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REVIEW ARTICLE

Congenital Disorders of N-Glycosylation Including Diseases Associated With O- as Well as N-Glycosylation Defects

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ABSTRACT: The congenital disorders of N-glycosylation (CDG), a steadily increasing group of multi-systemic disorders, have severe clinical implications in infancy and early childhood. The various inborn errors responsible adversely affect N-glycosylation of lysosomal proteins because of either failing assembly of lipid-linked (LL) oligosaccharides (OS) in the endoplasmic reticulum, CDG Type I, or faulty processing of the asparagines (N)-linked OS in the ER and in the Golgi, CDG Type II. The overlap of phenotypes precludes specific clinical delineation. Isoelectric focusing (IEF) of plasma transferrin remains a valuable, albeit imperfect, screening tool. IEF of plasma ApoC-III protein, introduced O-glycosylation defects that delineated some new CDGs due to mutations of both N- and O-glycosylation. Only CDG-Ib is amenable to treatment with free mannose supplementation. Hence, early specific diagnosis of any one entity is crucial for genetic counseling and elective preventive measures. **(*Pediatr Res* 60: 643–656, 2006)**

The multi-system disorders previously termed the carbohydrate-deficient glycoprotein syndromes have been renamed congenital disorders of glycosylation (CDGs) (1). In most, either the assembly or the processing of N-linked oligosaccharides is adversely affected by mutation. The ensuing OS deficiency results in incomplete utilization of the glycosylation sites in proteoglycans. The large majority of patients have a multi-system disorder with the CNS, often the main site of clinical involvement.

The CDGs have been subdivided into disorders of N- and of O-glycosylation. Diseases due to defects in mannose-α-O-glycosylation are not the subject of this review. In N-glycosylation defects, if O-glycosylation deficiency has been documented the subject will be addressed. Classic disorders of N-glycosylation are due to enzyme defects either in the assembly stage or in the subsequent trimming stage of the OS. The co-translational assembly of the OS or glycan structure occurs in the ER. Trimming and maturation start off in the ER and follow a divergent course in the Golgi cisterns. The Golgi apparatus is the intracellular site of O-glycosylation, a sequential post-translational process.

N-GLYCOSYLATION DEFICIENCY SYNDROMES DUE TO OS ASSEMBLY DEFECTS

N-glycosylation of proteins is conserved in eukaryotes. Oligosaccharide (OS)-type glycans are N-linked to proteins by the covalent amide bond between asparagine (Asn) in specific amino acid sequences (called sequons) in the nascent polypeptides, and the N-acetylglucosamine (GlcNAc) residue at their reducing end. The oligosaccharide to be transferred to nascent polypeptides is assembled in a step-by-step fashion while linked to dolichyl-phosphate (Dol-P-), an oligoisoprenyl type phosphorylated lipid, embedded in the ER membrane. Most of the already delineated congenital disorders of N-glycosylation are the result of mutations in genes encoding enzymes catalyzing either a monosaccharide interconversion or activation step, or functioning as monosaccharide transferases in the OS assembly. Because the defects result in hypoglycosylation of proteins, they are associated with a common abnormal profile obtained by plasma transferrin (Tf) isoelectric focusing (IEF) called the Type I Tf-IEF profile. The disorders associated with it are termed CDG-Type I. The Type II Tf-IEF profile is an inconsistent feature of the second group of CDGs as in half of them the normal plasma Tf-IEF profile is obtained. A few newly characterized types of CDG-II are associated with abnormal intra-Golgi enzyme distribution and their ineffective and uncoordinated action of deficient N- as well as O-glycosylation is the result.

Co-Translational Assembly of the Major OS; Transfer to Nascent Proteins

N-glycosylation has been studied extensively in the budding yeast, *Saccharomyces cerevisiae* (*S. cer.*). Findings in

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Abbreviations: CDG, congenital disorder of glycosylation; **CHO cells,** Chinese hamster ovary cells; **CMP,** cytidine monophosphate; **COG,** complex conserved oligomeric Golgi complex; **(DD-) OST,** (dolichyl-diphospho-) oligosaccharide protein glycosyl transferase; **Dol-P (-PP),** dolichyl-phosphate (-pyrophosphate); **GDP,** guanosinediphosphate; **GlcNAc,** N-acetylglucosamine; **IEF,** isoelectric focusing; **MIM,** Mendelian inheritance in man; **OS,** oligosaccharide; **Tf,** transferrin; **UDP,** uridinediphosphate

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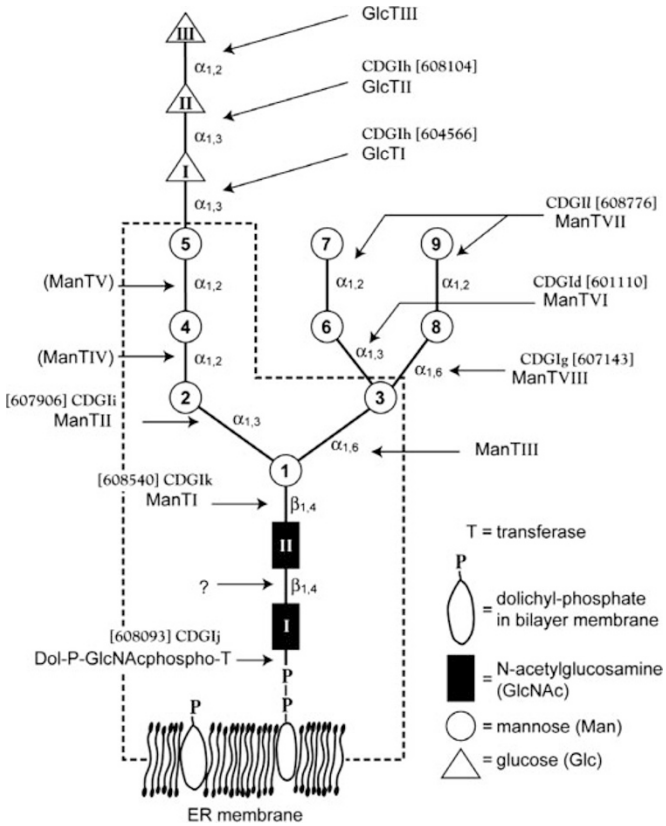

Figure 1. Dolichyl-pyrophosphate bound high-mannose type OS, end-product of assembly stage in N-glycosylation: Gly3Man9GlcNAc2-PP-Dol. Covalent glycosidic linkages shown and sequence of addition of building blocks indicated by Roman (GlcNAc and Glc) or Arabic (Man) numerals inside trivial monosaccharide symbols. Metabolic blocks causing corresponding CDG-I phenotypes (MIM # within square brackets) represented by arrow at appropriate assembly step. Encoding genes listed in Table 1. N-glycan part assembled at cytoplasmic side of ER membrane enclosed inside dashed line. (?): assembly enzyme unknown.

this organism have been invaluable in elucidating the molecular basis of the CDGs (2–7).

The end-product of the LLOS assembly is the glycan Glc3Man9GlcNAc2, composed of 3 glucose, 9 mannose and 2 N-acetylglucosamine residues covalently linked and ordered from the nonreducing to the reducing end (Fig. 1).

The biosynthetic pathway involves at least the following:

1. Dolichylphosphate (Dol-P), ER membrane lipid carrier of the OS during its assembly. Dol-P is a poly-isoprenol lipid. Dolichol kinase, encoded by the orthologous *sec59* gene, is important in yeast. In *sec59* mutants Dol-P is rapidly depleted and lethal. Dol-P is available by *de novo* biosynthesis and is also the substrate of recycling enzymes active in the glycosylation pathway.

2. Monosaccharide building blocks:

a) Nucleotide-activated donor substrates include UDP-N-acetylglucosamine (UDP-GlcNAc), GDP-mannose (GDP-Man) and UDP-glucose (UDP-Glc) (Figs. 1 and 2). They are building block substrates for OSs under construction at the cytoplasmic side of the ER. The latter two are also substrates in the synthesis of Dolichyl-phosphate-mannose (Dol-P-Man) and Dol-P-Glc.

- The biosynthesis of UDP-GlcNAc is initiated from fructose-6-phosphate. In humans no disorder has yet been described due to mutations affecting UDP-GlcNAc biosynthesis (Fig. 2).
- GDP-Man is interconverted in several steps and is also from fructose-6-P. Fructose-6-P is changed into Man-6-P by phosphomannose isomerase (PMI1) (MIM # 154550) (Database OMIM; http://www.ncbi.nlm.nih.gov/Omim/) encoded by the *PMI* gene. (See CDG-Ib [MIM # 602579]). Man-6-P in turn is converted to Man-1-P by phosphomannomutase, encoded by *PMM2* (MIM # 601785) (the orthologous gene in yeast is: *sec53* or *alg4*). Mutations in *PMM2* cause CDG-Ia (MIM # 212065).

