MUTATION UPDATE

Human Mutation

OFFICIAL JOURNAL
HGVS
HUMAN GENOME VARIATION SOCIETY
www.hgvs.org

Congenital Disorders of Glycosylation: An Update on Defects Affecting the Biosynthesis of Dolichol-Linked Oligosaccharides

Micha A. Haeuptle and Thierry Hennet*

Institute of Physiology, University of Zürich, Zürich, Switzerland

Communicated by William S. Sly

Received 5 June 2009; accepted revised manuscript 28 August 2009.
Published online 27 October 2009 in Wiley InterScience (www.interscience.wiley.com). DOI 10.1002/humu.21126

ABSTRACT: Defects in the biosynthesis of the oligosaccharide precursor for N-glycosylation lead to decreased occupancy of glycosylation sites and thereby to diseases known as congenital disorders of glycosylation (CDG). In the last 20 years, approximately 1,000 CDG patients have been identified presenting with multiple organ dysfunctions. This review sets the state of the art by listing all mutations identified in the 15 genes (*PMM2*, *MPI*, *DPAGT1*, *ALG1*, *ALG2*, *ALG3*, *ALG9*, *ALG12*, *ALG6*, *ALG8*, *DOLK*, *DPM1*, *DPM3*, *MPDU1*, and *RFT1*) that yield a deficiency of dolichol-linked oligosaccharide biosynthesis. The present analysis shows that most mutations lead to substitutions of strongly conserved amino acid residues across eukaryotes. Furthermore, the comparison between the different forms of CDG affecting dolichol-linked oligosaccharide biosynthesis shows that the severity of the disease does not relate to the position of the mutated gene along this biosynthetic pathway.
Hum Mutat 30:1628–1641, 2009. © 2009 Wiley-Liss, Inc.

KEY WORDS: glycosylation; endoplasmic reticulum; CDG; dolichol; glycoprotein

Introduction

N-glycosylation is an essential form of posttranslational modification in eukaryotes. Several types of N-glycosylation disorders have been described over the last decade, thereby expanding the list of congenital disorders of glycosylation (CDG) [Freeze, 2006]. Looking at the N-glycosylation disorders identified so far, it is reasonable to predict that all genes involved in the biosynthesis of N-glycans are likely to be once associated with a form of CDG. Despite the achievement of the last years, the identification of novel N-glycosylation disorders remains challenging, mainly because of their rarity and because of their rather nonspecific clinical pictures [Leroy, 2006]. The widespread application of a simple isoelectric focusing (IEF) test allowing the detection of underglycosylated serum transferrin [van Eijk et al., 1983] has been instrumental in pointing at potential cases of N-glycosylation disorders. The pattern of transferrin glycoforms obtained by IEF usually allows differentiating between defects of N-glycosylation site occupancy and defects of N-glycan trimming and elongation. These two groups of defects have been originally defined as CDG type-I (or CDG-I) and CDG type-II (or CDG-II), respectively [Aebi et al., 1999]. Defects in other classes of glycosylation and in sugar-nucleotide transporters have also been designated as CDG-II [Lubke et al., 2001; Topaz et al., 2004; Martinez-Duncker et al., 2005]. However, the recent description of glycosylation disorders caused by defects of vesicular transport [Wu et al., 2004; Foulquier et al., 2006, 2007], which by definition fall in the category of CDG-II, has prompted for a revision of the CDG nomenclature. Along this line, it has been suggested to abandon the differentiation between CDG-I and -II, and to name the glycosylation disorders by using the official abbreviation of the defective gene [Jaeken et al., 2008].

