The clinical relevance of glycobiology

Commentary

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The study of protein-bound glycans dates back to the 19th century (1). Until recently these macromolecules have played second fiddle to their cousins, the nucleic acids and proteins. This is not surprising in view of the stunning advances during the second half of the 20th century in the DNA-RNA-protein paradigm, which Francis Crick called the Central Dogma. Since information transfer is a key ingredient of this dogma, it is relevant to point out that the diversity of linkages and branching patterns between monomer building blocks confers on carbohydrates the ability to carry an enormous amount of information in very compact structures (2). These structures therefore carry "more information bang for the buck" than do the other, simpler polymers. The cell surface is covered with protein- and lipid-bound glycans. These structures vary significantly between cell types and at different stages of mammalian development and probably play important roles in the interaction of a cell with its cellular and fluid environment (3–5). Glycoproteins and proteoglycans are essential for normal development in mice (6–16), *Drosophila melanogaster* (17–20), and *Caenorhabditis elegans* (18, 21–26). Table 1 lists mice with null mutations in genes required for glycosylation; other null mutant mice are described in reviews by Stanley (9) and Varki and Marth (11).

In spite of all the evidence showing the importance of glycans for metazoan development, glycobiology did not earn the respect it deserves until the recent description of several human congenital diseases with defects in the glycosylation of proteins (27–32). Since at least 0.5–1% of the transcribed human genome is devoted to the production of proteins involved in the synthesis, degradation, and function of glycoconjugates (11), it is likely that we have seen only the tip of the iceberg. Two papers in this issue of the *JCI* (Schenk et al., ref. 33; and Kranz et al., ref. 34) support this suggestion. These papers describe a congenital disorder of glycosylation (CDG) in which the defective gene encodes an unusual protein with a role in glycan synthesis that is not as clearly defined as were the defects in the previously described human CDGs shown in Table 2.

Congenital disorders of glycosylation

The CDGs (previously known as carbohydrate-deficient glycoprotein syndromes) are a group of congenital diseases, often with severe multisystemic defects, characterized by defective *N*-glycosylation. The papers in this issue describe four unrelated patients with a new variant of CDG group I (CDG-If). Group I CDGs involve deficiencies in the assembly of the dolichylpyrophosphate-linked oligosaccharide *N*-glycan precursor (Figure 1) and/or its transfer to asparagine residues on the nascent polypeptide (35). Since complete loss of *N*-glycosylation is lethal in both yeast and mammals, all CDG-I patients have "leaky" mutations. This is indicated by the presence of appreciable amounts of $Glc_3Man_9GlcNAc_2$-pyrophosphate-dolichol (Figure 1) in the cells of all CDG-I patients. The four CDG-If patients all show severe psychomotor retardation, seizures, and other multisystemic abnormalities consistent with CDG-I. The serum transferrin isoelectric focusing patterns are similar to the pattern observed in most CDG-I patients. However, the major abnormal dolichol pyrophos-

Table 1
Glycosylation-deficient mutant mice that show developmental abnormalities

Missing enzyme	Biochemical role	Phenotype	References
β1,2-GlcNAc-transferase I	Addition of GlcNAc to the Manα1-3 arm of the complex *N*-glycan core	Mice cannot make complex *N*-glycans. Embryonic-lethal at embryonic days 9–10	7, 8
β1,2-GlcNAc-transferase II	Addition of GlcNAc to the Manα1-6 arm of the complex *N*-glycan core	Perinatal lethality; a few survivors (about 1%) mimic CDG-IIa (see Table 2)	6
β1,6-GlcNAc-transferase V	Addition of GlcNAc to the Manα1-6 arm of the complex *N*-glycan core	Viable mice. Suppression of tumor growth and metastasis	10
Dol-P GlcNAc-1-phosphate transferase	Transfers GlcNAc-1-phosphate to Dol-P to make GlcNAc-PP-Dol	Mice cannot make any LLOs. Embryonic-lethal at embryonic days 4–5	12
Ser/Thr O-GlcNAc-transferase	Adds GlcNAc in O-glycosidic linkage to Ser/Thr of many intracellular proteins	Deletion of this X-linked gene causes loss of embryonic stem cell viability	13
β1,3-Gal-transferase III	Adds Gal to GlcNAc-R; homologue of *Drosophila* Brainiac	Embryonic lethality prior to implantation	14
α3,6-mannosidase II	Removes 2 Man residues from $GlcNAc_1Man_5GlcNAc_2$-Asn-X	Defective synthesis of complex *N*-glycans Mimics congenital dyserythropoietic anemia type II (HEMPAS) Causes autoimmune disease (see Table 2)	15, 16

HEMPAS, hereditary erythroblastic multinuclearity with a positive acidified serum lysis test.