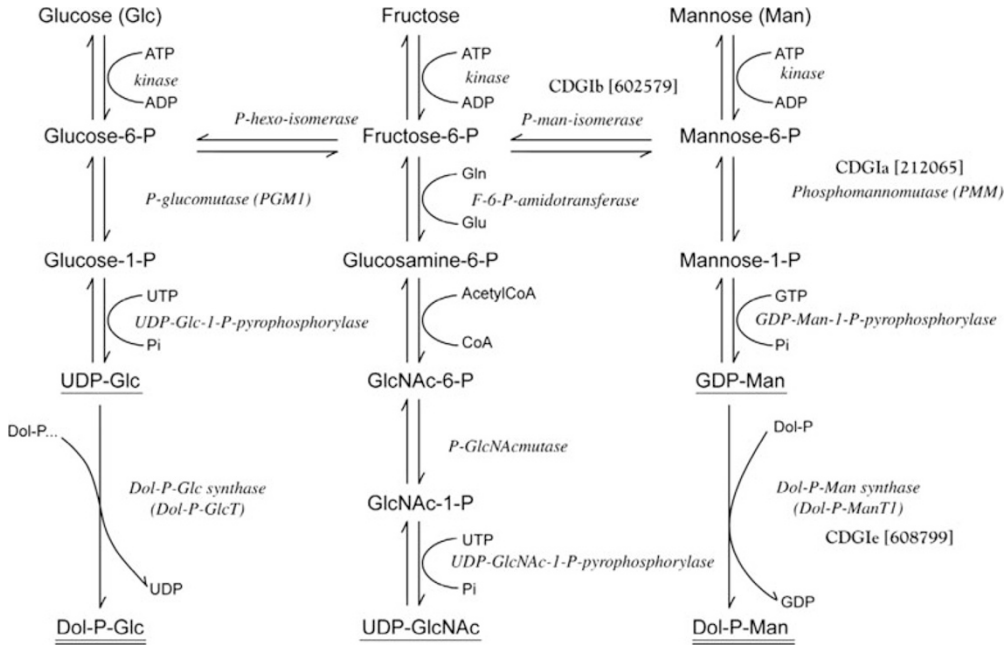

Figure 2. Enzymatic interconversions in hexose monophosphate pool. Nucleotidemonosaccharides, donor substrates at cytoplasmic stage in LLOS, singly underlined. Dolichyl-P-mannose (Dol-P-Man) and Dolichyl-P-glucose (Dol-P-Glc), donor substrates in the assembly within the ER, have double underlining. Enzyme defects associated with CDG-I types (MIM # within square brackets) indicated at appropriate metabolic step.

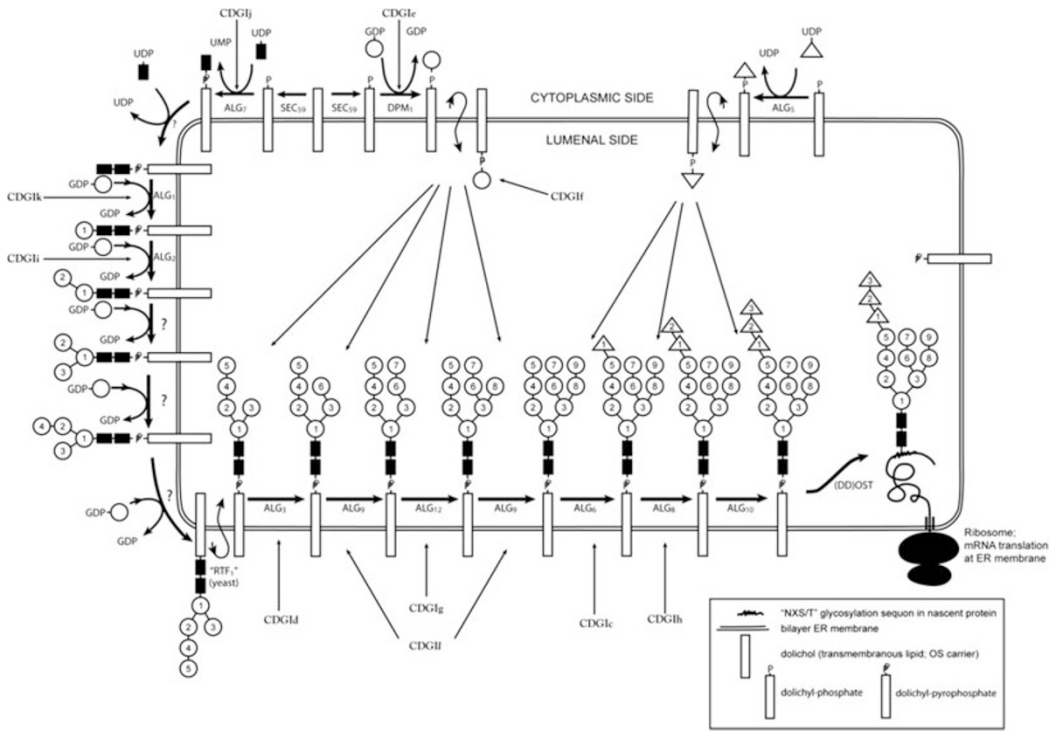

Figure 3. Step-wise assembly of the asparagine (N)-linked OSs. Drawing adapted from Fig. 2 in (7). Genes encoding enzymes catalyzing individual steps are in capital letters: ALG (asparagine-linked glycosylation) gene. Metabolic steps blocked shown by arrows exiting from the corresponding CDG-I entity.

● UDP-Glc synthesis starts from glucose-6-P. a) From the Glc-1-P intermediate UDP-Glc is generated by UDP-Glc pyrophosphorylase encoded by the *UDP1* gene (Fig. 2).

b) Dolichyl-phosphate activated monosaccharides (Figs. 2 and 3). Dol-P-Man and Dol-P-Glc serve as donor substrates in the assembly steps at the lumenal side of the ER membrane. Because they are synthesized at the cytoplasmic side of the ER membrane, timely intra-membrane switching to the lumenal side is required.

● Dol-P-Man is formed from GDP-Man by Dol-P-Man synthase, also called dolichyl-P mannosyltransferase I. This enzyme consists of the catalytic subunit (DPM1) (MIM # 603503) and the regulatory subunits DPM2 and DPM3. The *DPM1* gene is known to be mutated in CDG-Ie (MIM # 608799) patients.

● Dol-P-Glc synthase, with the alternate name dolichyl-P glucosyltransferase, encoded by *ALG5* (MIM # 604565), human ortholog of *alg5* in *S. cer.*., catalyzes the conversion of UDP-Glc into Dol-P-Glc.

3. Glycosyltransferases (Fig. 3). Synthesis of the lipid-linked OS starts at the cytoplasmic side of the ER membrane by covalently linking GlcNAc-P to Dol-P. The biocatalyst, GlcNAc-phosphotransferase (GPT), is encoded by the gene *ALG7* (MIM # 191350), also termed *DPAGT1*, human orthologue of *alg7* in yeast. This transferase is inhibited by tunicamycin, a known inhibitor of N-linked glycosylation. The product of this first assembly step, GlcNAc-P-P-Dol, serves as the acceptor for the next step.

The addition of the second GlcNAc residue in β-1, 4-linkage, and hence formation of GlcNAc2-P-P-Dol, is catalyzed by a still-unknown enzyme. Cloning of the human *ALG1* gene has been greatly helped by the detection of temperature-sensitive *alg1* mutants in yeast, known to accumulate the product of the previous assembly step. *In vitro* expression studies have demonstrated that *ALG1* (MIM # 605907) encodes a β-1,4-mannosyltransferase instrumental in adding the first mannose residue (mannose N° 1) to the acceptor GlcNAc2-P-P-Dol. Mutations in *ALG1* cause CDG-Ik (MIM # 608540) because of failure to synthesize ManGlcNAc2-P-P-Dol (Fig. 3).

Subsequently, the step-wise action of no less than four mannosyltransferases adds four more mannoses in α -linkage to the LLOS. Mannosyltransferase II adds the second mannose residue to the first one in α-1,3-linkage. The detection of a patient with CDG type Ii (MIM # 607906) with mutations in *ALG2* (MIM # 607905) confirms this fact. The third mannose is added (catalyzed by mannosyltransferase III) to the first one, but this time in α-1,6 linkage. Hence bifurcation is initiated at the nonreducing end of the growing OS.