Since the first description of mutations in the phosphomannomutase-2 (*PMM2*) gene as causing CDG [Matthijs et al., 1997], 25 additional disorders of N-glycosylation have been identified [Jaeken et al., 2008]. Clinically, most of these disorders lead to psychomotor retardation with variable neuromuscular involvement and additional features like hormonal abnormalities and coagulopathies [Leroy, 2006]. The severity of these symptoms often varies tremendously, ranging from slight mental retardation to multiorgan dysfunctions often associated to infantile lethality. A typical question arising when comparing the clinical manifestations described is the following: does the severity of a CDG case relate to the position of the defect along the N-glycosylation biosynthetic pathway or rather to the degree of inactivation conferred by the mutation underlying the gene defect? This question can be particularly well examined when considering the glycosylation disorders caused by defective assembly of the dolichol-linked oligosaccharides since this biosynthetic pathway is sequential (Fig. 1). Moreover, the assembly of dolichol-linked oligosaccharides is strongly conserved among eukaryotes, thereby enabling the application of asparagine-linked glycosylation (ALG) mutant strains of the yeast *Saccharomyces cerevisiae* as tools for investigating the functional impact of human mutations [Westphal et al., 2001a; Grubenmann et al., 2004; Haeuptle et al., 2008].

The aim of this review is to define the state of the art for the known disorders of dolichol-linked oligosaccharide biosynthesis and especially to discuss the effect of known mutations on the functions of the affected proteins. To date, 15 gene defects have been described in this group [Matthijs et al., 1997; Niehues et al., 1998; Imbach et al., 1999, 2000b; Körner et al., 1999; Schenk et al., 2001; Chantret et al., 2002, 2003; Thiel et al., 2003; Wu et al., 2003; Frank et al., 2004; Grubenmann et al., 2004; Kranz et al., 2007b; Haeuptle et al., 2008; Lefeber et al., 2009]. Common for all of these defects is the insufficient

Additional Supporting Information may be found in the online version of this article.

*Correspondence to: Thierry Hennet, Institute of Physiology, University of Zürich, Winterthurerstrasse 190, CH-8057 Zürich, Switzerland. E-mail: thennet@access.uzh.ch

