Congenital Disorders of Glycosylation:

Diagnostic steps

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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

- Liver (transferrin)
- Brain (MAG, P0, neurexin)
- 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
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, fructoseemia
  - 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**

**Endoplasmatic Reticulum**

**Cytoplasm**

**Trans Golgi**

**Median Golgi**

**Cis Golgi**

- **Ia** to **Ib**: Man-6-P → Man-1-P → GDP-Man → GDP-Man-6-P
- **Ic** to **Id**: UDP-Man → GDP-Man → GDP-Man-6-P
- **Ie** to **If**: UDP-Galactose → GDP-Galactose
- **Ig** to **Il**: UDP-N-acetylglucosamine → GDP-N-acetylglucosamine
- **Ih** to **Im**: UDP-Sialic acid → GDP-Sialic acid

**M** = dolichol-PP
**= dolichol**
**= GlcNAc**
**= mannose**
**= glucose**
**= galactose**
**= sialic acid**
**= fucose**
**= COG complex**
CDG patients with different profiles

Type I; ER defects

Type II; Golgi defects

4
2
0

control patient

patients

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

CDG-I
CDG-II
Walkers 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. Fructoseemia
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
Stage 2: CDG-I diagnostic work-up

Short LLO: normal

Lipid-linked Oligosaccharide (LLO) analysis
Stage 2: CDG-II diagnostic work-up

N-Glycan structural analysis

MGAT2
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 apolipoprotein C-III
Core 1 mucin type O-glycan in position Thr-94

Profile types of ApoCIII in CDG type II patients

Transferrin

ApoCIII

ApoCIII-0 profile

ApoCIII-1 profile

control

group 1

group 2

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

1. Options for a combined N+O glycosylation defect

2. 6 steps

3. Exit

Cytoplasm

GoIgi

M

M

N

O
Option 4: Trafficking in the secretory pathway

a. Indirect COG
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

Morava J Hum Genet 2007
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