In the name of GOD
CONGENITAL GLYCOSYLATION DEFECTS BIOCHEMICAL APPROACH

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

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

This number has increased to more than 70 disorders.

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).

CDG syndromes have a worldwide occurrence.

The incidence of CDG 1: 20000 TO 1: 40 000?

All known CDGs have a recessive inheritance except EXT1/EXT2-CDG, which is AD and MGAT1-CDG which is X-linked.
Introduction

• About half of the body proteins contain carbohydrate chains essential for structure and function.
• The term “protein glycosylation” describes the post-translational linkage of oligosaccharide moieties onto newly synthesized proteins which is a post-translational process in all animals, plants, and even bacteria.
• Glycosylation affects a variety of physicochemical properties of proteins in terms of their stability, solubility, and polarity.
• Glycoproteins play an important role in biological processes such as growth & differentiation, organ development, signal transduction, homeostatic and immunologic process.
Congenital disorders of glycosylations (CDG) are rare genetic defects in the assembly, attachment, and processing of glycans in ER or Golgi apparatus.

In man, the glycosylation machinery comprises more than 100 proteins that are located in different cellular compartments such as the cytosol, endoplasmic reticulum, and Golgi apparatus.

At least 40 steps (enzymes) are required for the synthesis of oligosaccharides, transfer to polypeptide, and subsequent modifications.

About 200-300 genes (~1-2% of the human genome) are involved in glycosylation processes.
Classification

- CDG disease family may be classified into protein and lipid-glycosylation disorders.
- The glycans on proteins are either N-linked (to the amide group of asparagine via an N-acetyl-glucosamine residue) or O-linked (to the hydroxyl group of serine or threonine via an N-acetyl-galactosamine or a xylose residue).
- 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- and O-glycosylation pathways.
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-Acetylgalactosamine-
Classification

- The N-glycosylation pathway embraces three cellular compartments: the cytosol, the endoplasmatic reticulum (ER) and the Golgi.
- The protein N-glycosylation disorders have been 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,...)
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 = PMI-CDG).

According to the novel classification, CDGs are divided into:

1. Protein N-glycosylation
   - CDG Ia: 90% of all CDG
   - CDG Ib: 5%

2. Protein O-glycosylation

3. Lipid glycosylation and glycosylphosphatidylinositol anchor glycosylation

4. Defects in multiple glycosylation pathways and in other pathways

In addition, several CDGs of so far unknown etiology (CDG-x) have been recognized.
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.
- Dolichol is also used for the synthesis of the glycan intermediate (Man$_5$GlcNAc$_2$-PP-Dol).
N-glycosylation ER pathway

N-glycan biosynthesis in cytoplasm and endoplasmatic reticulum

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N-glycosylation Golgi pathway

Glycosylated proteins from ER

- Cis Golgi
  - UDP
  - High Mannose glycoconjugates
  - To lysosome
  - Hybrid structures

- Medial Golgi
  - UDP
  - MGAT2
  - COG-complex
  - COG-CDG

- Trans Golgi
  - UDP
  - SLC35C1
  - CDG-IId
  - ATP6VOA2
  - ATP6VOA2-CDG
  - B4GALT1
  - CDG-IId
  - Exit
Clinical pictures of CDG

- CDG may present with involvement of any organ system at any age to any degree of severity.
- 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.
- 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.
- 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.
Biochemical findings

In CDG patients, pathological results in common biochemical tests has been seen like:

- Abnormal liver function tests
- Low cholesterol
- Low cholinesterase activity
- Proteinuria
- Hypoalbuminemia
- Hypoglycemia with increased insulin production
- Increased AST with normal ALT (characteristic for CDG-II)
- High Glycine in plasma and CSF
- High ferritin
Biochemical findings

- Thrombocytopenia
- Elevated activity of plasma lysosomal hydrolases like aspartylglucosaminidase (CDG-I) and β-hexosaminidase in amniotic fluid (CDG-Ia)
- Altered the glycoproteins level in plasma like α1-antitrypsin, TBG, transferrin
- Subnormal T4, T3, and rT3.
- Low levels of numerous clotting factors including factors V, IX, II, X, anti trombin III, proteins S,C, and heparin cofactor II.
- The level of these factors increases with age and later stabilize.
**Biochemical findings**

