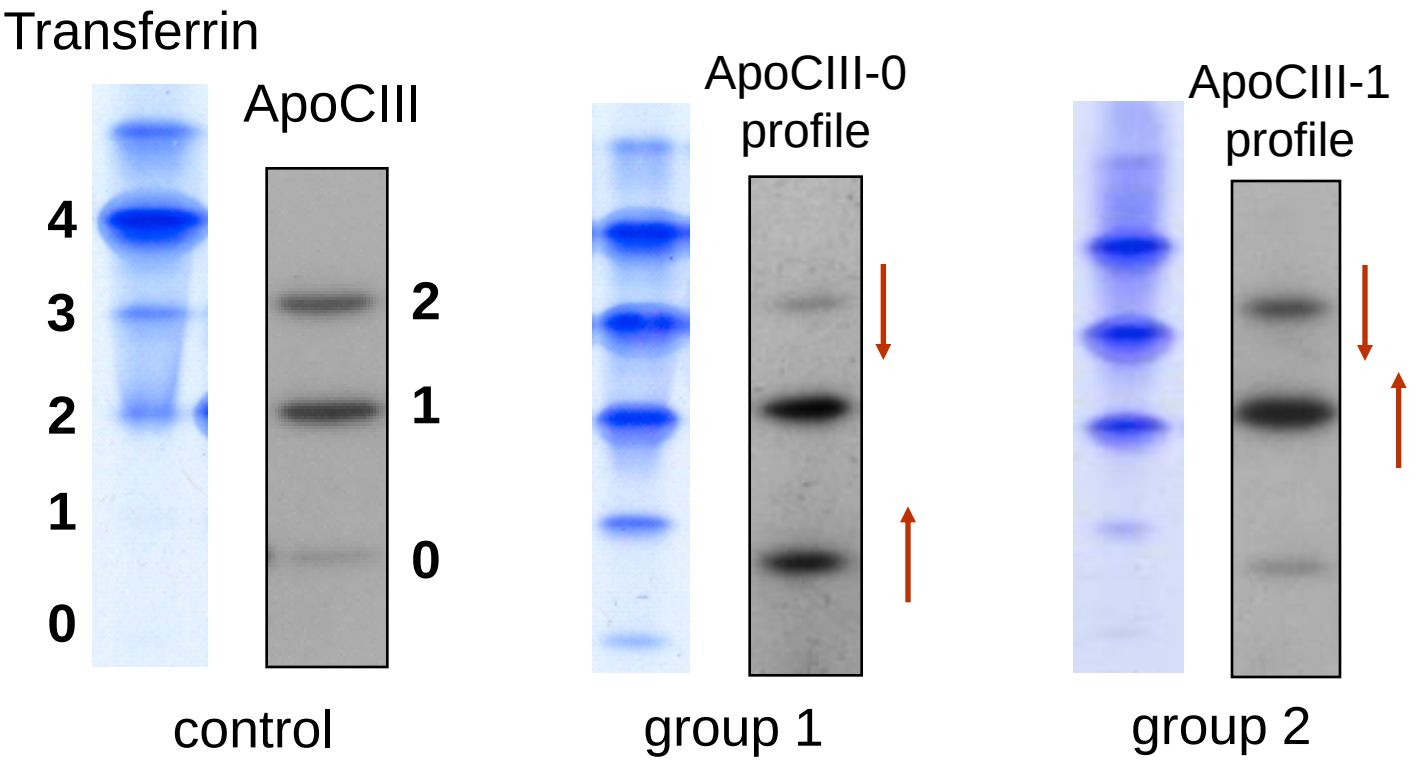
Core 1 mucin type O-glycan in position Thr-94





Wopereis et al. Clin Chem 2003;49(11):1839-45.