GDP-mannose serves two more times as donor substrate in the step-wise assembly. Both enzymatic additions of mannose occur in α-1,2-linkage to mannose residue N°2. The mannosyltransferase(s) involved, sometimes called mannosyltransferase IV and V, are insufficiently characterized. The encoding genes remain elusive. At this point the assembled OS is: Man5GlcNAc2-P-P-Dol, an asymmetric intermediate product. This OS intermediate is then switched or "flipped" from the cytoplasmic to the lumenal side of the ER membrane, a process catalysed by an ATP-independent bidirectional flippase. The corresponding yeast RFT1 is an integral ER membrane protein with 10 transmembrane domains. Dol-P-Man and Dol-P-Glc become consecutively the donor substrates to four more mannosyltransferases and to three glucosyltransferases in the ER.

The *ALG3* (MIM # 608750) gene encodes the Dol-P-Man dependent α-1,3-mannosyltransferase (VI) that initiates further mannosylation of the OS. The sixth mannose is added to the α-1,6-linked mannose N° 3. Hence it elongates the second oligomannosyl branch in the intermediate Man6GlcNAc2-P-

P-Dol. In *alg3* yeast mutants the asymmetric intermediate Man5GlcNAc2-PP-Dol accumulates. This compound also accumulated in the fibroblasts of patients with CDG-Id (MIM # 601110) ascribed to mutations in *ALG3*.

The next, or seventh mannose residue, is added in α-1,2-linkage to the sixth one by the Dol-P-Man dependent α-1,2-mannosyltransferase encoded by *ALG9* (MIM # 606941). It yields the intermediate LLOS, Man7GlcNAc2-PP-Dol. It completes the second oligomannosyl branch. *Alg9* mutants in yeast store the LLOS, Man6GlcNAc2-PP-Dol. Recently, mutations in the human ortholog *ALG9* were shown to cause CDG type I*l* (MIM # 608776).

The penultimate, or eighth mannose residue, is catalytically linked in a α-1,6 bond to mannose N° 3 in the LLOS and initiates the third oligomannosyl branch in the growing OS. This mannosyltransferase is encoded by the *ALG12* gene (MIM # 607144), and requires the completion of the second branch before it can effectively perform the catalytic action: Man7GlcNAc2-PP-Dol → Man8GlcNAc2-PP-Dol. Mutations in the *ALG12* gene cause CDG-Ig (MIM # 607143). Study of the stored OS products in *ALG9* mutants indicates that the *ALG9* encoded mannosyltransferase also adds the last mannose residue (N°9) in α-1,2 linkage. Following completion of the third oligomannosyl branch, the LLOS, Man9GlcNAc2-PP-Dol is nearly completed.

Effective transfer of the nascent LLOS to the glycosylation site(s) in any proenzyme polypeptide is ineffective without the addition of three glucose residues to mannose N° 5 at the nonreducing end of the first oligomannosyl branch (Figs. 1 and 3). Dol-P-Glc is the donor substrate in each of the three, enzymatic transfers. The first glucose is linked up in α-1,3 configuration by the *ALG6* (MIM # 604566) encoded glucosyltransferase. (See CDG-Ic [MIM # 603147]). Also, the second glucose residue is added to the first one in α-1,3 linkage and is catalyzed by the *ALG8* gene encoded glucosyltransferase (MIM # 608103). In yeast *alg8* mutants the OS intermediate Glc1Man9GlcNAc2 accumulates as it does in cell cultures from patients with CDG-Ih (MIM # 608104) due to mutations in *ALG8*. The glucosyltransferase proteins, pALG6 and pALG8, have structural similarities, a not unexpected finding given the catalysis of similar α-1,3 glycosidic bonds. In contrast, the gene product of *ALG10* (MIM # 603313), the α-1,2-glucosyltransferase that catalyzes the addition of the third and last glucose, has no sequence similarity to either glucosyltransferase enzyme. It has not yet been implicated in any type of CDG.

All glycosyltranferases instrumental in the step-wise build-up of the LLOS, are integral membrane proteins comprising multiple transmembrane domains.

4. "En bloc" transfer of the LLOS, Glc3Man9GlcNAc2 from its PP-Dol linked state to the Asn glycosylation site(s) in nascent protein. This cotranslational transfer is catalyzed by the putative enzyme complex oligosaccharyl-transferase (OST) or dolichyl-diphospho-oligosaccharide: protein glycosyltransferase (DDOST) (MIM # 602202). The multimeric complex is composed of several subunits with multiple transmembrane domains, eight in number in *S. cer.* cells. Most subunits have homologues in the mammalian DDOST complex. The terminal α-1,2 linked glucose is crucial for the effective transfer of the LLOS. The DDOST enzyme scans the still unfolded polypeptide for glycosylation sites that comprise Asn, consistent component in the N-glycosylation sequon of amino acids (NXS/T = Asn-X-Ser/Thr; X may be any amino acid except proline). The amino acid X strongly influences the occupancy rate of the glycosylation sequons. The OST catalyzes the covalent N-glycosidic amide bond.

The completed and completely glucosylated OS is by far most effectively transferred to the nascent proteins. It is not completely understood why in various tissues in the normal subject some glycosylation sequons are fully, some only partially and others not glycosylated at all by the OST.

The Type I Congenital Disorders Of N-Glycosylation (CDG-I)

CDG type Ia (CDG-Ia). This severe autosomal recessive disease, often with onset in early infancy and impressive involvement of the CNS, was soon recognized as a multisystem disorder (5,8–11). The natural course carries a 20% fatality risk in infancy. Following an uneventful pregnancy and delivery, the neonate with CDG-Ia has a normal birth weight and occipitofrontal circumference (OFC). Failure to thrive is often an early source of concern. Features of dysmaturity, limb-wasting, poor suckling and grunting noises have been noticed soon after birth, as have truncal floppiness, delay in neuromotor and physical development and episodes of lethargy and hypothermia. Roving eye movements, squinting and lack of social contact are among the parental complaints. Restriction of mobility in the large joints, specifically deficient extension, may be evident. Life-threatening episodes of severe disease with vital organ failure requiring urgent hospitalization include cardiac failure with pericardial effusion and/or hepatic insufficiency with coagulopathy. Later in infancy the so-called "typical dysmorphic" facies, with bitemporal narrowing, high nasal bridge, prominent jaw, full cheeks and large ears may become apparent. Inverted and laterally displaced nipples are less consistent features. In some patients the distribution of s.c. fat is abnormal over the buttocks and suprapubic region and lipodystrophic skin changes may develop in the pelvic region and over the thighs (10,12). However, in many patients abnormal physical features are absent. By the end of the first year there is considerable delay in gross neuromotor development but no longer progression of neuromental disease. Also, social contact and hand coordination improves as well. Axial hypotonia remains significant and signs of apparently nonprogressive cerebellar ataxia become more overt. There is moderate to severe mental deficiency. Lower motor neuron impairment is readily demonstrated and deep tendon reflexes cannot be elicited. Nerve conduction velocity is moderately reduced. Children with CDG-1a may have incomplete tapetoretinal degeneration that does not lead to frank blindness. From early childhood the course stabilizes except for episodes of stupor, or even coma, usually triggered by intercurrent infections. Sometimes stroke-like episodes cause sudden loss of consciousness, transient blindness, seizures and/or hemiplegia.

In adolescence the survivors enter the so-called leg-atrophy stage (10) and live as nonambulatory, speechless, moderately to severely retarded and more or less physically crippled individuals. This clinical course applies to the majority of CDG-1a patients. However, mild and unusual phenotypes are on record (13–15). The clinical diagnosis of CDG-Ia represents a true challenge because of the multi-system nature, the pleiomorphic crises-ridden first year of life and the inconsistent phenotypic changes throughout the natural course. It is however the most frequent CDG.

MRI or CT of the brain consistently reveal frank pontocerebellar atrophy from infancy. It is associated only with mild supratentorial cerebral "atrophy" in older patients. The few postmortem studies available (10) confirm this overall pontocerebellar atrophy. Complete loss of Purkinjé cells and partial loss of granular cells is detected throughout the cerebellar cortex and severe gliosis in all infratentorial brain structures. Peripheral neuropathy is confirmed histologically. Liver biopsy specimens obtained early in the disease show nonprogressive steatosis and fibrosis. Lysosomal vacuolar structures with concentric membrane-like inclusions and distorted ER may be found in hepatocytes by EM observation. Nephromegaly has been observed ultrasonographically (12). Cystic changes have been documented in kidney parenchyma (16). The generalized osteopenia and scoliosis often observed in older patients are likely secondary consequences of the neurologic syndrome. IEF of plasma sialotransferrin (TfIEF) shows an increase of the non- and poorly sialylated cathodal fractions and a decrease of the more anodal, normally sialylated Tfs. In a large portion of serum Tfs up to two of the normal four N-linked trisaccharides are absent (9,10,17,18). CDG-Ia shares this TfIEF profile with the other CDGs due to defects in the assembly stage of N-glycosylation, which identified them as CDG-I entities (9). TfIEF is an important screening tool. A number of other secretory glycoproteins similarly lack sufficient oligosaccharides in CDG-Ia. This finding confirms the multi-systemic adverse effect of hypoglycosylation and explains the unique coagulopathy in CDG-Ia patients, who have a marked deficiency of factor XI and of the anticoagulation glycoproteins antithrombin, protein C, protein S and heparin cofactor II (8,19). The consistent hypoalbuminemia in CDG-Ia may be related to liver dysfunction, to protein-losing enteropathy and other unknown factors. The increased levels of at least some hypophyseal hormones in CDG-Ia are probably due to hypoglycosylation of the corresponding integral receptor proteins. IEF of sialoTf or other glycoproteins in amniotic fluid cannot be used in prenatal diagnosis (20) that must be based on metabolic and molecular data.