- **Increase**
  - Fibrinogen
  - Glycine (no ketones)
  - Transaminases
  - Lysosomal enzymes: $\beta$-hexosaminidase, glucosidase, glucosyltransferase
  - Tubular proteinuria
  - Intermittent trombocytosis
  - Insulin

- **Decrease**
  - Hypoproteinemia
  - Cholinesterase, $\beta$-glucuronidase
  - Clotting factors II, V, IX, X, XI, AT III
  - Protein C, S
  - TBG, T₃, T₄, rT₃, ferritin, $\alpha_1$-AT
  - Hormones (PRL, GH, FSH)
  - Hypoglycemia

- Normal range

**Typical for CDG Ia**
- CDG Ib
- CDG IIb

- APTT, PT
- Haptoglobin

- Total TF
- Apolipoprotein
- TBG, AT

- CDT

**CDG Ia**

**CDG Ib**

**CDG IIb**

- Newborn
- Infant
- Child
- Adolescent
- Adult
Diagnostics Methods

- The diagnostic work up for CDG should start with analysis of glycosylation pattern of Transferrin by Isoelectric focusing (TIF).
- The isoelectric focusing needs to be performed on serum because EDTA plasma may cause false negative results due to iron chelation.
- 8 isoforms of TF exist: asialo-, mono-, di-, ..., octasialoTF.
- The main physiological isoform is 2 glycans + 4 sialic acids residues = tetrasialoTF.
Transferrin (Tf) is a glycoprotein known to be the most important iron-transporting protein in humans.

It consists of three substructural domains:
1) a single polypeptide chain,
2) two independent metal iron-binding sites (one within the N-terminal and the other within the C-terminal domain)
3) two N-linked complex glycan chains.

Thus, Tf is not a homogeneous molecule, but shows a distinct micro-heterogeneity, attributable to various Fe3+ loads, different N-glycan.
Diagnostics Methods

defects of synthesis lead to underglycosylation;

↓ tetrasialoTF

↑ (asialo- monosialo- + disialoTF)

control  CDG patients  control

4  2  0

normal  missing chain  altered processing

Sialic acid  N-acetylglucosamine
Galactose  Mannose
**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 2 pattern (CDG-II):**
indicates a processing defect after glycan transfer in the ER or during Golgi glycosylation.
Diagnostic Methods; IEF

• IEF is the most widely used method for CDG screening due to its low sample volume requirement (1µL), low cost, and ability to be performed in most laboratories.

• IEF is a method that separates the proteins according to their isoelectric points.

• With this technique the isoforms of Tf, TBG, Anti trombine-III, αAt, Hexosaminidase, haptoglobin, α1-acid glycoprotein, α2 antiplasmin, APOC-III plasminogene and … could be detected.

• Serum, cerebrospinal fluid and whole blood serum-dry spots on Guthrie-type filter paper are suitable specimens for CDG screening.

• Other suitable specimens include plasma, urine, or delipidated liver biopsies homogenates.
Diagnostic Methods; kits

- Bands of interest then is quantitated by densitometric scanning.
- Detection by immunofixation is performed immediately after IEF by exposure to anti-Tf antibody at room temperature and subsequent Coomassie Brilliant Blue or silver staining.
- A Tf IEF commercial kit is available now (Servalyte Precotes TM, Heidelberg, Germany).
Diagnostics Methods; CZE

- Serum TIF is the original and still most widely used method.
- However, it is a labor-intensive and time-consuming technique not suitable.
- Capillary electrophoresis (CZE) is another technique successfully applied in the determination of serum Tf isoforms.
- Determination of Tf by CZE with UV detection is a reliable and rapid method (15 min) which provide absolute concentration of sialo-Tf fractions. This method is suitable for automation, but it should be kept in mind that the profiles can show (CRP).
- This test is also normal in those CDG not accompanied by a deficiency of sialic acid.
Diagnostic Methods; SDS-PAGE

- In addition to IEF, SDS-PAGE (sodium dodecyl sulphate polyacrylamide gel electrophoresis) followed by Coomassie blue staining or Western blotting, also can detect Tf and other glycoproteins for screening of N-glycosylation defects and APOC-III for O-glycosylation disorders.

- The use of an antibody cocktail also could identify different glycoproteins (Tf, αAT, and haptoglobin).
More recently, the use of other charge separation methods and electrospray-mass spectrometry (ESI-MS) has proven valuable in detecting CDG defects.