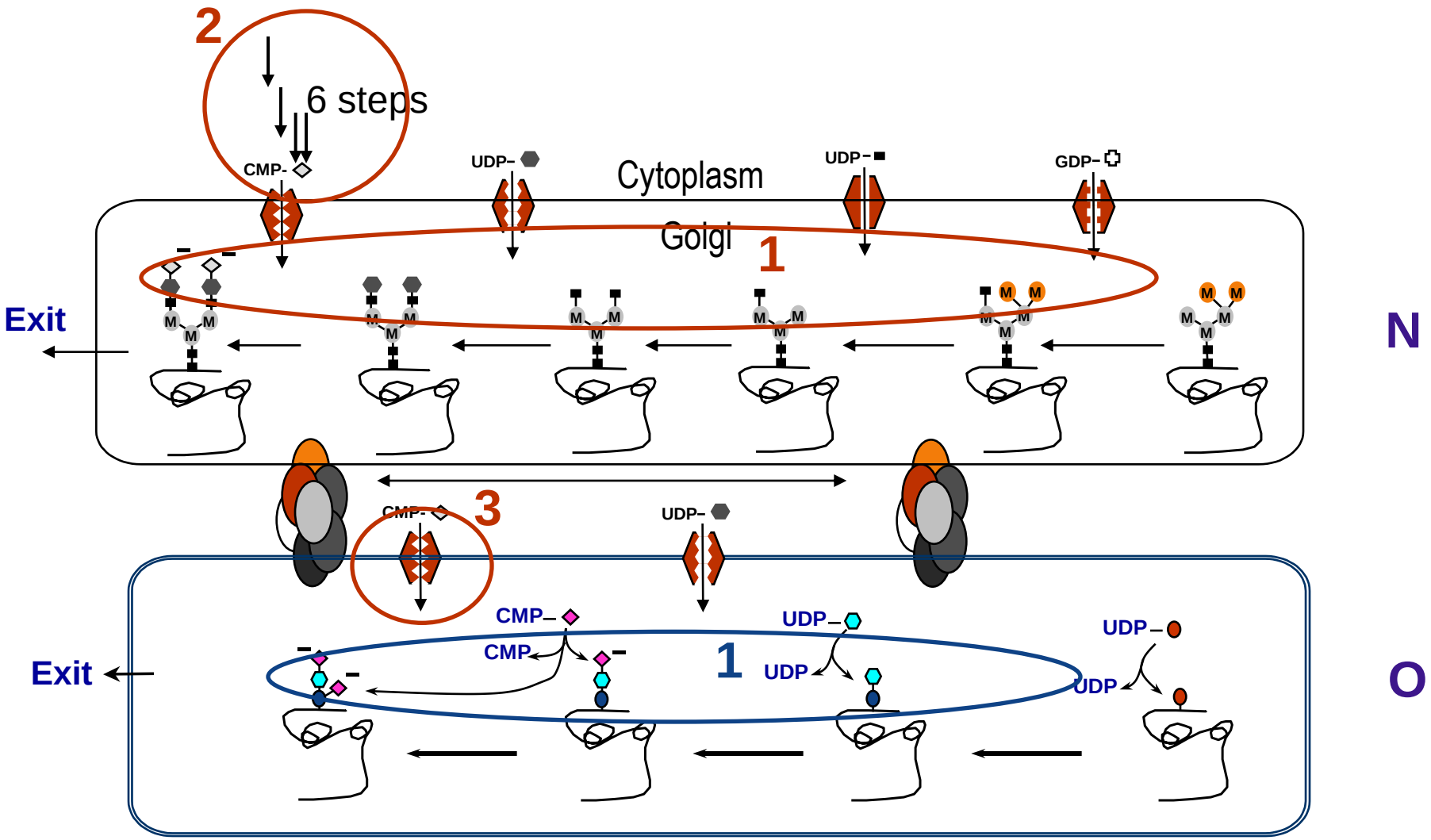
Profile types of ApoCIII in CDG type II patients