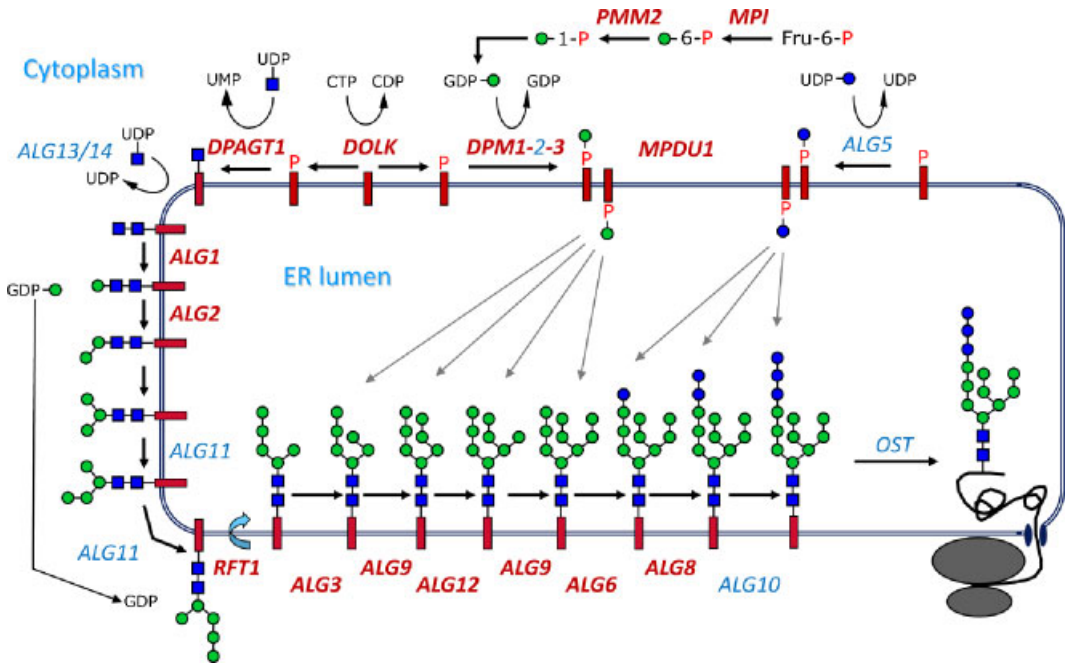


Figure 1. Pathway of dolichol-linked oligosaccharide biosynthesis. After phosphorylation (P) of the lipid carrier dolichol (red bar), two GlcNAc (blue box), nine Man (green circle), and three Glc (blue circle) units are successively added by various glycosyltransferases. Nucleotide-activated monosaccharides serve as donor substrates for the cytosolically-oriented enzymes. After being translocated into the endoplasmic reticulum (ER) lumen, the intermediate dolichol-PP-$GlcNAc_2Man_5$ is further extended by luminally acting mannosyl- and glucosyltransferases using dolichol-P activated Man and Glc as sugar building blocks. The complete structure dolichol-PP-$GlcNAc_2Man_9Glc_3$ is transferred to selected asparagines on newly synthesized glycoproteins by the oligosaccharyltransferase (OST) complex. The gene symbols are indicated next to the catalyzed reactions. The 15 genes associated with CDG are marked in red.

supply of dolichol-linked oligosaccharide precursor, which leads to decreased occupancy of N-glycosylation sites. The defective glycosylation reactions can be organized in five categories based on enzymatic activity and on subcellular localization (Fig. 1). The first category comprises the cytosolic enzymes PMM2 and mannose phosphate isomerase (MPI) [Matthijs et al., 1997; Niehues et al., 1998]. The second category includes the N-acetylglucosaminyl- and mannosyltransferase enzymes involved in the assembly of the dolichol-linked oligosaccharide at the cytosolic side of the endoplasmic reticulum (ER) membrane [Thiel et al., 2003; Wu et al., 2003; Grubenmann et al., 2004]. The third and forth categories include the mannosyltransferase and glucosyltransferase enzymes that elongate the luminally-oriented dolichol-linked oligosaccharide [Imbach et al., 1999; Körner et al., 1999; Chantret et al., 2002, 2003; Frank et al., 2004], respectively, whereas the last category comprises the proteins that modify dolichol or affect the availability of dolichol-linked carbohydrates to the assembly pathway [Imbach et al., 2000b; Schenk et al., 2001; Kranz et al., 2007b; Haeuptle et al., 2008; Lefeber et al., 2009].

Over the last decade, close to 1,000 CDG patients were diagnosed with a disorder of dolichol-linked oligosaccharide assembly, thereby unraveling more than 200 mutations in 15 genes (Table 1). The present review provides a comprehensive overview of the pathway by mapping these mutations on model representations of the affected proteins. By integrating the clinical features associated with each mutation, this overview enables a discussion of the importance of individual proteins in the context of N-glycosylation output and human physiology.

Group 1: Cytosolic Enzymes

The glycosyltransferase enzymes of the N-glycosylation pathway use nucleotide- or dolichol-activated monosaccharides as donor substrates. Defects in the biosynthesis of these substrates lead to CDG. The formation of guanosine diphosphate–mannose (GDP-Man) occupies a central stage in the process considering the nine Man residues constituting the N-glycan core (Fig. 1). Accordingly, the cytosolic enzymes PMM2 and MPI, which catalyze the conversion of Man-6-P to Man-1-P and of fructose-6-P to Man-6-P, respectively, are essential members of the N-glycosylation pathway. Noteworthy, PMM2 and MPI deficiencies affect additional glycosylation pathways, such as O-mannosylation and glycosylphosphatidylinositol-anchor biosynthesis, which also rely on Man-based donor substrates.