Methods based on MS/MS analysis of the charged Tf isoforms using Ion-trap or TOF (time of flight) have recently been introduced.

By this method hypoglycosylated Tf are reliably identified and allows to discriminate CDG-I and CDG-II correctly.

There are different MS methods like MALDI-MS (matrix assisted laser desorption/ionization-MS), and SELDI-MS (surface enhanced laser desorption/ionization-MS) which are screening tools for altered glycoproteins.

ESI-MS of Tf is preferred screening method due to its sensitivity (10 µl sample volume), analytical speed (10 min per sample), and relatively simple operation.
Diagnostic Methods; MS

Transferrin capture

Mass spectrometry (TripleQ/QTOF)

Mild CDG-Ia
LLO, NLG and glycopeptide analysis could be differentiate the subtypes of CDG-II whereas the MALDI-MS or ESI-MS can not reliably show the defect in the sugar chain of intact glycoproteins.

In order to obtain structural data, the N-glycans enzymaticlly or chemically cleaved and with various procedures include SDS-PAGE, CZE, or LC-MS and preferably TOF-MS could be recognized.
Diagnostic Methods; Others

- Two dimensional (2-D) electrophoresis combines protein separation via charge (IEF) and molecular weight (SDS-PAGE) and help to study various glycoproteins.

- CDT may also be detected by high performance liquid chromatography (HPLC) and commercially available assays. HPLC analysis of Tf isoforms can be automated for large sample numbers but need large sample volume (200 µL), and higher cost.

- Thin layer chromatography can be used for analysis of urine oligosaccharides

- An abnormal tetrasaccharide band, Glc3Man is characteristic finding in CDG-IIb whereas the TIF is completely normal in blood and CSF.
For some rare CDG types with fucose defects (IIc and IIIf, not detectable by the sialyl-based Tf-IEF), the membrane abnormality is the only CDG screening marker.

Lost expression of some glycoproteins, such as blood-group antigens (Bombay phenotype, assayed by serological testing) on erythrocytes, or sialyl LewisX (CD15s) antigen on neutrophils (e.g. by flow cytometry using monoclonal antibodies) should be assessed.

Abnormal ApoC-III profile is indicative of combined O-glycosylation defect.

Unfortunately by these screening tests all CDG subtypes might be detected correctly.
The large genetic polymorphism of glycoproteins has to be taken into consideration.

False results in very young individuals (fetal and neonatal period) and some proven CDG individuals.

False-positive results in secondary glycosylation defects (Galactosemia, Fructose intolerance, alcohol abuse, haemolytic-uraemic syndrome, very young age), Hashimoto thyreoiditis, epilepsy, some non-specific seizures, Liver failure (liver cirrhosis, fibrosis, chronic active hepatitis, carcinoma), Hemochromatosis, and Cystic fibrosis.

Also false positive results are seen in pregnancy, Estrogens, antiepileptics, β-blockers, Low ferritin, High total transferrin, Strong hemolysis, Storage error.
Enzyme assay preferably in leukocytes for PMM and PMI confirm the diagnose of CDG-Ia and Ib.

The are false high PMM residual activity in rapidly dividing fibroblast. Fibroblast is suitable analysis other enzymes.

Molecular genetic methods play an important role in CDG diagnosis and if the functional mutation is found it would be confirmed at genomic level in the afflicted patient, parents, and sometimes in sibling.

PND is possible by enzyme assay for CDG-Ia and Ib or mutation analysis for other types.
**Diagnostics algorithm**

- **IEF Tf**
  - normal
  - abnormal
    - does not exclude CDG
      - exclude secondary CDG (galactosemia, alcohol abuse, hereditary fructose intolerance)
        - CDG type I
        - CDG type II
          - CDG-Ix
            - enzymatic analysis (PMM2 and MPI)
              - deficient
                - mutation analysis
                  - LLO analysis
                    - abnormal
                    - normal
                      - enzymatic analysis
                        - dolichol-phosphate +/- mutation analysis
                          - pathway defects?
                      - normal
                        - mutation analysis
                          - ApoC-III IEF
                            - normal
                            - abnormal
                              - complementary assay to IEF Tf (ATP6V0A2 gene, COG1-8 subunit genes)
Thank you for your patience