In the name of GOD
CONGENITAL GLYCOSYLATION DEFECTS

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The CDGs, congenital glycosylation defects (formerly called Carbohydrate-deficient Glycoprotein disorders) was first described in 1980 by Professor Jaak Jaeken.

In 1999, only 6 CDG were known, and in these 10 years, increased rapidly.

This number has increased to more than 45 disorders.

Although CDGs have been known for more than 30 years, the pathophysiology of the CDGs identified thus far is widely unexplored.
Epidemiology

- CDG syndromes have a worldwide occurrence.
- All known CDGs have a recessive inheritance except EXT1/EXT2-CDG which is AD and MGAT1-CDG which is X-linked
- Incidence of CDG < 1 : 40 000 ?
- In recent years more than 900 proven CDG patients have been reported, most of them with CDG Ia (>600 cases) and CDG Ib (>30 cases).
- However, since 2009, most of the researchers use a novel nomenclature based on the name of the affected gene (e.g. CDG-Ia = PMM2-CDG, CDG-Ib = MPI-CDG).
**Introduction**

- “protein glycosylation” describes the post-translational linkage of oligosaccharide moieties onto proteins.
- Glycoproteins play an important role in protein stability, solubility, and polarity, and in biological processes such as growth & differentiation, organ development, signal transduction, immunologic defense, and hormonal activities.
- In man, the glycosylation machinery comprises more than 100 proteins in at least 40 steps.
- Near to 200 genes (near to 1% of whole genome) are involved in glycosylation!
**Classification**

- CDG disease family may be classified into protein and lipid-glycosylation disorders.
- The protein glycosylation are either N-linked (to the amide group of asparagin via an N-acetyl-glucosamine residue) or O-linked (to the hydroxyl group of serine or threonine via an N-acetyl-galactosamine, mannose, or xylose residue).
The synthesis of the N-glycans encompasses a much longer pathway than that of the O-glycans.

In humans, most protein glycosylation disorders are due to defects in the N-glycosylation pathway.

The remaining ones affecting the O-glycosylation disorders or combined N- & O-glycosylation pathways.
Pathophysiology

- Synthesis of N-Glycans proceeds in these stages:
  1- formation of nucleotide–linked sugars.
  2- assembly
  3- attachment
  4- processing

- Synthesis of O-Glycans involve assembly and attachment but not processing and mainly in Golgi apparatus.

- It forms a diversity of structures:
  - O-Xylosyl-glycans
  - O-Mannosyl-glycans
  - O-N-Acetyl galactosamine glycans

Cytosol & ER
ER & Golgi

N-glycan (ER)
N-glycan (Golgi)
O-GalNAc
O-Man
EGF O-Fuc
TSP O-Fuc
The N-glycosylation divided into two groups:

1) **CDG-I**; diseases caused by defects in the assembly of glycans and their attachment to proteins (occurring in the cytosol and the endoplasmic reticulum).

2) **CDG-II**; caused by defects in the processing of the glycans (in the ER and the Golgi).

The different diseases designated by small letters in the order of discovery of the basic defect. (like Ia, Ib,...)
Pathophysiology: N-glycosylation
Cytosolic pathway

1) Assembly:

- In the cytosol, the mannose donor, GDP-mannose, is synthesized from fructose 6-P, an intermediate of the glycolytic pathway.

- Dolichol formed from plastid- and mevalonate-derived IPP is used for the synthesis of Dol-P-Man, and used for synthesis of the glycan intermediate (Man$_5$GlcNAc$_2$-PP-Dol) in ER.
Pathophysiology: N-glycosylation ER pathway

- At the cytosolic side of the ER membrane, the oligosaccharide dolichol-PP-GlcNAc2Man5 is synthesized.
- Dol-P-Man and Man$_5$GlcNAc$_2$-PP-Dol are subsequently translocated to the ER lumen by unknown flippases.
- Dol-P-Man is subsequently used for the biosynthesis of GPI (Glycosyl-phosphatidyl-inositol) anchored proteins and assembled glycan Glc$_3$Man$_9$GlcNAc$_2$, (LLO)
Pathophysiology: N-glycosylation
ER Pathway

- The oligosaccharide chain is further elongated by different mannosyl- and glucosyl-transferases using dolichol-phosphate-mannose and -glucose as donor substrates.
- All reactions outside and inside the ER are catalyzed by different glycosyltransferases.

II) Attachment:
- The GlcNAc2Man9Glc3 is transferred to the nascent glycoprotein by an oligosaccharyl-transferase complex.