Deficiency of phosphomannomutase (PMM) is the enzyme defect in CDG-Ia (21). Isomerization of mannose-1 phosphate (Man-1-P) to Man-6-P is necessary to supply sufficient GDP-mannose and Dol-P-Man, building block donor substrates for LLOS assembly. The gene *PMM2* encoding the enzyme protein has been mapped to chromosome 16p13 (22,23). Causal involvement of *PMM2* in CDG-Ia has been rigorously proved by the demonstration in a large series of CDG-Ia patients of several dozen mutations, nearly all of the missence type and mostly in compound heterozygous constellation. The patients were of different geographic and ethnic origins (24,25). Remarkably, even the most frequently encountered R141H amino acid substitution has only been observed in compound heterozygotes (26). Prenatal diagnosis has been successfully performed (27).

There is no effective treatment for CDG type I, with the obvious *a priori* recurrence risk of one-quarter to full sibs of patients. Autosomal recessive inheritance is also the mode of hereditary transmission in all other forms of CDG- I.

CDG-Ib. Delineated independently by several groups in six patients altogether (28–30), this disorder is clinically distinct from CDG-Ia and from most other CDGs. It does not primordially affect the CNS and is, contrary to all other forms of CDG-I, amenable to mannose supplement substitution treatment.

The few patients reported were normal at birth. Soon in infancy, cyclic episodes of vomiting and/or diarrhea with threatening dehydration were frequent and often urgent reasons for hospitalization. Initially, the bouts of gastrointestinal upset were not recognized as the expression of a protein-losing enteropathy. Hypoglycemia and hyperinsulinemia have been the presenting symptoms of the disorder (31,32). The recurrent episodes in infancy and early childhood may be associated with convulsions, apnea, decreased consciousness or coma. Patients may have generalized edema. The consistently moderate but firm liver enlargement was associated with signs of liver failure, such as hypoalbuminemia, hypocholesterolemia, and abnormally low levels of clotting and anti-clotting factors. Liver biopsy specimens showed hepatic cytolysis, fibrosis in portal spaces, microvesicular steatosis and thrombosis. Mild hypoproteinemia and moderate hyperaminoaciduria have been observed intermittently. Whether the renal disease was also a primary expression of the mutant gene or only a secondary complication is not yet clear. The diagnosis of CDG-Ib has been delayed until adolescence in the event of the somewhat milder phenotype (28). Remarkably, psychomotor development has been normal or near normal in these severely troubled children with the CNS at great risk for the adverse effects of severe dehydration and disturbed electrolyte homeostasis. Oral treatment with the recommended amount of mannose (1 g/kg body weight/d, divided over 5 doses) (6) is well tolerated. Higher doses may induce hyperosmotic diarrhea. This treatment resulted in clinical improvement within weeks, but much later in gradual but significant metabolic and functional normalization (32). The TfIEF type I profile did not normalize until several months later. Mannose is directly converted into Man-6-P by the specific hexokinase. Hence, the free Man treatment bypasses (29) the metabolic block caused by the deficient MPI. (Fig. 2).

The diagnosis of CDG-Ib is formally established by showing the severely deficient specific activity in leukocytes or fibroblasts of mannosephosphate isomerase (MPI) (EC 3. 5.3.1.8) (Fig. 2). The enzyme, a dimeric protein active in the cytosol, has been purified from placenta. Full length *MPI* cDNA was isolated and characterized. Northern blot analysis revealed a single 1.8 kb *MPI* mRNA that is ubiquitously expressed (33). Niehues and colleagues detected the first

mutation in a CDG-Ib patient (30). The 656G>A transition resulted in the missense mutation, Arg219Gln. Sequencing of gDNA has shown that the second mutation of the compound heterozygote is an insertion of C between the nucleotides 166 and 167. This results in a reading frame shift and probably a much truncated and unstable translation product (34). The *MPI* gene spans 5 kb of gDNA and contains 8 exons (33,34). In the familial protein-losing enteropathy with hepatic fibrosis originally reported from Quebec, Canada in siblings with the Saguenay-Lac St-Jean (SLSJ) syndrome (35), the heterozygous Arg295His missense mutation (due to a 884G>A transition in the *MPI* gene) has been found in both parents (36). This proves that this familial disorder is a mild variant of CDG-Ib. The same mutation was homozygous in an ethnically related CDG-Ib patient (36). A list of selected allelic mutations of the wild-type *MPI* gene is provided in OMIM.

CDG-Ic. A few dozen infants and patients at toddler age have been reported since the metabolic and molecular delineation of this disorder (37,38). It is milder than CDG-Ia and so is its natural course. There are no significant dysmorphic changes, no cerebellar atrophy and no peripheral neuropathy. Nevertheless, some of the affected have shown axial and appendicular ataxia. The patients' plasma TfIEF profile is indistinguishable from that in CDG-Ia. Low levels of serum cholesterol, coagulation factor XI, antithrombin and protein C were found.

In fibroblasts from CDG-Ic patients, the dolichol-linked precursor $Man_9GlcNAc_2$ is the main accumulated OS intermediate. Characterization of it has led to the identification of the primary enzyme defect in CDG-Ic (37–39): Dol-P-Glc: Man9GlcNAC2-PP-Dol α-1,3-glucosyltransferase I. (Fig. 3). This enzyme transfers the first glucose residue in covalent α 1,3-linkage onto the first oligomannosyl antenna in the LLOS. These findings have confirmed earlier results obtained in *S. cer.* strains, mutant in the orthologous *alg6* gene, and hence helpful for the isolation of the complete *ALG6* cDNA. The latter contains an open reading frame (ORF) predicting a protein of 505 amino acids (40). The *ALG6* gene is located at 1p22.3. The initial patients observed in an inbred family were homozygous for the Ala333Val missence substitution.

CDG-Id. In one of the patients with CDG-Id, originally described as a fourth subtype of CDG (41), the biochemical and molecular defect was first elucidated (42) to be the deficient Dol-P-Man: Man5GlcNAc2-PP-Dol α-1,3-mannosyltransferase, encoded by the *ALG3* gene, located at 3q27 and human orthologue of the *alg3* locus in *S. cer*. The homozygous mutation was the 353G>A transition resulting in the Gly118Asp missence substitution. The "wild-type" enzyme catalyzes the transfer of the sixth mannose residue onto the LLOS, the first mannose residue transfer after the LLOS has been switched from the cytoplasmic to the lumenal side of the ER. The acceptor substrate, the intermediate glycan Man5GlcNAc2-PP-Dol, accumulates in the mutant fibroblasts. (Fig. 3).

More recent patient reports (43–45) confirm that CDG-1d, apparent from the early weeks of life, is a severe encephalopathy, reminiscent of CDG-Ia and associated with dysmorphic facial and limb features that point at the prenatal expression of this inborn error of metabolism. In one patient the OFC at birth was small (43) and in another it was large (44). However, from late infancy secondary microcephaly was a consistent finding. Retinal changes such as optic nerve "atrophy" were associated with poor vision in the survivors. Iris coloboma was found only in one of the original patients (41). Hypotonia, severe in infancy, persisted throughout the clinical course. Poorly controllable seizures were a major concern in most subjects, but were not evident in the patient reported by Sun *et al.* (43). Psychomotor development was severely impaired. Even sitting without support and elementary speech were not achieved in patients aged 5 y and more. Brain MRI showed frank cerebral atrophy but a rather intact cerebellum. Motor nerve conduction velocity was normal. Protein-losing enteropathy, hypoalbuminemia, hepatic fibrosis and hyperinsulinemic hypoglycemia, reminiscent of both CDG-Ia and CDG-Ib, contributed to the early demise in one patient (43) but were not recorded in the course of the others (41,44–46).

The Tf-IEF profile resembled that in CDG-Ia and hypoglycosylation of other plasma proteins was readily shown. Denecke *et al.* (44) offer a useful discussion on fetal *versus* postnatal hypoglycosylation, as the former was shown to be less pronounced in the affected sibling fetus than in their index patient (44). They prove that prenatal diagnosis also of CDG-Id must depend solely on molecular screening of the *ALG3* mutations. For details regarding the more recently reported mutant alleles, reading the original reports (41–46) and consultation of OMIM is recommended.