PMM2 (PMM2-CDG, CDG-Ia)

More than 800 patients have been identified with mutations in the *PMM2* gene (PMM2-CDG), thereby constituting the largest group of CDG cases (Table 1). Clinically, neurologic symptoms, including psychomotor retardation, developmental delay, epilepsy, ataxia, cerebellar hypoplasia, and visual impairment, are predominant. Furthermore, symptoms of coagulopathy, hypotonia, cardiomyopathy, and gastrointestinal and hepatic problems are also frequently observed. Strong dysmorphic features including severe skeletal deformities are found in most cases [Mizugishi et al., 1999; Westphal et al., 2001a; Briones et al., 2002; Tayebi et al., 2002; Ono et al., 2003; Coman et al., 2005; Schollen et al., 2007; Vermeer et al., 2007; Wurm et al., 2007; Truin et al., 2008; Perez-Duenas et al., 2009; Thong et al., in press; Vega et al., 2009]. A mortality rate of over 20% within the first years of life is frequent in cases presenting with low residual PMM2 activity [Matthijs et al., 2000]. The essential role of PMM2 is supported by finding that disruption of this gene in mice leads to early embryonic lethality [Thiel et al., 2006].

Table 1. Gene Defects Leading to Deficient Assembly of Dolichol-Linked Oligosaccharides

Gene	OMIM[a]	Enzyme	Disorder[b]	Disorder[c]	OMIM[a]	Mutations	Patients
PMM2	601785	Phosphomannomutase 2	CDG-Ia	PMM2-CDG	212065	103	>800
MPI	154550	Mannose phosphate isomerase	CDG-Ib	MPI-CDG	602579	18	>25
DPAGT1	191350	GlcNAc-1-P transferase	CDG-Ij	DPAGT1-CDG	608093	3	3
ALG1	605907	Mannosyltransferase 1	CDG-Ik	ALG1-CDG	608540	4	7
ALG2	607905	Mannosyltransferase 2	CDG-Ii	ALG2-CDG	607906	2	1
ALG3	608750	Mannosyltransferase 6	CDG-Id	ALG3-CDG	601110	9	11
ALG9	606941	Mannosyltransferase 7–9	CDG-Il	ALG9-CDG	608776	2	3
ALG12	607144	Mannosyltransferase 8	CDG-Ig	ALG12-CDG	607143	11	8
ALG6	604566	Glucosyltransferase 1	CDG-Ic	ALG6-CDG	603147	20	>36
ALG8	608103	Glucosyltransferase 2	CDG-Ih	ALG8-CDG	608104	12	9
DOLK	610746	Dolichol kinase	CDG-Im	DOLK-CDG	610768	2	4
DPM1	603503	Dolichol-P mannosyltransferase 1	CDG-Ie	DPM1-CDG	608799	6	8
DPM3	605951	Dolichol-P mannosyltransferase 3	CDG-Io	DPM3-CDG	612937	1	1
MPDU1	604041	Man-P-dolichol utilization defect 1	CDG-If	MPDU1-CDG	609180	5	5
RFT1	611908	RFT1 homolog (*S. cerevisiae*)	CDG-In	RFT1-CDG	612015	5	6

[a]http://www.ncbi.nlm.nih.gov/sites/entrez?db = OMIM.
[b]According to the recommended nomenclature of 1999 [Aebi et al., 1999].
[c]According to the recommended nomenclature of 2008 [Jaeken et al., 2008].

PMM2-CDG is the most frequent form of CDG in respect to the number of mutations identified to date, which sums up to 103 (Supp. Table S1) [Matthijs et al., 1997, 1999, 2000; Mizugishi, et al., 1999; Grunewald et al., 2001; Westphal et al., 2001a; Briones et al., 2002; Schollen et al., 2002, 2007; Tayebi et al., 2002; Callewaert et al., 2003; Ono et al., 2003; Coman et al., 2005; Le Bizec et al., 2005; Vuillaumier-Barrot et al., 2006; Vermeer et al., 2007; Wurm et al., 2007; Truin et al., 2008; Perez-Duenas et al., 2009; Thong et al., in press; Vega et al., 2009]. Three of them (p.Q37H, p.E197A, and p.A233T) occur only in combination with two other heterozygous mutations and are therefore assumed to be single nucleotide polymorphisms. The mutations are scattered over the *PMM2* gene and yield a broad range of protein defects (Fig. 2; Supp. Table S1). A total of 80 missense mutations affect 68 different amino acid residues. Five nonsense mutations introduce an early stop codon and lead to truncated PMM2 proteins. The seven base deletion and insertion mutations identified to date lead to frameshifts and thus to truncated proteins. A total of 10 splicing defects have been described, whereas the causative mutations were not only found at the splicing sites, but also as far as 15 kb in intronic regions [Schollen et al., 2007; Truin et al., 2008; Vega et al., 2009]. Finally, one patient displayed the complete loss of exon 8 due to a deletion mediated by an Alu retrotransposition [Schollen et al., 2007].