III) Processing:
- During the subsequent reactions, the oligosaccharide GlcNAc2-Man9Glc3 undergoes trimming of the three glucose residues and of one mannose residue.
Finally, the glycan of the newly formed glycoprotein is processed first in the ER, further in the Golgi, by trimming off six mannoses and replacing these with two residues each of N-acetylglucosamine, galactose, and sialic acid.

This process is crucial for the quality control of protein folding.

Newly synthesized glycoproteins are transferred to the Golgi apparatus by vesicular transport.

The shortened oligosaccharide is elongated by N-acetylglucosaminetransferases, Galactosyltransferases, sialic acid transferases, and fucosyltransferases before the mature glycoproteins are transported to their destination point.
**Diagnostics**

- The diagnostic work up for CDG should start with analysis of glycosylation pattern of Transferrin by Isoelectric focusing (TIF).

- 8 isoforms of TF exist: asialo-, mono-, di-, ..., octasialoTF

- the main physiological isoform is 2 glycans + 4 sialic acids residues tetrasioloTF

- Plasma –EDTA causes false positive results.

- Serum, CSF and DBS on Guthrie card, urine, or delipidated liver biopsies are suitable specimens for CDG screening.
**Diagnostics**

**The type 1 pattern (CDG-I):**
points to an assembly or transfer defect of the dolichol-linked glycan (in the cytosol or ER glycosylation pathway).
The type 1 pattern is characterized by a decrease of anodal (tetra-, penta-, hexa-) and an increase of the cathodal di- and a-sialotransferrins primarily indicating a loss of complete glycans (CDG-I).

**The type 2 pattern (CDG-II):**
indicates a processing defect after glycan transfer in the ER or during Golgi glycosylation.
The type 2 pattern shows also an increase of trisialotransferrins and/or monosialotransferrins, primarily indicating structurally abnormal glycans.
Diagnostics Pitfalls

• Limitations of these methods including:

• 1- false-negative results in very young individuals (fetal and neonatal period) and some proven CDG individuals

• Also there is false negative result in first 2 months of life and in high clinical suspicion it should be repeated after 2-3 months of age.

• **TIF is not applicable for PND due to false negative results.**

• 2-false-positive results in secondary glycosylation defects Galactosemia, Fructose intolerance, alcohol abuse, hemolytic-uremic syndrome, Hashimoto thyreoiditis, some non-specific seizures, Liver failure (liver cirrhosis, fibrosis, chronic active hepatitis, carcinoma), Hemochromatosis, and Cystic fibrosis, transferrin protein polymorphisms, bacterial infections, pregnancy, Estrogens, anti-epileptics, β-blockers, Low ferritin, High total transferrin, Strong hemolysis, Storage error
Diagnostics

• Broad spectrum of methods may be used for CDG screening and diagnostics:
  • CDTect assays (CDT-RIA, CDT-TIA, %CDT immunassays, %CDT-HPLC);
  • IEF (Tf, apo C-III)
  • Capillary zone electrophoresis (CZE)
  • Agarose gel electrophoresis, SDS-PAGE
  • Lipid-linked oligosaccharide (LLO) analysis,
  • HPLC, TLC,
  • MS/MS
  • MALDI-TOF
  • Enzyme essay
  • Genetic analyses
Clinical pictures of CDG

- CDG is an enormous challenge for clinicians, as CDG is often associated with significant morbidity and mortality, especially in early infancy.
- CDG may present with involvement of any organ system at any age to any degree of severity.
- Accordingly, CDG should be considered in every patient with an unexplained syndrome.
- On the other hand, CDG may mimic other metabolic diseases like mitochondriopathies.
- There are CDG that affect only one or a few organ systems, for example congenital muscle dystrophies in association with migration disorders of the brain.
Clinical pictures of CDG

- Most CDG are multisystem diseases comprising more or less severe brain involvement.
- In most CDG brain is involved, because glycans play essential roles among others in development, regeneration, and synaptic plasticity.
- Except for CDG-Ib all affect the brain besides many other organs.
- CDG screening should be considered in:
  - 1) any unexplained neurological syndrome, particularly when associated with other organ disease
  - 2) any unexplained syndrome even without neurological involvement.
Clinical pictures

• The main organ involvements are:

• **1-CNS involvements:** MR or NDD, hypotonia, cerebellar atrophy, epilepsy, Ataxia, neuronal migration disorders, stroke-like episodes, poly-neuropathy, microcephaly,

• **2-ophtalmologic symptoms:** abnormal eye movement, strabismus, optic atrophy, coloboma, retinitis pigmentosa, cataract

• **3-GI disorders:** protein-losing enteropathy, ascitis, cyclic vomiting, chronic diarrheha, hepatomegaly, hepatic like diseases, liver fibrosis with Gly or lipid vacuoles, pancreatitis.