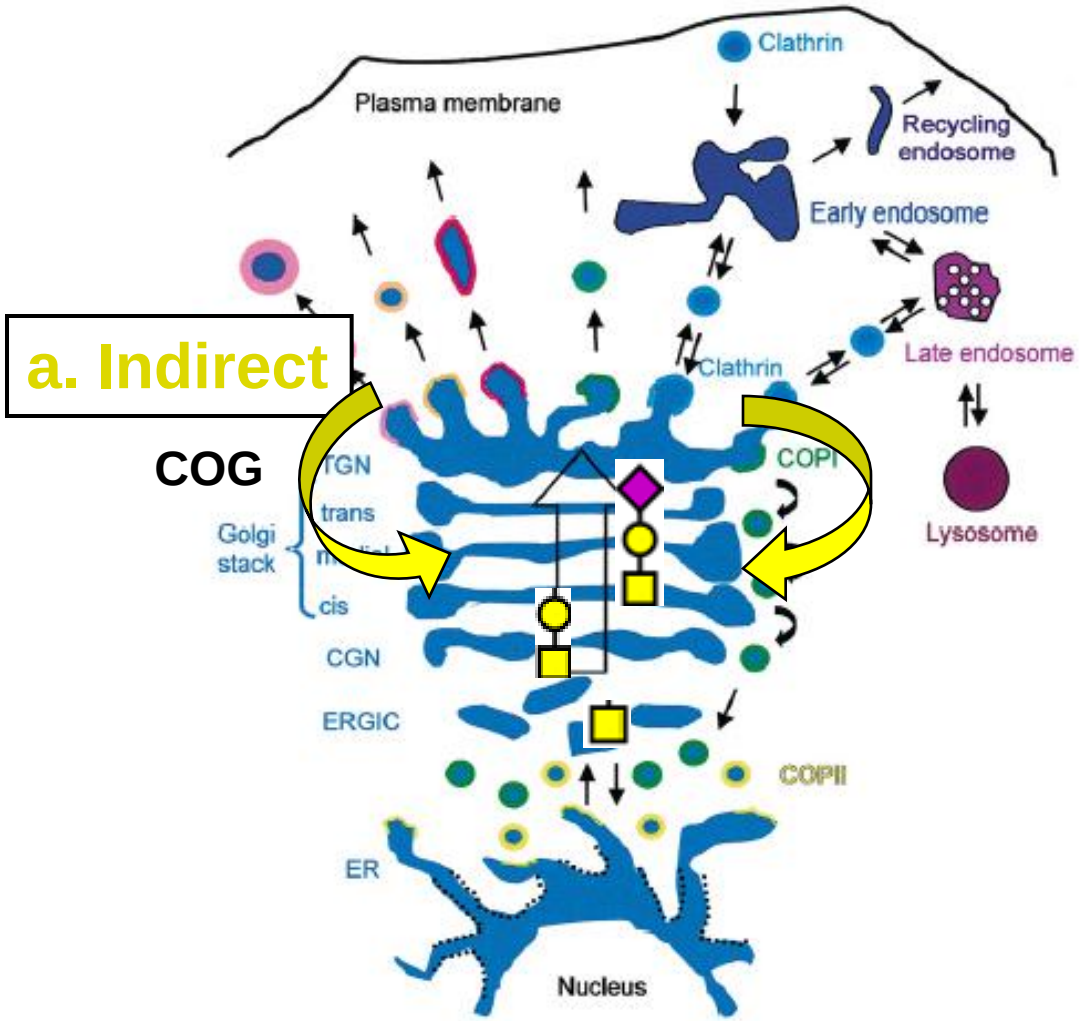
46 CDG-II: 15 N glycosylation
10 N+O glycosylation group 1
21 N+O glycosylation group 2