The human PMM2 enzyme is 246 amino acids long and shows a very high level of amino acid conservation among eukaryotes (Fig. 2). The crystal structure of its isozyme PMM1 has been solved [Silvaggi et al., 2006], which by extrapolation contributes to assign the impact of mutations on PMM2 protein function and stability. Interestingly, none of the amino acids constituting the active center have been found to be mutated in CDG. However, several mutations have been identified leading to declined substrate or cofactor binding. The arginines at position p.R150, p.R123, and p.R28 in PMM1 (equivalent to p.R141, p.R123, and p.R21 in PMM2) were shown to be involved in binding either the phosphate or the C2 hydroxyl group of the substrate [Silvaggi et al., 2006]. Substitution of these amino acids in PMM2 (Fig. 2) results in a severe clinical phenotype and it has been shown that homozygosity for the p.R141H mutation is not compatible with life [Matthijs et al., 1998]. The homodimeric interaction of PMM1 is mediated by a hydrophobic core, supported by surrounding hydrogen bonds and salt bridges [Silvaggi et al., 2006]. The mutations p.L104V, p.F119L, and p.I120T in PMM2 might disrupt the analogous hydrophobic core (p.L113, p.F128, and p.I129 in PMM1). Furthermore, the mutations p.E93A and p.N101K (p.E102 and p.N110 in PMM1) are suspected to impair the homodimeric interaction by deletion of a hydrogen bond and a salt bridge (Fig. 2) [Silvaggi et al., 2006]. Other mutations have been shown to destabilize the overall protein fold, thereby leading to reduced catalytic activity [Pirard et al., 1999].

MPI (MPI-CDG, CDG-Ib)

MPI-CDG can be detected biochemically by measuring MPI activity in patients' fibroblasts or leukocytes [Jaeken et al., 1998]. In doing so, 25 patients have been identified so far, exhibiting 18 different mutations in the *MPI* gene [Jaeken et al., 1998; Niehues et al., 1998; Babovic-Vuksanovic et al., 1999; de Lonlay et al., 1999; Schollen et al., 2000a, 2002; Westphal et al., 2001b; Vuillaumier-Barrot et al., 2002; Vuillaumier-Barrot, 2005; Penel-Capelle et al., 2003]. The clinical presentation of these patients is unique due to the fact that neurological symptoms are usually absent. Mostly affected are the gastrointestinal tract and the liver with symptoms such as diarrhea, vomiting, gastrointestinal bleeding, protein-losing enteropathy, hepatomegaly, and hepatic fibrosis. Additionally, coagulopathy, hypoglycemia, and thrombotic events have been observed in moderate to severe cases, including at least six lethal outcomes [Jaeken et al., 1998; Niehues et al., 1998; Babovic-Vuksanovic et al., 1999; de Lonlay et al., 1999; Westphal et al., 2001b; Vuillaumier-Barrot et al., 2002; Penel-Capelle et al., 2003]. Interestingly, MPI-CDG can effectively be treated by Man supplementation [Niehues et al., 1998; Babovic-Vuksanovic et al., 1999; de Lonlay et al., 1999; Westphal et al., 2001b; Penel-Capelle et al., 2003]. The orally-applied Man could be phosphorylated by hexokinases yielding Man-6-P, thereby enabling the functional bypass of the defective isomerase step [Panneerselvam and Freeze, 1996].