CDG-Ie. The few patients reported had a subacute encephalopathy from birth with hypotonia, intractable seizures and cortical blindness. The severe neurodevelopmental delay was associated with delay in myelinisation shown by neuroimaging. Secondary microcephaly was noticed later in infancy. Some facial dysmorphism was reported in addition to small hands with tapering fingers and hypoplastic nails. Serum transaminases and creatine kinase were increased, coagulation and/or anti-coagulation plasma proteins decreased (47,48). The TfIEF pattern closely matched that in CDG-Ia.

In [2-^{3}H]-mannose labeled fibroblasts, the primarily stored LLOS species was Man5GlcNAc2 instead of the fully assembled glycan. Hence, clinically and metabolically CDG-Ie appears indistinguishable from CDG-Id. However, in the former the enzyme Dol-P-Man synthase 1 was found to be functionally deficient with causal mutations in the *DPM1* gene (47,48). DMP1 exists in a heteromeric protein complex, in which the DPM2 and DMP3 proteins, structurally unaffected in CDG-Ie, are also components.

CDG If. From early infancy patients with this type of CDG-I have severe hypotonia, nystagmus and seizures. Blindness was noticed by six months of age. Neuromotor and staturo-ponderal development were much delayed. The skin was remarkably dry, hyperkeratotic and scaling with erythrodermia in several areas of the body. Severe axial ataxia and hypotonia precluded unaided walking. The few single words already gained were lost from the third year of life (49,50).

The IEF pattern of serum Tf closely resembled that in CDG-Ia. *In vitro* experimental studies following [2-^{3}H] -mannose labeling

Table 1. *Congenital disorders of N-glycosylation: CDG-I*

CDG-I (refs)	Enzyme Defects	MIM #	GENE		
			Symbol	MIM #	Chromosome Location
a (8–27)	Phosphomannomutase	212065	*PMM2*	601785	16p13.3-p13.2
b (28–36)	Mannose-P isomerase	602579	*MPI*	154550	15q22
c (37–40)	Dol-P-Glc:Man9GlcNAc2PP-Dol α1,3-glucosyltransferase	603147	*ALG6*	604566	1p22.3
d (41–46)	Dol-P-Man:Man5GlcNAc2PP-Dol α1,3-mannosyltransferase	601110	*ALG3*	608750	3q27
e (47,48)	Dol-P-Man synthase	608799	*DPM1*	603503	20q13.13
f (49,50)	Dol-P-Man utilization factor	609180	*MPDU1*	604041	17p13.1-p12
g (51–54)	Dol-P-Man:Man7GlcNAc2PP-Dol α1,6-mannosyltransferase	607143	*ALG12*	607144	22
h (55–57)	Dol-P-Glc:Glc1Man9GlcNAc2PP-Dol α1,3-glucosyltransferase	608104	*ALG8*	608103	11pter-p15.5
i (58)	GDP-Man:Man1GlcNAc2PP-Dol α1,3-mannosyltransferase	607906	*ALG2*	607905	9q22
j (59)	Dol-P-GlcNAc-phosphotransferase	608093	*DPAGT1 (ALG7)*	191350	11q23.3
k (60,61)	GDP-Man:GlcNAc2PP-Dol β1,4-mannosyltransferase	608540	*ALG1*	605907	16p13.3
l (62,63)	Dol-P-Man:Man(6)(8)GlcNAc2PP-Dol α1,2-mannosyltransferase	608776	*ALG9*	606941	11q23

of patient fibroblasts showed accumulation of the Man5GlcNAc2 intermediate in addition to Glc3Man9GlcNAc2, Man9GlcNAc2 and the unusual Glc3Man5GlcNAc2. The latter compound lacks all four mannoses added by the Dol-P-Man dependent mannosyltransferases. The set of OS species stored was found to be congruent to that stored in *Lec35* mutant Chinese Hamster Ovary (CHO) cells. In either system, both the Dol-P-Glc synthase and the Dol-P-Man synthase were functioning normally. The hypothesis of a functionally deficient protein encoded by the *MPDU1* gene was explored and shown to be correct. Mutations in this Dol-P-Man utilization factor were identified as the cause of CDG-If (49,50).

The most recently delineated CDG-I entities; Remark on CDG-Ih. Several more disorders of N-glycosylation of Type I (CDG-I) have been detected and characterized in recent years following the detection in plasma of the abnormal TfIEF Type I profile. In each one of the "new" metabolic entities the cause was identified as the failure of a specific step in the OS assembly, different from that in previously characterized types of CDG-I. Given the extensive pathway of N-glycosylation, the discovery of still more type I CDGs may be predicted. The newly delineated entities termed alphabetically according to time of discovery (CDG-Ig through CDG-I*l*) are listed in Table 1 with references to the original reports (51–63) and to the OMIM database. The infants with any one of these "new" CDG diseases type I are sick from birth and indistinguishable from the CDG-I cases with major CNS involvement reviewed above, including CDG-Ia, or from one another (5). In all instances overall description of the clinical phenotype must await the reports of more patients.

The first report of a patient with CDG-Ih is that of a still-surviving patient, who from infancy had protein-losing enteropathy with bouts of severe diarrhea and threatening dehydration, episodic generalized edema, ascites, hypoalbuminemia, hepatomegaly and plasma coagulation factor anomalies. There were some dysmorphic features but apparently normal psychomotor development. This observation prompted the clinical alignment of this disorder with CDG-Ib (5,55). The subsequent report of three young infants with a more aggressive form of the same disorder, also shown to be due to the deficiency of Dol-P-Glc: Glc1Man9GlcNAc2-PP-Dol α 1,3 glucosyltransferase, trivially named α 1,3 glucosyltransferase II and encoded by the *ALG8* gene on chromosome 11pter-p15.5, was considered to support the view that CDG-Ih may be the second type of CDG-I without primordial involvement of the CNS. These infants succumbed to refractory anasarca already in early infancy and "normal" neurologic and cranial neuro-imaging results obtained early in the disease course cannot rule out primary CNS disease (56). As pointed out in the most recent report of a fifth CDG-Ih patient to manifest neurodevelopmental delay and cerebral atrophy (57), most other CDG-Ih patients probably also have had an early onset encephalopathy. The apparently milder disease in the first patient may represent a more leaky *ALG8* mutant phenotype. Comparison with CDG-Ib needs rigorous clinical and scientific scrutiny and obviously the reports of more patients. CDG-Ih is the only CDG-I type that consistently features renal disease and failure (57). Comparative studies between the clinical expression of the closely related defects in CDG-Ih and CDG-Ic also deserve special attention when more observations are available.

Diagnosis in CDG-I

The formal diagnosis of CDG-I and of the specific subtype is a multi-step laboratory procedure. IEF of plasmaTf, the hallmark of introductory screening for the CDGs, detects the abnormal TfIEF profiles I and II that differ from the profile in control plasma. In the latter, the tetrasialo-Tf fraction is by far the most prominent one. The disialo-Tf is a minor fraction and asialo-Tf is often undetectable. In the type I abnormal profile the cathodal fractions, asialo- and especially disialo-Tf, are significantly increased at the expense of the normal tetrasialo-Tf and the still more anodal bands of more highly

sialylated Tf molecules (4,8). The TfIEF type 1 profile is associated with the cytoplasmic and the ER defects in the Dol-PP-linked OS assembly pathway, but does not provide information about the recognition and subclassification of the various CDG-I entities. Moreover, the diagnosis of CDG cannot be excluded if a normal TfIEF profile is obtained in a relevant patient. Unusual variants of the Tf protein need to be ruled out either by studying the pateint's parents or by having neuraminidase treatment precede the TfIEF. A type I TfIEF profile is found also in galactosemia and in hereditary fructose intolerance. Finding a type I TfIEF profile should prompt the assays of PMM and/or MPI. If the former assay reveals significantly deficient specific activity, the diagnosis of the CDG-Ia is the conclusion. If the clinical phenotype calls instead first for the assay of MPI and if it is found to be nearly inactive, CDG-Ib is diagnosed. Leukocytes are the preferred material for either assay as mutant cultured fibroblasts often have more residual activity. These enzyme assay based diagnoses require confirmation by mutation analysis of the relevant gene. If no deficiency is detected by either enzyme assay, help should be solicited in laboratories with specific research interest and experience in this branch of glycobiology. *In vitro* studies using [2-^{3}H] mannose or [6-^{3}H] glucose labeling of mutant cultured fibroblasts in the presence and absence of specific enzyme inhibitors, allows the identification of the type of stored glycan by appropriate chromatographic techniques. Characterization of the accumulated OS intermediate is a prerequisite for designing the appropriate assay of the assembly enzyme inactivated by the causal mutation. *In vitro* expression tests in mutant yeast or fibroblast cell strains following transfection with patient and with control cDNA confirm the genetic defect. Detection of the homozygous or compound heterozygous mutations in the relevant human gene brings ultimate confirmation of the causal diagnosis.