Wopereis, Glycobiology 2005

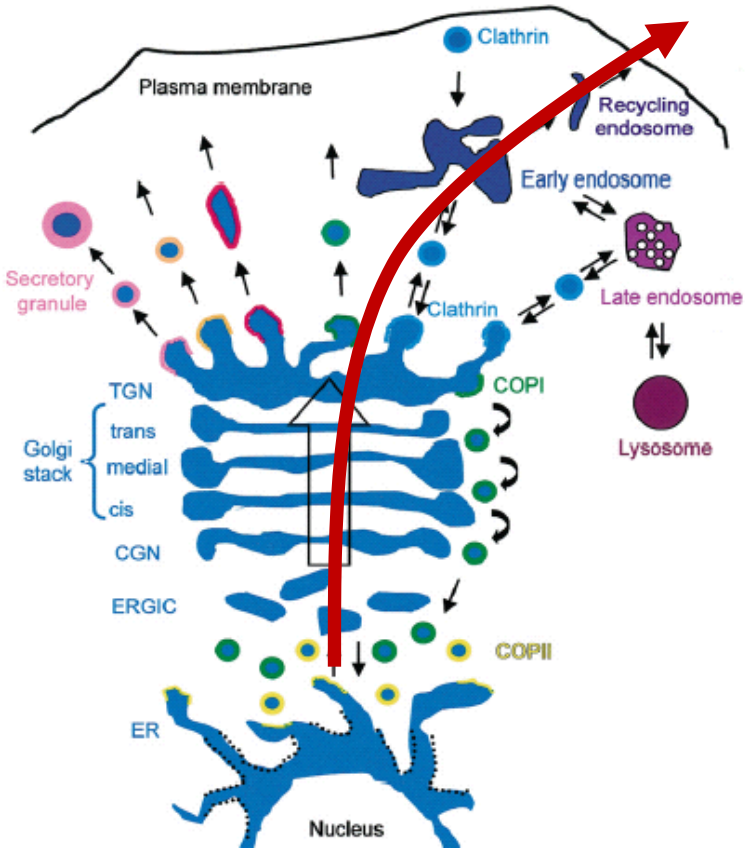
Options for a combined N+O glycosylation defect