The human MPI enzyme is a soluble cytosolic protein of 423 amino acids (Fig. 3). The crystal structure of *Candida albicans* MPI [Cleasby et al., 1996] suggests that the protein is a metalloenzyme containing one zinc atom. Whereas most MPI domains show a rather low conservation status among eukaryotes, the central domain, forming the catalytic cleft, is highly conserved [Cleasby et al., 1996]. The human MPI shares 42% sequence homology with its *C. albicans* ortholog and is therefore assumed to present a similar protein fold.

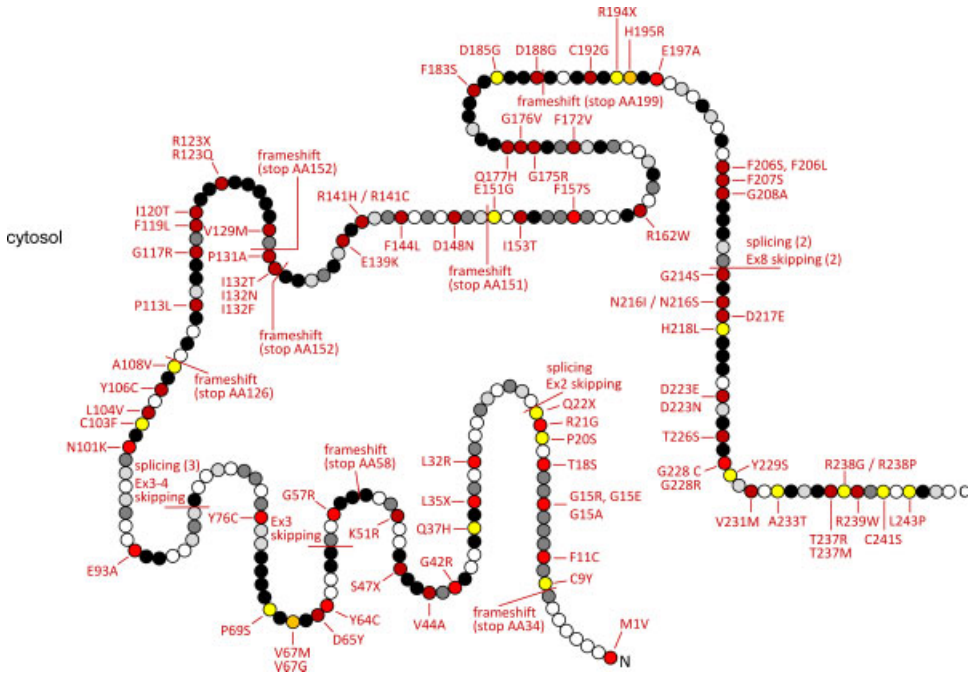


Figure 2. Schematic model of PMM2. PMM2 protein sequences were aligned using the ClustalW program [Thompson et al., 1994]. The *Homo sapiens* sequence (NP_000294.1) was compared to the rodent *Mus musculus* (NP_058577.1), to the zebra fish *Danio rerio* (NP_956378.1), to the fruit fly *Drosophila melanogaster* (NP_648589.1), to the nematode *Caenorhabditis elegans* (NP_502698.2), and to the budding yeast *Saccharomyces cerevisiae* (NP_116609.1). Sequences were obtained from the GenBank (www.ncbi.nlm.nih.gov/GenBank/index.html). Black dots represent strictly conserved amino acids, and dark gray, light gray, and white, represent those amino acids with a conservation of 83%, 67%, and less than 67%, respectively. Mis- and nonsense mutations are marked next to the affected amino acids. The dark red, light red, orange, and yellow dots mark the mutated amino acids that are conserved at 100%, 83%, 67%, and less than 67%, respectively. Splicing defects are marked with a bar and explicitly entitled in the case of assigned exon skipping. Further or unknown implications on the protein level of the affected enzyme are termed with splicing. Deletion or insertion mutations causing a frameshift are marked with a bar as well and marked as frameshift, whereas the position of the resulting premature stop codon is given in brackets.

