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

- Mutations in genes encoding enzymes involved in glycoprotein biosynthesis: congenital disorders of glycosylation (CDGs)

- Mutations in genes encoding enzymes involved in the catabolism of glycosylated molecules: lysosomal storage disorders (LSDs)

- Defects in mannose 6-phosphate biosynthesis: mucolipidosis II (MLII) or I-cell disease (both a CDG and LSD)
Congenital Disorders of Glycosylation (CDGs)

- clinically heterogenous, autosomal recessive, hypomorphomic

- 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* Haeuptle 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

Possible defects

Sequence one or more cDNAs

FUNCTIONAL ANALYSIS

Over-express and determine activity

Complement glycosylation-defective yeast or mammalian cells

$^3$H-Mannose metabolic label
analyze glycans, LLO, small molecule precursors
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
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
- MPI
- CDG-Ib
- PMM2 CDG-Ia
- Man-1-P
- GDP-Man
- Underglycosylation of proteins

**CDGs + flux-based therapies**

- Mannose
- metformin
- MPI inhibitors
- CDGs + flux-based therapies
- Man-6-P
- Fru-6-P
- Glycolysis
- PMM2 activators
- GDP-Man
- LLO
- Increased protein glycosylation

**Treat CDGs by Increasing Substrate Flux**

- Zaragozic acid A
Diversion of Lipids From Cholesterol to Dolichol as a Therapeutic Approach for CDG

from Improvement of dolichol-linked oligosaccharide biosynthesis by the squalene synthase inhibitor zaragozic acid
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 mutation into the pmm2-null background results in normal mice

- knock-in of another common pmm2 mutation (F119L) was embryonic lethal

- 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”
Overview of Lysosomal Storage Disorders

- group of roughly 40 disorders typically caused by defects in lysosomal enzymes and proteins

- characterized by the intralysosomal accumulation of various macromolecules, including glycolipids, GAGs and glycoproteins

- many of these diseases represent genetic mutations that affect glycosidase enzymes responsible for the breakdown of oligosaccharides in lysosomes; these enzymes are monosaccharide- and linkage-specific

- overall incidence worldwide: 1 in 5000 live births

- many lysosomal storage disorders were identified in the early 20th century but molecular bases not defined until much later
Overview of Lysosomes and Lysosomal Biogenesis/Function

- small intracellular vesicles found dispersed throughout the cytosol

- enriched with acid hydrolases that degrade proteins, sugars, DNA; involved in digestion of both extracellular and intracellular components

- glycoproteins and glycolipids are retrieved from the cell surface by endocytosis and sent to lysosomes for recycling

- acidic pH; created by resident proton (H⁺) pumps

- formed by maturation of late endosomes into lysosomes

- related structures include secretory lysosomes that are subject to fusion with the plasma membrane during membrane repair and for specialized functions within the immune system
The Etiology of Lysosomal Storage Disorders is Diverse

- hydrolytic enzymes – glycosidases, proteases
- enzymes that aid in targeting hydrolases to lysosomes – M6P targeting
- enzymes that modify/activate hydrolases – SUMF1
- integral membrane proteins that support lysosomal integrity and formation – LAMP2
- metabolite/ion transporters – cholesterol efflux, amino acid and monosaccharide transport
- Niemann-Pick type C proteins involved in the transport of cholesterol out of the lysosome

- galactosialidosis caused by defects in cathepsin A (also called protective protein); forms multimeric complex that helps target and protect neuraminidase and β-galactosidase during transport to lysosomes
Clinical Features of Lysosomal Storage Disorders

- highly variable depending on type of disorder (most LSDs have mild subtypes)

- most have neurological involvement (i.e. severe mental retardation, neurodegeneration, etc.)

- skeletal abnormalities and muscular weakness common

- kidney failure, stiffness of joints, enlarged livers and spleens

- corneal clouding

- severe forms result in death before two years

- late-onset or adult forms are milder (often find residual enzyme activity)
Pathogenesis of Lysosomal Disease: more than just storage?

- impact of intralysosomal storage
- effects on intracellular trafficking
- activation of secondary biochemical and cellular pathways
- changes in gene expression
Biogenesis of Lysosomal Enzymes

- most lysosomal enzymes (i.e. cathepsin D) are synthesized as *preproenzymes*

  the prepiece refers to the signal sequence that directs the enzyme into the lumen of the ER

  the propiece may have several functions:
  1) keeps protease inactive until it is sorted (don’t want an active protease eating away at secretory cargo!)
  2) cleavage of propiece may lead to stabilization of enzymes in the acidic lysosomal environment
  3) propiece may contain sorting information
Targeting of Lysosomal Enzymes is Mediated by Sugars

- lysosomal enzymes (as well as membrane glycoproteins and secretory proteins) undergo co-translational glycosylation of specific asparagine residues

* oligosaccharides on secretory and membrane glycoproteins processed to complex-type

* oligosaccharides on lysosomal enzymes processed to high mannose structures with mannose-6-phosphate residues

*these Man-6-P residues serve as the essential component for targeting of the enzymes to the lysosomes; first example that sugars contain “information”*
Mannose 6-Phosphate (M6P) Biosynthesis Occurs Via a Two-Step Pathway in the Golgi

- GlcNAc-1-phosphotransferase is a heterohexameric enzyme encoded by two genes (GNPTAB and GNPTG)
- GNPTAB is processed into the alpha/beta subunits (responsible for catalysis) and GNPTG encodes the gamma subunit (function unclear)
Basis for GlcNAc-1-phosphotransferase Specificity

- evidence suggests that phosphotransferase recognizes a three-dimensional protein domain common to all lysosomal enzymes

  1) heat-denatured or proteolyzed lysosomal enzymes do not bind to phosphotransferase

  2) isolated high mannose oligosaccharides are also poor substrates (protein-protein interaction)

- although gamma subunit of phosphotransferase was originally believed to mediate specificity, current data suggests that the alpha/beta subunit serves this functions along with catalysis
Defects in Mannose 6-Phosphate (M6P) Biosynthesis Result in Mucolipidosis II and III

- developmental delay
- craniofacial abnormalities (course facial features)
- cardiac defects and upper respiratory infections
- abnormal skeletal development and restricted joint movement

Some bone and cartilage phenotypes are noted at birth, reflecting defects in early development; others are rapidly progressive in early childhood
Lysosomal enzymes lacking mannose 6-phosphate on their N-linked oligosaccharides are secreted from the cell.
Therapeutic Options for Lysosomal Storage Disorders
Basis for Enzyme Replacement and Gene Therapy Approaches

- enzymes can be made in the lab or expressed in transduced tissues

- if glycans are modified with M6P residues, circulating enzymes can be picked up by other organs due to the fact that some M6P receptors are present at the cell surface

- enzymes are then delivered to the lysosome, where they can replace the defective protein

Caveats: M6P-modified enzymes are too efficiently cleared by M6P and mannose receptors in the liver and not available to other tissues; all transduced tissues may not produce M6P-modified enzymes
Basis for Pharmacological Chaperone Therapy

- unlike enzymes, these molecules can easily cross the blood-brain barrier

Caveats: molecules are potent inhibitors of the enzymes so they must be released in lysosomes to work!

### Basis for Substrate Reduction Therapy

#### Diseases with Primary GSL Storage
- Gaucher types 1, 2 and 3
- Fabry
- GM2 gangliosidoses (Tay–Sachs and Sandhoff)
- GM1 gangliosidosis

#### Disease with Secondary GSL Storage
- Niemann–Pick type C

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![Chemical reaction diagram](image)

Caveats: inhibition of GSL biosynthesis can affect normal function of tissues.