Option 4: Trafficking in the secretory pathway



Golgi defects

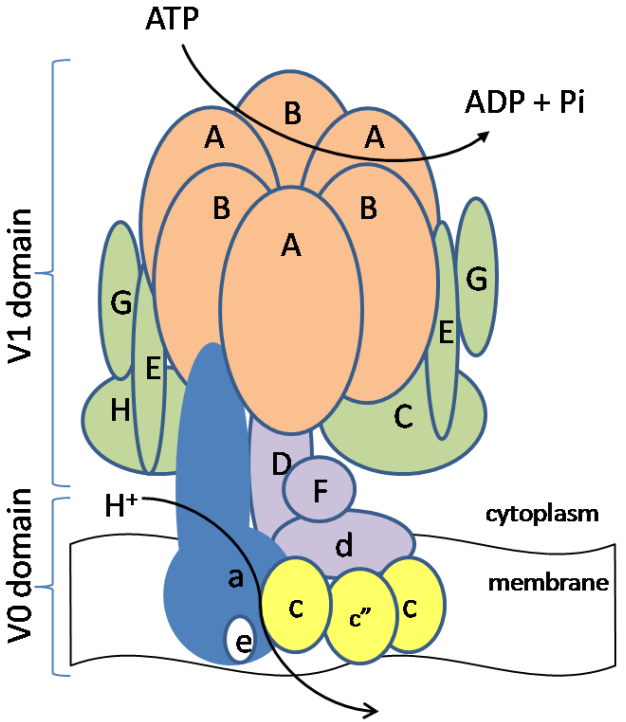


Conserved Oligomeric Golgi (COG) complex

- Transport between Golgi vesicles

Cutis laxa

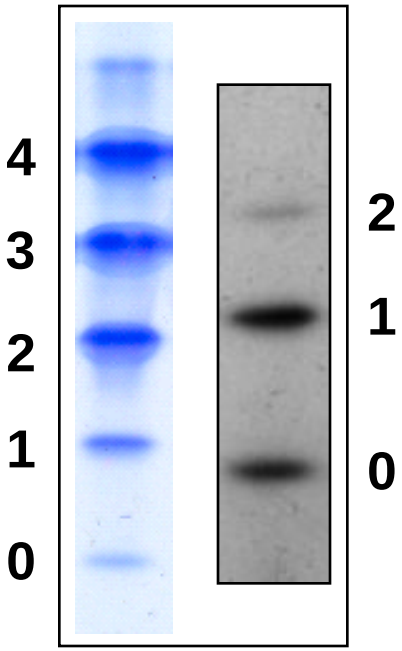
- ATPase defect influencing Golgi pH



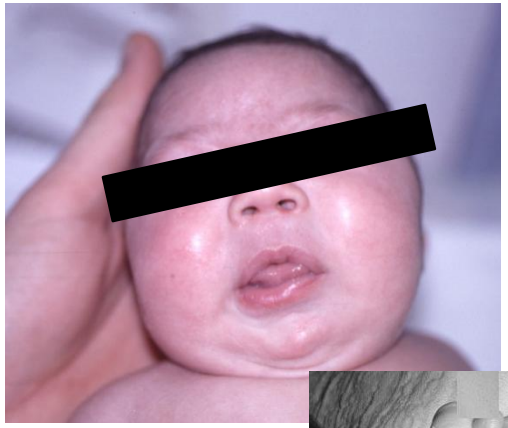
Group 1: COG defect in 5/10 patients

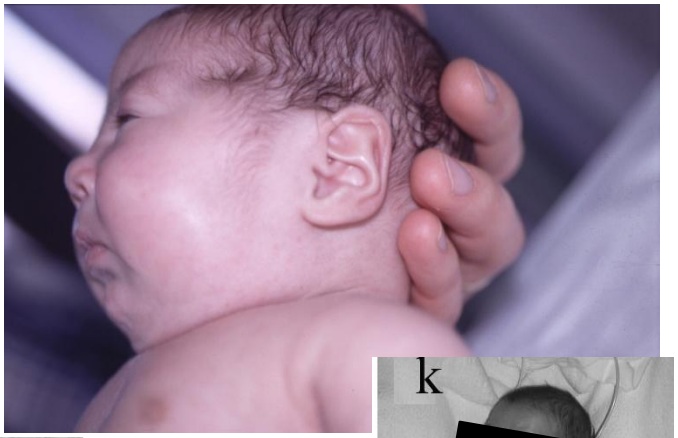
COG7:

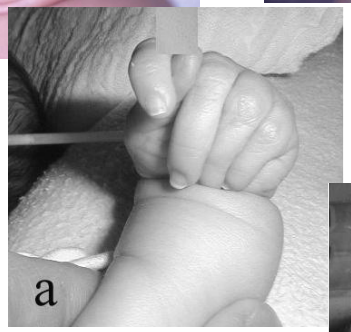
- Microcephaly, adducted thumbs, growth retardation, VSD, episodes of hyperthermia, early fatal