Table 2
Human congenital disorders with defective glycosylation

(1) CDG Group I: Defects in *N*-linked protein glycosylation due to deficiencies in the assembly of the dolichylpyrophosphate-linked oligosaccharide and/or its transfer to asparagine residues on the nascent polypeptides.

CDG type	Enzyme defects	Gene	OMIM[A]	Locus Link[A]	Acronym
Ia	Phosphomannomutase 2	*PMM2*	212065 601785	5373	CDG-Ia
Ib	Phosphomannose isomerase	*MPI*	602579 154550	4351	CDG-Ib
Ic	Dolichyl-P-Glc:$Man_9GlcNAc_2$-PP-dolichyl α1,3-Glucosyltransferase	*ALG6*	603147 604566	29929	CDG-Ic
Id	Dolichyl-P-Man:$Man_5GlcNAc_2$-PP-dolichyl α1,3-Mannosyl-transferase	*ALG3* *NOT56L*	601110	10159	CDG-Id
Ie	Dolichol-P-Man synthase 1	*DPM1*	603503	8813	CDG-Ie
If	Dolichol-P-Man utilization defect 1; Lec35	*MPDU1*	604041	9526	CDG-If
Ix	Genetic basis unknown		603585 212067		CDG-Ix

(2) CDG group II: Defects in the processing of *N*-glycans or addition of other glycans to proteins.

CDG type	Enzyme defect	Gene	OMIM[A]	Locus Link[A]	Acronym
IIa	UDP-GlcNAc:α6-D-mannoside β1,2-*N*-acetylglucosaminyltransferase II (GnT II)	*MGAT2*	212066 602616	4247	CDG-IIa
IIb	α1,2-Glucosidase I	*GCS1*	601336	7841	CDG-IIb
IIc	GDP-fucose transporter I	*FUCT1*	266265 605881	55343	CDG-IIc LAD II

(3) Other.

Name of disease	Enzyme defect	Gene	OMIM[A]	Locus Link[A]	Acronym
HEMPAS	α3.6-Mannosidase II; other unknown defects	*MAN2A*	224100 154582	4124	CDA II

[A]OMIM, Online Mendelian Inheritance in Man; Locus Link summarizes all the known information on a particular gene. Both databases can be accessed at http://www.ncbi.nlm.nih.gov/. LAD II, leukocyte adhesion deficiency type II. CDA II, congenital dyserythropoietic anemia type II.

phate oligosaccharides (lipid-linked oligosaccharides; LLOs) in the patient fibroblasts are $Man_5GlcNAc_2$ (Figure 1c) and $Man_9GlcNAc_2$ (Figure 1b), a novel pattern suggesting defects in both dolichol phosphate mannose-dependent (Dol-P-Man–dependent) mannosylation and dolichol phosphate glucose-dependent (Dol-P-Glc–dependent) glucosylation, respectively. Surprisingly, the two dolichol phosphate monosaccharide precursor synthases and the respective glycosyltransferases are all normal. In fact, whereas all five of the previously described CDG-I types (CDG-Ia to -Ie) are due to defects in enzymes within the synthetic pathways that lead to $Glc_3Man_9GlcNAc_2$-pyrophosphate-dolichol, the mechanism of action of the protein (Lec35p) responsible for CDG-If has not yet been determined.

The name Lec35 derives from a lectin-resistant Chinese hamster ovary (CHO) mutant cell line that accumulates $Man_5GlcNAc_2$-pyrophosphate-dolichol (Figure 1c) (36). The Lec35 protein was shown to be required for the utilization of both Dol-P-Man and Dol-P-Glc and, consequently, for the mannosylation and glucosylation of LLOs, the mannosylation of glycosylphosphatidylinositols, the *C*-man-

