Failure to properly synthesize glycoproteins and other glycosylated molecules results in human disease

- mutations in genes encoding enzymes involved in glycoprotein biosynthesis: congenital disorders of glycosylation (CDGs)
Congenital Disorders of Glycosylation (CDGs)

- clinically heterogenous, autosomal recessive, hypomorphic

- first described in 1980 by Jaeken in Belgium as a multisystem disorder that presents in infancy

- clinical features include:
  1) developmental and neurological abnormalities
  2) hypotonia and axatia
  3) liver and renal failure
  4) cardiac insufficiency
  5) hematological and gastrointestinal complications
  6) skeletal manifestations

Type I - involve defects in the biosynthesis of the lipid-linked oligosaccharide precursor

Type II - involve defects in oligosaccharide processing
CDGs represent nearly all the genes in the lipid-linked oligosaccharide biosynthetic pathway.

_from_ Hauuptle MA, Hennet T. Hum Mutat. 2009 Dec;30(12):1628-41
CDGs are very rare but may be underdiagnosed

* nomenclature was recently changed to incorporate growing number of defects
Location of Defects in Known Type II CDG Cases

- CDGs caused by trafficking proteins (i.e. COG) are classified as Type II CDGs as are proteins involved in vesicle acidification (V-type ATPase subunits)

- the list of Type II CDGs will likely continue to grow as more proteins involved in Golgi function are identified as affecting glycosylation

*from* Essentials in Glycobiology, 2*nd* edition
Type I CDGs affecting sugar phosphate metabolism: CDG-Ia (PMM2-CDG) and CDG-Ib (MPI-CDG)

* both PMM and PMI deficiencies lead to less GDP-mannose, less oligosaccharide precursor, and, therefore, underglycosylation of proteins; clinical features are distinct
Known mutations in PMM2 – the cause of CDG-Ia
Type I CDGs affecting dolichol sugar metabolism: CDG-Ie (DPM1-CDG) and CDG-Io (DPM3-CDG)

- due to defects in making Man-P-Dol, LLO with only 5 Man residues accumulates

- Man5 can be transferred to proteins but not nearly as well as Man9 (Man5 also lacks the glucose residues needed for proper folding of glycoproteins)

- these defects will also affect O-mannosylation and GPI biosynthesis
Dolichol-P-mannose synthase is a oligomeric complex of three subunits (DPM1-3)

- the one DPM3 patient is more mildly affected compared to the DPM1 patients
Type I CDGs affecting dolichol linked oligosaccharides: CDG-If (MPDU1-CDG) and CDG-In (RFT1-CDG)

* the ability to utilize Man-P-Dol and Glc-P-Dol is compromised in CDG-If; this was the first example of a protein involved in the use (not the biosynthesis) of oligosaccharides
Defects in Dolichol Biosynthesis Can Also Cause CDG-like Disorders

from Cantagrel et al. “SRD5A3 is required for converting polyprenol to dolichol and is mutated in a congenital glycosylation disorder” Cell 2010 Jul 23;142(2):203-17
Traditional Diagnostic Platform for CDGs

**IN THE CLINIC**

- CDG Patient
- Clinical Features
- Transferrin Analysis (Tf)

**IN THE LAB**

- Enzymatic Assays
- Structure of Tf Glycans
- $^3$H-Mannose metabolic label
- analyze glycans, LLO, small molecule precursors

Possible defects

Sequence one or more cDNAs

**FUNCTIONAL ANALYSIS**

- Over-express and determine activity
- Complement glycosylation-defective yeast or mammalian cells
Isoelectric focusing of transferrin, which measures the total charges (sialic acids) present on the two biantennary oligosaccharides, is the most common diagnostic method for identifying CDG patients.
Why Aren’t Fibroblasts Always Useful for CDG Diagnosis?

- besides lymphoblasts, dermal fibroblasts are the only available cell type from CDG patients

- these cells can be used to aid in the diagnosis of specific CDG subtypes but often don’t exhibit the same glycosylation defects that are seen in hepatocyte-derived glycoproteins

- mechanisms that govern cell type-specific differences in glycosylation defects are poorly understood
- identification of new CDG causes has increased significantly through the use of whole exome sequencing technology

- these newly identified defects expand the proteins and enzymes that impact N-glycosylation

- geneticists were necessary looking for new CDGs

- ALG14 and ALG2 identified as novel genes in which mutations cause a congenital myasthenic syndrome
- Congenital myasthenic syndromes are heterogeneous, inherited disorders that arise from impaired signal transmission at the neuromuscular synapse and fatigable muscle weakness
- Through analogy with yeast, ALG14 is thought to form a multiglycosyltransferase complex with ALG13 and DPAGT1 that catalyses the first two committed steps of asparagine-linked protein glycosylation

DPM2-CDG: a muscular dystrophy-dystroglycanopathy syndrome with severe epilepsy

A novel congenital disorder of glycosylation type without central nervous system involvement caused by mutations in the phosphoglucomutase 1 gene
J Inherit Metab Dis. 2012 Sep 14
Oligosaccharyltransferase-subunit mutations in nonsyndromic mental retardation
Am J Hum Genet. 2008 May;82(5):1150-7

A defect in the TUSC3 gene is associated with autosomal recessive mental retardation
Am J Hum Genet. 2008 May;82(5):1158-64

DDOST mutations identified by whole-exome sequencing are implicated in congenital disorders of glycosylation
Pathophysiology of CDGs

- not much known to date regarding pathology in affected tissues

- presumably the underglycosylation of proteins leads to their reduced function or stability (i.e. blood clotting factors) and causes disease

* one vs. many?
* function vs. stability?
* pecking order for glycoproteins?