THE TYPE II CONGENITAL DISORDERS OF GLYCOSYLATION: DEFICIENT OS PROCESSING

The purpose of describing the processing pathways of the fully assembled N-linked OSs or Glycans is to better understand the various forms of CDG-II. Most of the information is from recent reviews on the subject matter (3, 5–8).

The Pathway(s) of N-glycan Processing

Glc3Man9GlcNAc is the N-glycan species most effectively transferred to the Asn containing sequons in nascent proteins. Transient glucosylation is not only a prerequisite for such effective transfer, but plays a pivotal role as well in the molecular folding process and quality control by chaperone type ER proteins.

Soon after the OST mediated transfer, the outermost α-1,2-linked glucose residue is removed by α -glucosidase I (Fig. 4). (See CDG-IIb.) The subsequent removal of the second glucose residue by α -glucosidase II strongly favors binding of the new glycoprotein to calnexin (CNX), a lectin-like integral ER membrane chaperone and to calreticulin (CRT), a related soluble ER resident. Thus, the glycoprotein is sequestered from premature degradation, granted opportunity to reach default polypeptide folding and interaction with other proteins recruited to this macromolecular complex that also includes ERp57, a thiol-disulfide oxidoreductase catalyst of disulfide bond formation.

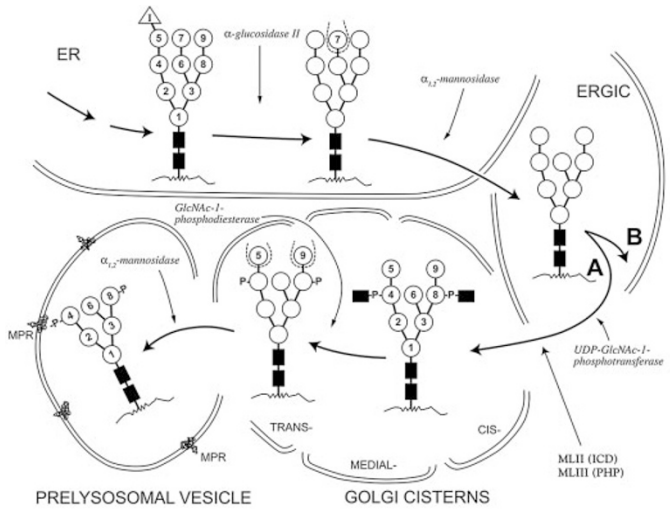

Figure 4. N-glycan processing pathway: (*A*) Lysosomal phosphorylation pathway failing in GlcNAc-phosphotransferase deficiency disorders, Mucolipidosis II (ML II) or I-cell disease (ICD) and Mucolipidosis III (ML III) or pseudo-Hurler Polydystrophy (PHP). Failure in Cis-Golgi cisterns to generate mannose-6-phosphate (M-6-P) recognition marker in the oligomannosyl type asn-linked OS, precludes binding of glycoprotein to M-6-P-receptors (MPRs), intracellular routing to the lysosomal compartment and hence, loss of hydrolases into intercellular space. Symbols like in Fig. 3.

When the remaining α-1,3-linked glucose is removed by α-glucosidase II, the normal glycoprotein dissociates from CNX and/or CRT and concomitantly looses mannose residue N° 7 by the action of the ER α-1,2-mannosidase I. It leaves the ER in vesicles collectively called ERGIC (ER to Golgi intermediate compartment) trafficking to the Cis-Golgi cisterns (Figs. 4 and 5). If, however, the normal molecular folding has not yet been achieved, UDP-glucose: glycoprotein glucosyltransferase, also a normal component in the large ER chaperone complex, reglucosylates the glycan for reiterative binding to the lectins. Hence, a new CNX/CRT quality control cycle and more opportunity is offered to reach the proper three-dimensional conformation. If the mutant glycoprotein cannot achieve normal folding it enters the pathway of ER-associated degradation (ERAD).

Further processing of the N-linked Man8GlcNAc2 glycan side chains occurs in at least two directions. Route A (Fig. 4) has been characterized by the study of the disorders of lysosomal enzyme phosphorylation and intracellular localization (64), also termed the GlcNAc-phosphotransferase deficiency disorders (65,66). 6-Phosphorylation of some mannoses (the n°4 and n°8 symbols in Fig. 4) in the oligomannosyl type N-glycan is achieved by the UPD-GlcNAc: lysosomal glycoprotein GlcNAc-1 phosphotransferase (EC N° 2.7.8.17) (trivial name: GlcNAc-phosphotransferase: GNPTA) (MIM # 607838 and # 607840). The transiently GlcNAc-protected phosphate groups are subsequently exposed by an α-N-acetylglucosaminidase and become crucial components in the

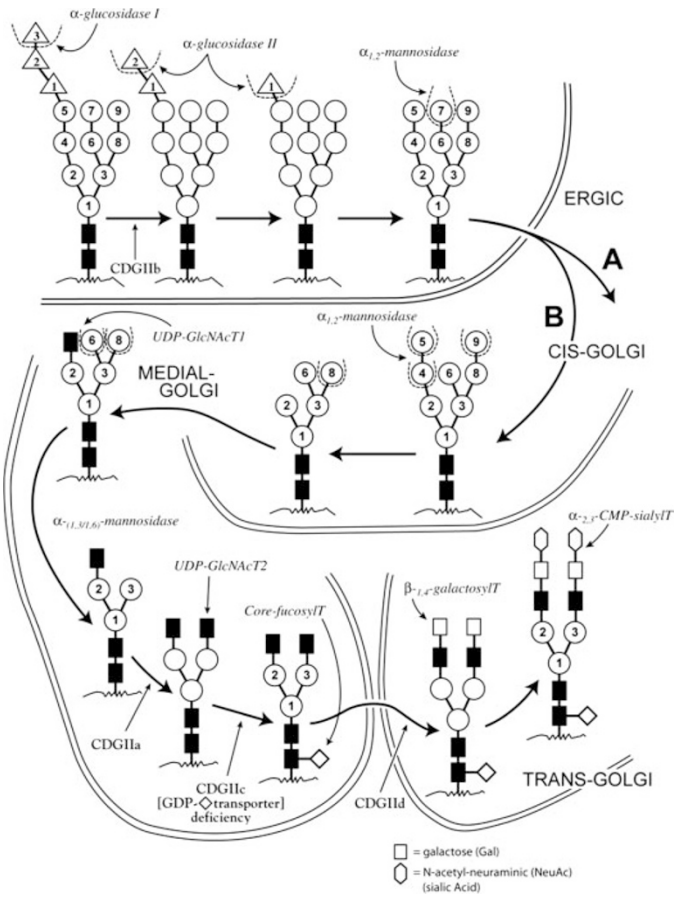

Figure 5. N-glycan processing pathway B: Step-wise synthesis of complex OS following formation of core N-linked glycan, Man3GlcNac2-Asn. Specific CDG-II entity shown by arrow at metabolic step, blocked by inactivity of corresponding mutant processing enzyme. Appropriately processed lysosomal enzymes (glycoproteins) traffick within intracellular vesicles to the plasma membrane, site of secretion or of perimembranous function. Symbols like in Fig. 3.

mannose-6-phosphate (M-6-P) recognition marker in lysosomal enzymes. This enzymatic processing occurs in the Trans-cisterns of the Golgi apparatus. Concomitantly, the mannose residues n°5 and n°9 are released by α-1,2-mannosidase I, thus generating the M-6-P bearing oligomannosyl type N-glycan, Man5GlcNAc2, in lysosome-bound glycoproteins. The recognition marker insures binding to the M6P receptors (MPRs) that are integral plasma membrane proteins in some of the postGolgi and/or prelysosomal vesicles. Thus, routing of the hydrolases to the lysosomes, site and environment of their function, is also insured (Fig. 4). I-Cell disease (ICD) or Mucolipidosis type II (ML II) (MIM # 252500) and pseudo-Hurler polydystrophy (PHP or ML III) (MIM # 252600 and # 252605) are due to failure of one of the subunits in the oligomeric GlcNAc-phosphotransferase. They are usually listed among the oligosaccharidoses (64–66), but may be considered also as members of the CDG-II group. They have been reviewed elsewhere (64–66).

The alternate pathway of enzymatic trimming and maturation of the N-glycan chains proceeds along route B (Fig. 5). By this alternate label the glycoproteins are routed either toward their site of function within the plasma membrane or made ready for secretion into the intercellular space.