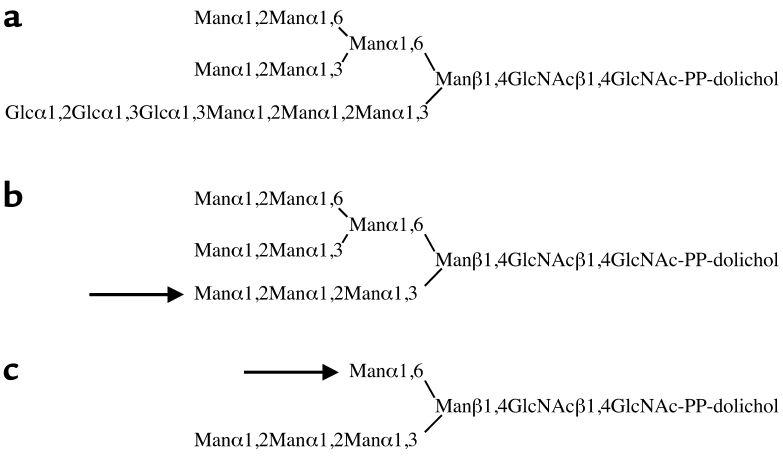

Figure 1
(**a**) The structure of $Glc_3Man_9GlcNAc_2$-pyrophosphate-dolichol. This compound is essentially the only LLO found in normal human fibroblasts. However, appreciable amounts of this material are also found in all CDG-I fibroblasts reflecting the "leaky" nature of the mutations in these patients. Based on animal studies, total lack of this LLO is not compatible with life. (**b**) The structure of $Man_9GlcNAc_2$-pyrophosphate-dolichol. This is one of the major LLOs found in CDG-If. The accumulation of this compound implies a defect in the addition of Glc in α1,3 linkage to the Man at the arrow. Since the synthesis of Dol-P-Glc and the activity of the relevant Dol-P-Glc–dependent α1,3-glucosyltransferase are both normal in CDG-If, the defect has been attributed to an inability to utilize Dol-P-Glc. (**c**) The structure of $Man_5GlcNAc_2$-pyrophosphate-dolichol. This is the other major LLO found in CDG-If. The accumulation of this compound implies a defect in the addition of four Man residues to the Man at the arrow by four Dol-P-Man–dependent mannosyltransferases. Since the synthesis of Dol-P-Man and the activities of the relevant mannosyltransferases are normal in CDG-If, the defect has been attributed to an inability to utilize Dol-P-Man.

nosylation of tryptophanyl residues, and protein *O*-mannosylation (37). These results show that Lec35p has an essential role for all known classes of dolichol phosphate monosaccharide-dependent glycosyltransferase reactions in mammals. The human *Lec35* ortholog has been named *MPDU1* (mannose phosphate dolichol utilization defect 1) and was mapped to 17p12-13. Sequence analysis of the *MPDU1* gene showed distinct mutations in all four CDG-If patients. The predicted amino acid sequence of Lec35p does not suggest an obvious function or mechanism. Lec35p is a 27-kDa endoplasmic reticulum membrane-associated protein with two putative transmembrane segments and is probably involved in the "flipping" of Dol-P-Man and Dol-P-Glc from the cytoplasm, where these molecules are synthesized, to the lumen of the endoplasmic reticulum, where glycosylation occurs. The use of mutant mammalian cell lines (such as lectin-resistant CHO lines) and yeast mutants has been essential for the elucidation of many CDG types, including CDG-If.

Phenotypic variability among the CDG individuals

The complexity of the various glycosylation pathways suggests that many congenital diseases of unknown etiology will turn out to be CDGs. One major reason is the diversity of clinical presentation and severity in the CDG spectrum. This is true even in a single type of CDG (for example, the four cases of CDG-If described in this issue of the *JCI* [refs. 33, 34]). The same diversity occurs in the broader spectrum of CDG-I even though all known types are due to defective synthesis of $Glc_3Man_9GlcNAc_2$-pyrophosphate-dolichol. For example, the enzymes responsible for CDG-Ia (phosphomannomutase 2) and CDG-Ib (phosphomannose isomerase) sit side by side in the synthetic pathway leading to Dol-P-Man, yet the clinical presentations of the two diseases differ enormously: CDG-Ia patients show severe psychomotor retardation, whereas CDG-Ib patients have no neurological defects. The biochemical findings within a single CDG type also tend to vary; for example, the lipid- and protein-bound oligosaccharide patterns can differ quite dramatically between patients. This is perhaps not surprising in view of the various deglycosylation and reglycosylation pathways involved (38–40). There are almost certainly other as-yet unknown factors — genetic, regulatory, environmental, etc. — that contribute to differences between CDG patients (28). Both under- and overglycosylation may cause disease, and it may be necessary to strike a healthy balance between the two (28). The relative frequency of certain CDG mutant alleles suggests that they may have advantages in the heterozygous state, e.g., protection against viral infection (28).

The search for new CDG types can benefit from animal models, which may show phenotypic changes that differ from the human clinical presentations. Indeed, comparison of Tables 1 and 2 shows that only two known mouse glycosylation defects correspond to identified human CDGs, namely CDG-IIa and the atypical CDG HEMPAS (hereditary erythroblastic multinuclearity with a positive acidified serum lysis test). Hence, there are almost certainly more CDGs to be described, and clinicians should think of CDG whenever they are faced with a puzzling congenital disease. Of the human diseases, only CDG-Ib and -IIc have, to date, responded to therapy — oral mannose and fucose, respectively. Further research may improve this picture as it reveals more fascinating facts about glycobiology.

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