- N-linked glycosylation is especially important in the spatiotemporal development of the brain, hence, most CDG patients have mental retardation
Other Factors Influencing Pathology of CDG

- different steps in oligosaccharide precursor biosynthesis may be rate limiting at different times or under different environmental conditions
- regulation of enzymes in LLO biosynthesis poorly defined (phosphorylation, etc.)
Involvement of ER Stress and the Unfolded Protein Response in Type I CDG Fibroblasts

Extension of lipid-linked oligosaccharides is a high-priority aspect of the unfolded protein response: endoplasmic reticulum stress in Type I congenital disorder of glycosylation fibroblasts
Shang J, Körner C, Freeze H, Lehrman MA.
Glycobiology. 2002 May;12(5):307-17

* Type I CDG cells have chronic ER stress
* compared with three other well-known UPR aspects (transcriptional activation, inhibition of translation, and cell death), LLO extension was the most sensitive to ER stress; and (2) Type I CDG cells had a mild form of chronic ER stress in which LLO extension was continuously stress-activated, but other aspects of the UPR were unchanged

Genome-wide analysis of the unfolded protein response in fibroblasts from congenital disorders of glycosylation type-I patients
Lecca MR, Wagner U, Patrignani A, Berger EG, Hennet T.

* tunicamycin elicited a strong transcriptional response typical for the UPR, CDG fibroblasts displayed a qualitatively similar yet moderate induction of genes encoding components of the UPR
* among these genes, the PERK kinase inhibitor DNAJC3/P58(IPK) gene showed the highest induction throughout all CDG-I types tested; paralleled by elevated expression of genes involved in amino acid biosynthesis and transport
Modifiers of the CDG Phenotype

- one puzzling feature of CDGs is that the phenotypic expression of the same mutation can have widely variable impact, even among affected siblings

- most likely explanation is differences in the genetic background

- a very frequent single-nucleotide polymorphism (SNP) in ALG6, the cause of CDG-Ic, has a barely discernible effect on glycosylation of a model protein in yeast and yet when examined in CDG-Ia patients (PMM2 deficiency), the SNP is twice as frequent in severe cases relative to mild cases

- a knockout mutation may be lethal in one highly inbred mouse strain, but not in another because compensatory pathways may exist

- the synergism of multiple simultaneous or sequential environmental insults on genetic insufficiencies may create a cascade leading to overt disease.
Selective Advantage of Decreased Glycosylation?

- the occurrence of PMM2 mutations noticed that the p.R141H mutation is very prevalent in the European population with a carrier frequency of about 1/70

- high frequency of mutations likely due to advantage of not having full glycosylation

- hepatitis viruses depend on N-glycosylation of their coat proteins to form infectious virions; modest decreases in oligosaccharide processing of a coat protein is enough to drop viral titers by nearly 100-fold, host not affected
Treatment Options for CDGs are Limited

- sugar supplementation is the only approved treatment for CDGs but its application is limited

-> adding mannose to certain Type I CDG fibroblasts can often correct the defect (drives the forward reaction)

-> only CDG-1b (PMI deficiency) responds to oral mannose therapy

-> CDG-IIc (GDP-fucose transporter deficiency) patients also respond to oral fucose therapy

Why doesn’t mannose supplementation work for all type I CDGs?
Treating CDGs by Increasing Substrate Flux

Type I CDGs

- Mannose
  - Mannose
  - Man-6-P
  - Man-1-P
  - GDP-Man
  - Underglycosylation of proteins

- MPI
  - CDG-Ib

- PMM2
  - CDG-Ia

- Glycolysis
  - Fru-6-P
  - Man-6-P
  - Man-1-P
  - GDP-Man

CDGs + flux-based therapies

- Mannose
  - metformin
  - MPI inhibitors
  - PMM2 activators

- Glucose
  - Fru-6-P
  - Man-6-P
  - Man-1-P
  - GDP-Man

- dolichol
  - Man-P-Dol
  - GDP-Man
  - LLO

- Increased protein glycosylation
  - zaragozic acid A

- LLO
Diversion of Lipids From Cholesterol to Dolichol as a Therapeutic Approach for CDG

Mouse Models of Type I CDG

- complete KO of pmm2 is embryonic lethal (E2.5-3.5) - *Thiel et al.*, *Mol Cell Biol* 2006

- knock-in of the common R141H human mutation into the pmm2-null background results in embryonic lethal

- knock-in of another common pmm2 mutation (F119L) resulted in normal mice

- when carrier mothers are fed mannose during pregnancy, pmm2 KO embryos survive longer (~ E6.0)
- complete KO of mpi is embryonic lethal (E11.5) - *DeRossi et al., J Biol Chem* 2006

- mannose supplementation actually increases lethality of these embryos

- added mannose accumulates as Man-6-P, inhibits glucose metabolism and depletes cellular ATP via “honeybee effect”