The first step in this pathway occurs in the Cis Golgi by catalytic removal of all α-1,2-linked mannose residues, hence N°5, N°9 and the remaining N°4 as well. According to the "stable compartments" model pertaining to the identity of the Golgi cisterns, the glycoprotein then travels to the Medial cisterns once again in membrane bound vesicles. In the "cisternal maturation" Golgi model however, the cargo glycoprotein stays within the cistern originally entered. Maturation of this cistern is achieved by Golgi enzymes received per retrograde vesicular transport from already more mature cisterns at the Trans side of the Golgi. At that stage UDP-GlcNAc transferase I adds a GlcNAc residue to the now uncovered mannose N°2. Subsequently, α-(1,3/1,6) mannosidase also called α -mannosidase II (or two different α -mannosidases) remove(s) both the α-1,3-linked mannose N°6 and the α-1,6-linked mannose N°8. The result: the asymmetric intermediate GlcNAcMan3GlcNAc2 N-glycan.

The processing continues by the UDP-GlcNAc transferase II mediated addition of a second GlcNAc residue onto mannose N°3. Hence, symmetry is restored at its bifurcated non-reducing ends. This UDP-GlcNAc transferase II is deficient in CDG type IIa. The normal enzyme may even add 1 to 3 extra GlcNAc residues, thereby creating multi-antennary glycan chains onto the glycoproteins (6).

Still in the Medial Golgi cisterns, a fucose residue is added to GlcNAc residue N°1 that is covalently linked by the amide bond to the Asn in the glycosylation "sequon" in the polypeptide. The mutant fucose transporter protein causes CDG-IIc. Assuming the stable compartments Golgi model, the glycoproteins traffic within vesicles to the Trans Golgi cisterns. In the Trans cisterns a galactose and a sialic acid residue are added symmetrically and sequentially to each of the nonreducing ends of the N-glycan antennas and synthesis of the complex type glycan is completed. The consecutively involved enzymes are: β-1,4-galactosyltransferase, deficient in CDG-IId, and α -2,3-cytidine monophosphate (CMP)-sialyltransferase. The latter enzyme has not been implicated directly in any human disorder. However, in CDG-IIf it cannot function effectively because CMP-sialic acid, its donor substrate, is poorly supplied due to the mutant CMP-sialyl transporter gene.

Plasma TfIEF and ApoC-III IEF: Orientation Toward Diagnosis of CDG-II?

In the type 2 abnormal TfIEF profile the disialoTf fraction is prominent and the tetrasialo-Tf decreased compared with the normal control. Also, the asialo- and the monosialo-Tf fractions are often apparent in the type 2 profile ascribed to the group of type II CDGs (4,8). However, the power of diagnostic orientation of the type 2 profile is limited as in the CDG subtypes -IIb, -IIc and -IIf. The normal plasma TfIEF profile is obtained because there is no hyposialylation of Tf. Recently the distinction has been reported between the "asialo profile" with asialo- and monosialo-Tf fractions more prominent (found in CDG-IId) and the "disialo profile" with increased disialo- and trisialo-Tf bands (observed in CDG-IIa) (67). In contrast to the LLOS assembly pathway, the trimming path-

way is affected also in some conditions with disturbed biosynthesis of the core 1 mucin type O-glycans. This was detected by the recently introduced screening IEF of plasma apolipoprotein C-III (Apo-CIII), a marker of O-glycosylation (68). The latter yields the normal plasma apoC-III in CDG-I, but the abnormal "apoC-III_0" or "apoC-III_1" profile in plasma of some CDG-II patients (67). Several nucleotide sugars, transferases and transporter proteins are common to the synthesis of both N- as well as O-glycans. Moreover, some causes of complex glycosylation failure are known to lie outside the "glycosylation machinery" proper (69).

Overview of the Known Types of CDG-II

CDG-IIa. This disorder, associated with a plasma TfIEF profile distinct from that in CDG-Ia, was first described by Jaeken *et al.* (70) and termed CDG-II. Presently, few patients are known. They had generalized hypotonia from infancy, moderate to severe neuromotor developmental delay and failure to thrive. Generalized seizures from early childhood, stereotypic hand wringing and head banging behavior, and poor visual contact were almost consistent findings. Few if any words were learned. Ambulation was acquired belatedly and remained broad-based and unsteady. The CDG-IIa patients had neither cerebellar atrophy nor peripheral neuropathy. The mild facial dysmorphism consistently reported had only inconsistent components. All patients had small stature, relative macrocephaly, pectus excavatum, kyphoscoliosis, hypotrophic legs and hypogonadism. Minor signs of liver dysfunction were also documented. The patients' bleeding tendency was explained by decreased intrinsic adhesiveness of the platelets (19). Some plasma glycoproteins, in particular coagulation factor XI, were decreased (71–73).

The enzyme defect: UDP-GlcNAc: α-6-D-mannoside-β-1,2-GlcNActransferase II (EC 2.4.1.143) only affects the Man-α 1,6 antenna of the glycan. The encoding *MGAT2* gene (MIM # 602616), located on chromosome 14q21 (74), has only a single exon. The mature enzyme has 447 amino acids. The mutations found in this gene were either homozygous (70) or compound heterozygous (73).

CDG-IIb. To date, only a single patient born to consanguineous parents has been reported (75). The neonate had hypotonia, hypomobility, generalized edema, and episodes of hypoventiation and apnea from birth. Seizures started at day 21 with suppression burst-type EEG. Mild dysmorphic features were clinically insignificant. There was gradual liver enlargement. The child made no measurable developmental progress. The MRI scan of the brain revealed no abnormality, but EMG findings supported peripheral neuropathy. The patient died on day 74 after ten days of intractable seizures and increasing stupor (MIM # 606056).

Postmortem findings included septal cholangiofibrosis with excessive bile duct proliferation and ectasia. The EM study, showed myelin-like lamellar profiles in hepatocytes. Enlarged perikarya in neurons of the cerebral cortex were identified as membrane-bound vacuoles surrounding the nuclei. The ER was visibly dilated in these cells.

The TfIEF in plasma yielded the normal profile. Excessive urinary excretion of the tetrasaccharide Glc(α-1,2)Glc(α-1,3)Glc(α-1,3)Man prompted confirmation of the α-glucosidase I deficiency hypothesis with a specific activity of less than 3% of the normal average in fibroblasts. The patient, although from consanguineous parents, was compound heterozygous for two missense mutations in the *GCS1* gene, located at 2p13-p12 (MIM # 601336) (75–77).

CDG-IIc: Leukocyte adhesion deficiency type II (LAD II). LAD II, first identified in 1992 (78), is characterized from infancy by recurrent bacterial infections, failure to thrive, deficient physical growth, microcephaly and moderate psychomotor retardation. The face is mildly dysmorphic. Body proportions are abnormal, allegedly because of shortened arms and legs. The plasma TfIEF profile is normal in CDG-IIc patients. The hematology findings were remarkable because of the highly elevated peripheral neutrophil and lymphocyte counts and because the sialyl-$Lewis^X$ antigen was lacking in glycoproteins and glycolipids on the surface of the neutrophils. Sialyl Lewis antigen, a normal fucose-containing carbohydrate ligand to the selectins known cell adhesion molecules, is crucial for recruitment of neutrophils to any inflammation site. Moreover, the patients also had the "Bombay" ABO blood group characterized by H-antigen absent on the RBC's surface and by anti-H antibody in plasma. In various somatic cell lines several fucosylated glycoproteins were absent. Consequently, the hypothesis of a more general defect in fucose handling was considered the more likely culprit than deficiency of any specific fucosyltransferase. Most patients with CDG-IIc are of Middle Eastern descent, where the disease was known as the Rambam-Hasharon syndrome (79). Recently, the reports of a Turkish patient with CDG-IIc (80) and of a patient of consanguineous Brazilian descent (81) were valuable.

In vitro exposure to fucose had restored surface fucosylation in mutant fibroblasts and lymphoblastoid cells in some patients (80,81). Oral fucose therapy (up to 1g/kg/d) has been clinically effective by lowering the circulating neutrophil count to normal values within the course of one day. By this treatment the sialyl-Le^X antigen reappeared and selectin ligand binding was observed again at the surface of the patient's neutrophils. Neutrophil rolling and halting by surface contact with the vascular endothelia was partially restored.