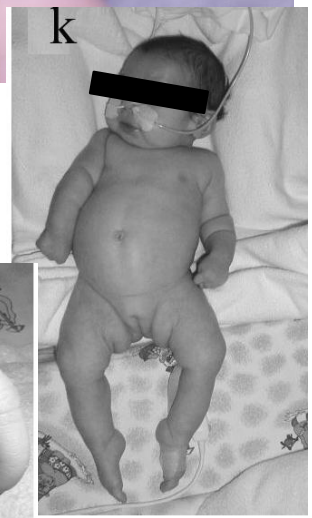


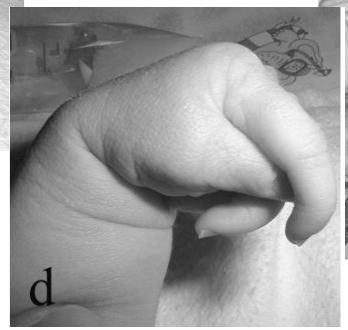
group 1: ApoCIII-0





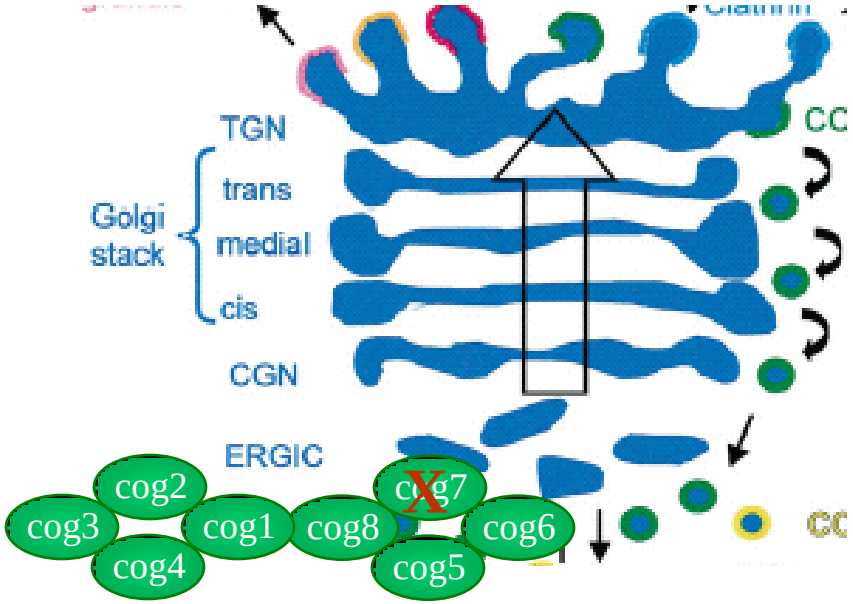


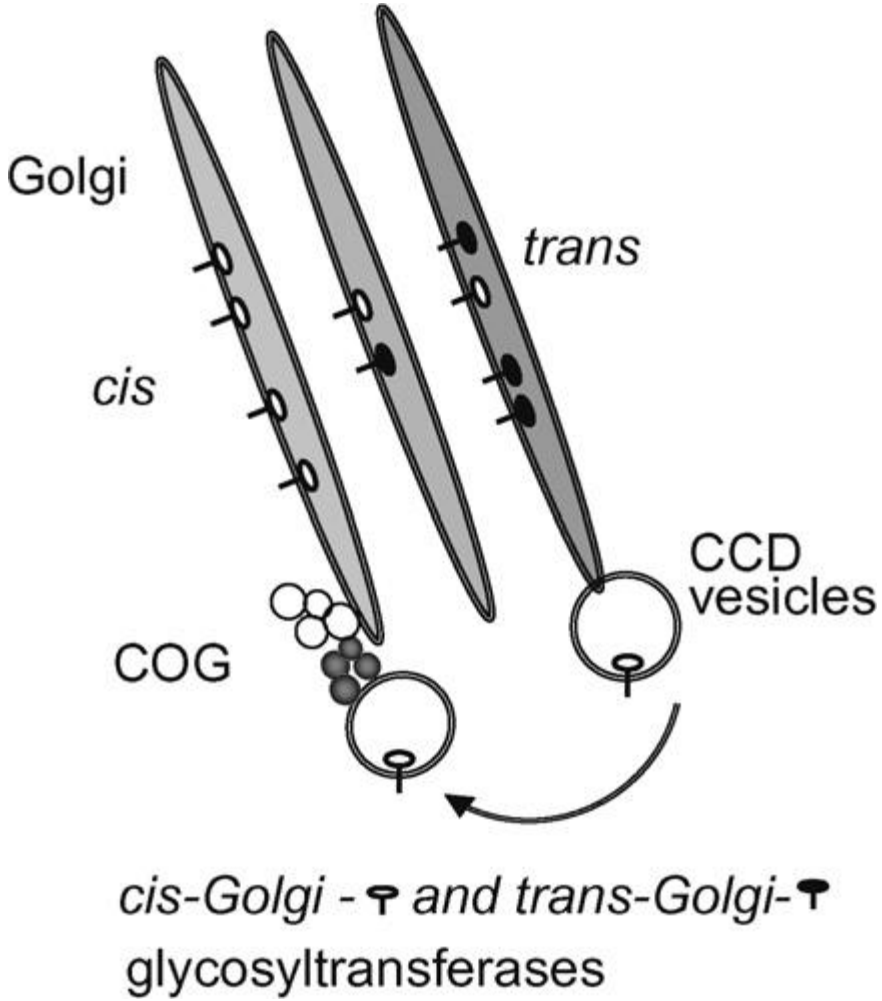




Morava J Hum Genet 2007

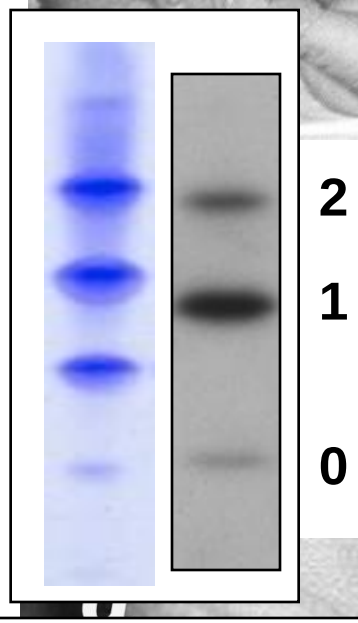