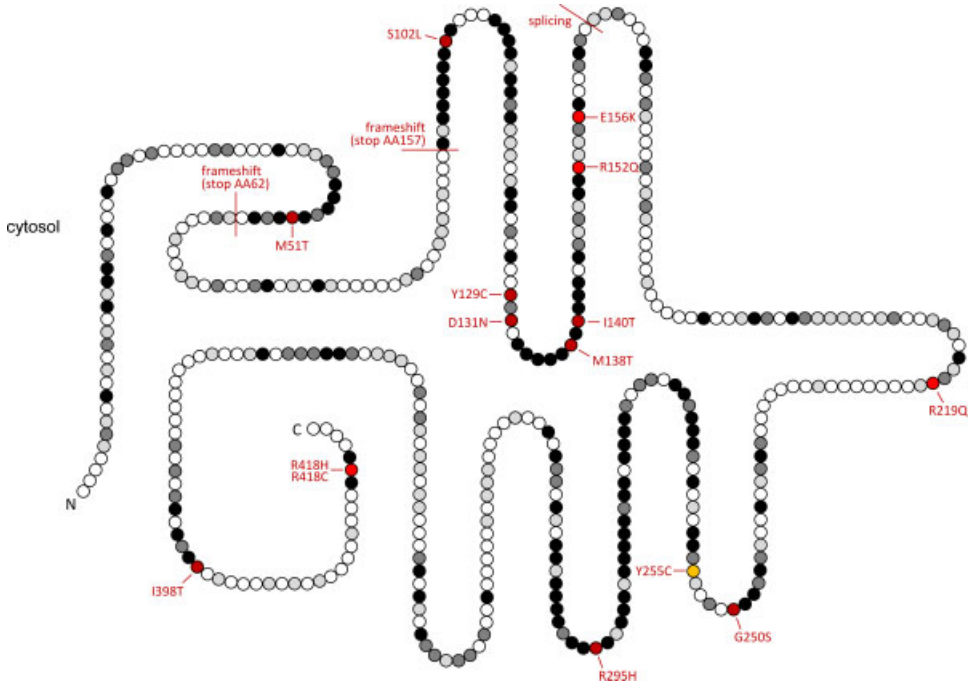


Figure 3. Schematic model of MPI. Mutations and the conservation of the MPI amino acids among the human (NP_002426.1), mouse (NP_080113.1), zebra fish (NP_001028282.1), fruit fly (NP_649940.1), nematode (NP_499174.3), and budding yeast (NP_010918.1) proteins were mapped as in Figure 2.

The 18 known mutations (Supp. Table S2) comprise a group of 15 point mutations, while only two frameshift-causing mutations and one splicing defect have been reported. Seven of these missense mutations map to the predicted catalytic domain, which ranges from cysteine p.C11 to phenylalanine p.F151 and from leucine p.L257 to arginine p.R322 based on the *C. albicans* MPI crystal structure (Fig. 3) [Cleasby et al., 1996]. The mutations of strictly conserved residues, such as p.M51T, p.S102L, p.M138T, and p.I140T, lie in closest proximity to the active site and might directly impede the enzymatic activity. The p.R295H mutation converts a strictly conserved arginine, which corresponds to p.R304 in the *C. albicans* enzyme. This positively charged residue is positioned at the border of the active site cleft and is proposed to be responsible for substrate phosphate binding [Cleasby et al., 1996]. The remaining missense mutations alter amino acids of various degrees of conservation, thereby most probably leading to a destabilization of the MPI protein fold (Fig. 3).