• **4-skin manifestations:** Ichtyosis, inverted nipples, abnormal fat pads, progeria-like syndromes, cutis laxa, lipodystrophies

• **5-cardiac problems:** cardimyopathies, pericardial effusion, neonatal pericarditis
Clinical pictures

• **6-orthopedic problems:** osteopenia, **contracted joints, exostosis,** Neurosyndromatic radio-ulnar synostosis, protrusion of the thorax and/or kyphoscoliosis

• **7-hormonal imbalances:** retarded growth, **hypogonadism,** delayed or missing puberty, **hyperinsulinism**

• **8-Renal disease:** proteinuria, **congenital nephrotic syndrome,** micro cyst, proximal tubulopathies in neonatal period

• **9-hematologic problems:** thrombosis, **bleeding tendency,** phelbitis, Neurological syndrome with Bombay blood group, Congenital dyserythropoietic anemia type II or HEMPAS

• **10-General symptoms:** hydrops fetalis, FTT, dysmorphism, Recurrent infections, high leukocytosis, Neurological syndromes with episodic hyperthermia
Clinical pictures

The ABC of clinical symptoms in CDG

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Biochemical findings

- In CDG patients, abnormal biochemical findings include:
  1. Low plasma cholesterol
  2. Hypoalbuminemia
  3. Hypoglycemia with inadequately increased insulin production
  4. High activities of aminotransferases are typical of CDG Ib
  5. Disproportionate aminotransferase activity, i.e., increased AST and normal ALT, appeared characteristic for CDG subtypes II.
  6. Elevated plasma activity of lysosomal hydrolases, like aspartyl-glucosaminidase, β-hexosaminidase,…
  8. T3, T4, and rT3 are mostly subnormal.
**Biochemical findings**

- Increase:
  - Fibrinogen
  - Glycine (no ketones)
  - Transaminases
  - Lysosomal enzymes: β-hexosaminidase, glucosidase, glucosyltransferase
  - Tubular proteinuria
  - Intermittent trombocytosis

- Normal range:
  - APTT, PT
  - Haptoglobin

- Decrease:
  - Hypoproteinemia
  - Cholinesterase, β-glucuronidase
  - Clotting factors II, V, IX, X, XI, AT III
  - Protein C, S
  - TBG, T₃, T₄, rT3, ferritin, α₁-AT
  - Hypoglycemia
  - Hormones (PRL, GH, FSH)
  - Cholesterol

- Typical for CDG Ia
  - CDG Ib
  - CDG IIb

- Total TF
- Apolipoprotein
- TBG, AT

- CDT
- Increase for typical for CDG Ib and CDG IIb

- Newborn, infant, child, adolescent, adult
There are an increasing number of known specific syndromes have been identified as glycosylation disorders. Like:
- Hereditary multiple exostosis
- Congenital muscular dystrophies, Hereditary inclusion body myopathy
- Leukocyte adhesion deficiency type II,
- The allelic disorders hyper phosphatemic familial tumoral calcinosis and cortical hyperostosis with hyperphosphatemia, spondylocostal dysostosis type 3 and cutis laxa type II,
- Congenital olivopontocerebellar atrophy, hereditary ataxia
- Some syndromes like Joubert, Walker-Warburg, Malouf,
- Muscle-Eye-Brain Disease
- Dyserythropoietic anaemia type –II (HEMPAS)
CDG-la; clinical

All CDG-la patients showed mental retardation, hypotonia, cerebellar hypoplasia, and strabismus, but the classical hallmarks, i.e., the inverted nipples and fat pads, are not always present.
CDG-la; clinical

Normal

Cerebellar hypoplasia
CDG; Treatment

• Last but not least is treatment the most important issue for the patient!
• In most CDG types no specific therapy is available
• Progress is slow; only one CDG (CDG-Ib) is efficiently treatable.
• Because there is a combination of increased tendency of bleeding and thrombosis in patients with recurrent strokes ASA, 1 mg/kg/d recommended.
• Dehydration should be avoided especially during illness, anesthesia, and surgery.
• During such episodes, electrolytes, Glc, coagulation factors should be monitored.
• It is hoped that the synthesis of membrane-permanent derivatives of mannose 1-phosphate may mark the beginning of a treatment for the most common N-glycosylation disease, CDG-Ia.
Thank you for your patience