Model of COG complex



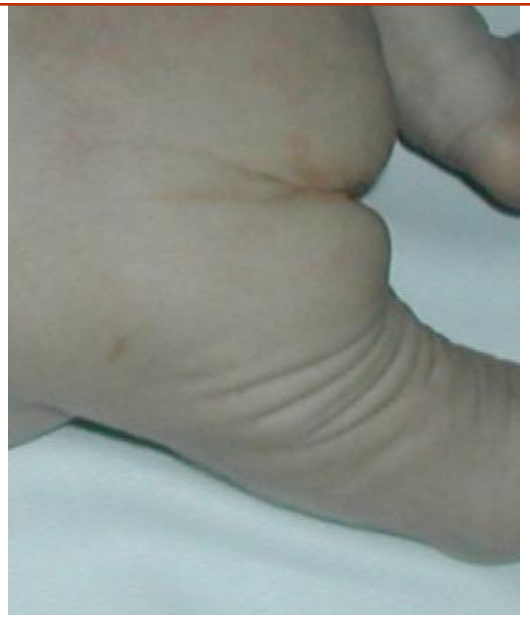


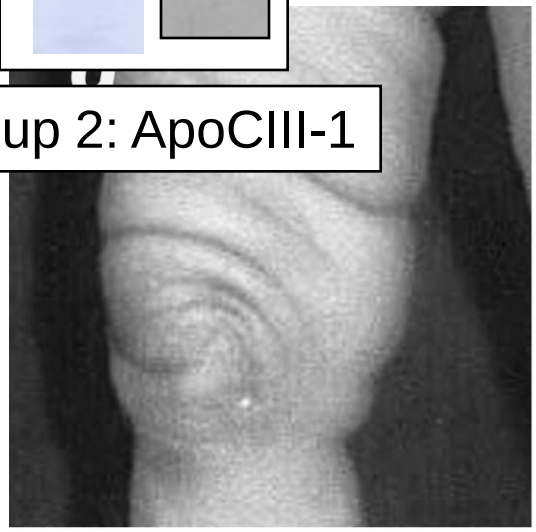
COG complex is required for recycling of glycosyltransferases

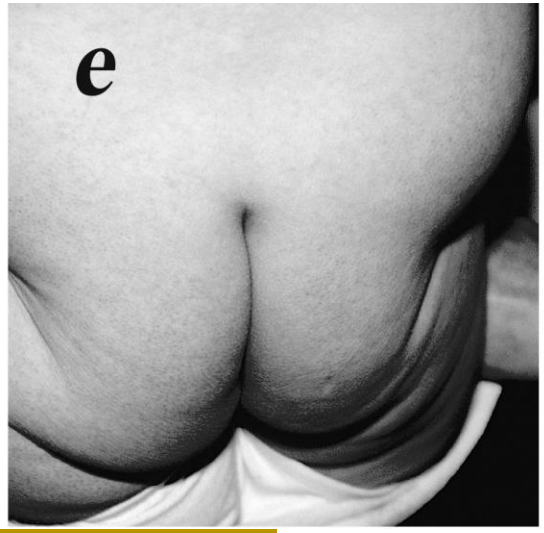
Group 2: 14/21 patients with cutis laxa phenotype