No favorable therapeutic effect was observed in the Arabic patients (79) and the psychomotor development of the patient treated with partial success did not improve (82) with fucose treatment. Potential dangers of the treatment have also been highlighted: 1) Generation of autoantibodies against neoantigens on the neutrophil surface and neutropenia as a consequence; the neutropenia observed could be controlled by lowering fucose intake; and 2) expression of fucosylated glycans on the erythrocyte surface, including H-antigen may induce hemolysis by binding of serum anti-H antibody. Fucose therapy, known to have worked in two instances, elicited H-antigen development in only one: No evidence of hemolysis was observed. Such potential dangers require close monitoring of patients during fucose treatment (81). None of the recent reports includes a rationale as to how fucose partially corrected the fucosylation defect in some patients.

The putative GDP-fucose transporter protein was identified (83–85) and characterized to be a Golgi resident protein with ten transmembrane domains. This was one of the results of the initial cloning and characterization of cDNA representing the GDP-fucose transporter 1 (*FUCT1*) gene (MIM # 605881). The reader is referred to the original reports (81,83–86) for details on the specific mutations that prove the hereditary nature of CDG-IIc. Obviously failing import of the donor substrate CDG-fucose by the mutant FUCT1 protein not only precludes normal core fucosylation of the maturing N-glycans in glycoproteins, but also fucosylation of O-linked glycoproteins.

CDG IId. One patient has been reported with deficiency of β-1,4-galactosyltransferase I, the cause of CDG-IId. The plasma Tf with type 2 TfIEF profile was found to lack both sialic acid and galactose residues (87). The patient, 2.5 y old at the time of report, had moderate psychomotor and physical developmental delay. There was general hypotonia and also muscular weakness associated with enhanced serum creatine kinase. The patient had macrocephaly and the Dandy-Walker type malformation. He had been followed for progressive hydrocephaly. The brain malformation may have been a coincidental finding (88).

The enzyme, named β-galactosyltransferase (EC 2.4.1.38), is known to catalyze the binding of UDP-galactose to GlcNAc in β 1,4 linkage. The more correct name is UDP-Gal: β-GlcNAc β-1,4-galactosyltransferase (MIM # 137060). The activity in the patient's leukocytes and fibroblast strain was reduced to about 5% (84). The residual enzyme protein in the mutant fibroblasts was considerably smaller than the normal product encoded by the wild-type *B4GALT 1* gene and also failed to reach the Trans Golgi cisterns, its normal site of catalytic action (89).

Full-length cDNA of *B4GALT1* was cloned (86) and shown to encode a 400 amino acid protein containing a N-terminal membrane-anchoring domain. The chromosome location of *B4GALT1* is 9p13 (90). It spans over 50 kb of gDNA and contains six exons (91). The patient was homozygous and both parents heterozygous for a single nucleotide insertion resulting in a premature stop codon and a truncated translation product (87).

CDG-IIe. Identified as CDGII/ Cog7 deficiency.

CDG-IIf. The single patient reported had initial cutaneous hemorrhages and an extensive vitreoretinal bleeding at 4 mo of age. The clinical course was compounded by more internal hemorrghages associated with severe thrombocytopenia, respiratory distress, local infections and sepsis. The unsuccessful bone marrow transplantation was complicated by graft-*versus*-host disease. The patient succumbed to the aggressive disorder at 37 mo. Sialyl-Le^x antigen was lacking on the neutrophils, but α-1,3-fucosyltransferase and α-2,3-sialyltransferase, involved in sialyl-Le^x antigen synthesis, were normal (92). Knowledge of identical biochemical features in *LEC2* CHO cells with mutations demonstrated in the CMP-sialic acid transporter gene prompted the detection of mutations in the human orthologous gene (MIM # 605634). Neither one of the patient's mutant alleles could complement the CHO mutations *in vitro*, whereas the transduced wild-type human gene fully restored sialyl transporter function in the *LEC2* cells. TfIEF of the patient's plasma yielded the normal profile, as the leaky mutations did not result in hyposialylation of Tf. However, the absence of sialyl-Le^x antigen pertains to the lacking CMP-sialic acid, donor substrate of sialyltransferase, by the failing CMP-sialic acid transporter (92). The latter, encoded by the *SLC35A1* gene (MIM # 605634), is a member of the large solute carrier family 35 of nucleotide-sugar transporters.

CDG-II Caused by Defects in the Conserved Oligomeric Golgi (COG) Complex with Implications for both N- and O-glycosylation

The major importance of the COG complex, also in the human species, has been illustrated by the recent reports of two multi-systemic disorders with early clinical onset (93,94). The COG complex is essential to establish and maintain cisternal identity and to coordinate the function of the Golgi apparatus, where the sequential modification of glycoproteins by Golgi enzymes and the nonuniform distribution thereof within the Golgi stacks remain intriguing features (69,95,96). The COG complex is a hetero-octamer of protein subunits peripherally attached to the Golgi membrane (96). Recently it was realized that the subunits discovered piece by piece over more than 20 years function most effectively as a unit. The specific roles of the various subunits and their subassembly pattern are still unknown. The COG complex with all components intact insures adequate retention and localization of Golgi transporters and enzymes, mediates retrograde vesicular inter-cisternal vesicular trafficking and effective biosynthetic pathways such as N- and O-glycosylation (95).

CDG-II/COG 7. The two sibling neonates recently described with a multi-systemic disorder had perinatal asphyxia, mild dysmorphic features, hypotonia, prolonged jaundice and hepatosplenomegaly with evidence of liver disease. Severe convulsions compounded the clinical picture in the last weeks of life. Refractory infection and cardiac failure were recorded as the apparent causes of death at 5 and 10 wk, respectively. The healthy consanguineous parents had previously lost another infant with a congruent clinical course. In the patients' plasma a type 2 TfIEF profile was found (93). Biochemical results obtained in the mutant fibroblasts that were at variance with findings in most types of CDG-II included: cell surface hyposialylation of O-linked glycans in addition to partially deficient N-glycan sialylation; partial defects of sialyltransferases and of sialic acid metabolism; reduced rate of transport of several nucleotide sugars into and within the Golgi cisterns; reduced activity of other glycosyltransferases pertaining to O-linked OS synthesis; partial impairment of intracellular vesicle trafficking, and particularly the membrane-bound cargo proteins, confirmed by *in vitro*, real-time fluorescent immunohistochemistry. In contrast, soluble lysosomal enzymes, such as cathepsin D were unaffected by the mutant gene (93). The transport defect was apparently selective and therefore strongly reminiscent of the defects known in *IdlB* and *IdlC* CHO cells with mutations in the COG-1 and the COG-2 subunits respectively (97).

The subunits COG-5, COG-6 and COG-8 were abnormally distributed, but COG-7 was undetectable in the patients' cultured fibroblasts. Sequencing of COG-7 cDNA revealed the deletion of the last 19 nucleotides in exon 1. In gDNA the homozygous intronic transversion (IVS1 + 4 A>C) was detected. It impairs splicing at the canonical site, but allows splicing at the conserved alternate splicing site near the first exon /intron boundary and results in the 19 bp deletion. In both patients the amounts of COG-7 mRNA and the COG-7 protein were reduced (93). (See also MIM # 606978 and # 608779.) In addition to plasma screening by Tf and ApoC-III IEF (68) that yielded the "disialo" profile and the Apo-$CIII_0$ profile (67), *in vitro* identification and characterization of the COG complex or the COG-sensitive proteins in patient fibroblasts may orient more effectively to the specific CDG-II/COG diagnosis (69,93,98).

CDG-II/COG 1. The proposita, the second child of healthy consanguineous parents, was born at 35 wk gestation by Caesarean section. The neonate was small but macrocephalic with some facial dysmorphic features. The clinical course of initial feeding problems with failure to thrive and generalized hypotonia was compounded by slowing rate of general and cephalic growth and by mild left ventricular hypertrophy and slight hepatosplenomegaly. MRI neuro-imaging at 21 mo revealed only mild cerebral and cerebellar atrophy. The child's psychomotor development was only slightly retarded (94). The plasma TfIEF type 2 profile and the ApoC-III_0 profile were indications of failing N- and deficient O-glycosylation respectively (4,8,67,68,94). Structural analysis of the N-glycans confirmed not only undersialylation but also undergalactosylation in plasma glycoproteins. The levels of the COG complex subunits 1–4 and 8, in mutant fibroblast lysates studied by Western blotting, were significantly decreased. Only the immunoreactive COG-1 subunit was found to be smaller than the wild-type counterpart. Sequencing of the *COG1* gene detected the homozygous insertion of a single cytosine (c2659-2660insC) in the patient's cDNA. Both parents were heterozygous for this mutation that predicted a truncated COG1 protein. The steady state levels of COG-2, -3, -4 and -8 were reduced, but COG-5, -6, and -7 were found in normal amounts. The effects of the truncated COG-1 subunit were multiple (94). In the original report, evidence is also presented for the direct functional correlation of COG complex deficiency and intracellular levels and/or localization of Golgi glycosyltransferases and for the highly probable role of the COG complex in controlling intra-Golgi trafficking of resident proteins.

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