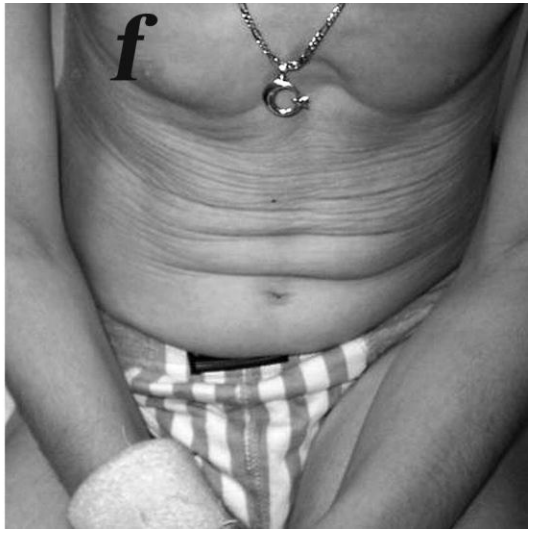


group 2: ApoCIII-1

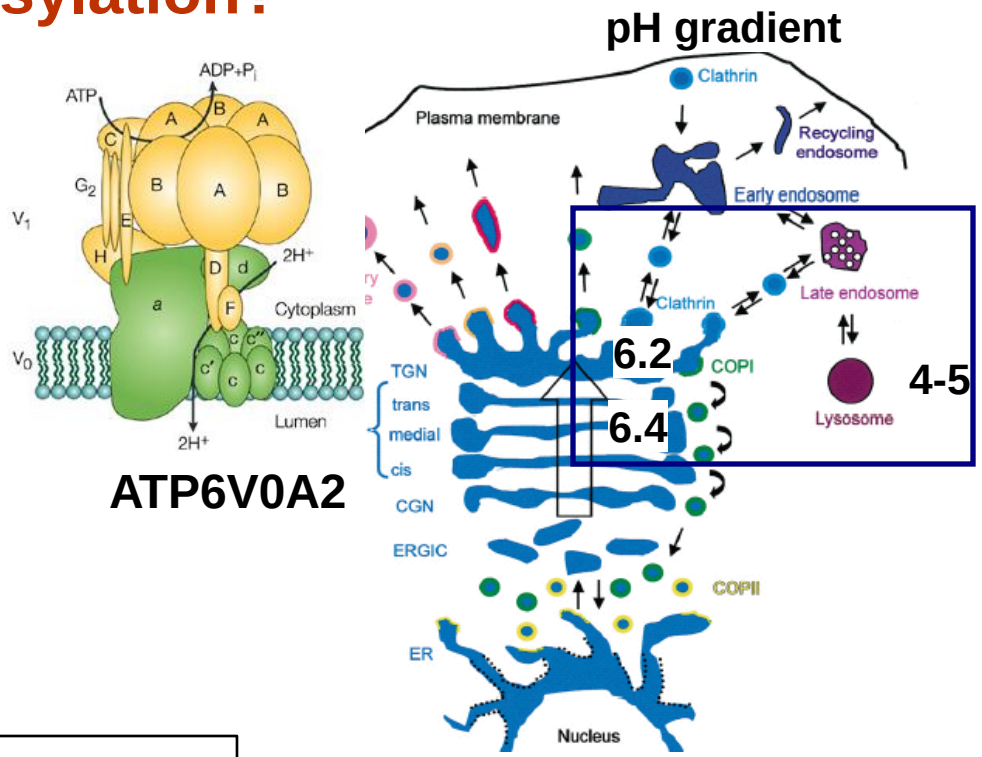








ATP6V0A2 and glycosylation?



- <6 months of age: isolated ApoCIII -1 profile
- Patients with normal ApoCIII exist

Step by step diagnostic approach for CDG

Stage 1:

- Interpretation of transferrin isofocusing gel
- Confirmation of generalized glycoprotein abnormality
- Exclude transferrin protein polymorphism
- Exclude secondary causes of N-glycan biosynthesis abnormalities
- Discriminate between CDG-I and CDG-II

Stage 2; CDG-I:

- PMM/PMI measurement; LLO analysis in fibroblasts
- Genetic tools & clinical information

Stage 2; CDG II:

- Check O-glycan abnormalities and N-glycan structure
- Genetic tools & clinical information