Group 2: Cytosolically-Acting Glycosyltransferases

The assembly of the dolichol-linked oligosaccharide required for N-glycosylation is initiated on the cytosolic side of the ER membrane and proceeds up to the formation of the intermediate dolichol-PP-$GlcNAc_2Man_5$ (Fig. 1). Mutations in genes encoding three involved glycosyltransferases, namely *DPAGT1*, *ALG1*, and *ALG2*, have been found to cause CDG.

DPAGT1 (DPAGT1-CDG, CDG-Ij)

Deficiency of the UDP-GlcNAc:dolichol-phosphate GlcNAc-1-P transferase initiating the biosynthetic pathway leads to DPAGT1-CDG [Wu et al., 2003]. With only three patients and three mutations identified, it represents a rare form of CDG [Wu et al., 2003; Vuillaumier-Barrot, 2005]. The clinical manifestations associated to DPAGT1 deficiency are unclear since only 1 of the 3 patients has been described clinically. This case presented with a developmental delay, microcephaly and exotropia, mental retardation, severe hypotonia, and intractable seizures [Wu et al., 2003]. Unfortunately, the clinical description of the two other DPAGT1-CDG patients, a pair of siblings, is not available [Vuillaumier-Barrot, 2005].

The human DPAGT1 is a hydrophobic protein of 408 amino acids that is predicted to span the ER membrane 10 times (Fig. 4). The five loops assumed to protrude to the cytosol are mostly conserved among eukaryotes, which supports their function as part of the active center. One of the two identified missense mutations alters a highly conserved isoleucine at position p.I297, which localizes to the last of the five cytosolic loops (Fig. 4; Supp. Table S3). The other point mutation (p.Y170C), which maps to the fifth transmembrane (TM) domain, was found in combination with a splicing defect originating from an unknown genetic reason [Wu et al., 2003]. The third identified mutation is another splicing defect, assigned to a mutation within the first intron (c.162–8G>A) [Vuillaumier-Barrot, 2005].

Mannosyltransferase 1 (ALG1-CDG, CDG-Ik)

The phenotype of ALG1-CDG is very severe, given that at least 4 out of 7 patients died in childhood. Common symptoms are dysmorphic features, microcephaly, intractable seizures, hypotonia, coagulopathy, and visual impairment [de Koning et al., 1998; Grubenmann et al., 2004; Kranz et al., 2004; Schwarz et al., 2004]. Individual patients present with nearly the complete set of CDG symptoms, including immunoglobulin G deficiency [Kranz et al., 2004] and recurrent nonimmune hydrops fetalis [de Koning et al., 1998].

The human *ALG1* gene encodes a β-1,4 mannosyltransferase, which catalyses the addition of the first Man to dolichol-PP-linked chitobiose. The 464–amino acid protein is predicted to have a type-I ER membrane topology with a large cytosolic C-terminal domain harboring the active site (Fig. 5). To date, four *ALG1* mutations have been described (Supp. Table S4) [de Koning et al., 1998; Grubenmann et al., 2004; Kranz et al., 2004; Schwarz et al., 2004]. However, 1 out of 3 heterozygous point mutations (p.D429E) mapped in a single CDG patient, most likely represents a single nucleotide polymorphism [Grubenmann et al., 2004]. The residual three-point mutations are spread all over the cytosolic domain, where they mainly convert weakly conserved amino acids (Fig. 5).

Mannosyltransferase 2 (ALG2-CDG, CDG-Ii)

The rarest form of CDG described to date is the ALG2 mannosyltransferase deficiency, with a single patient identified [Thiel et al., 2003]. This patient is only mildly affected with developmental delay, seizures, poor vision, coagulopathy, and delayed myelinization. Accordingly, it is not possible to draw any conclusion on the severity of ALG2-CDG from the description of this single case.

The α-1,3 mannosyltransferase ALG2 enzyme is 416 amino acids long and is predicted to form a type-I TM protein (Fig. 6). Like the ALG1 mannosyltransferase, its active site is cytosolically oriented and uses GDP-Man as donor substrate. Two heterozygous mutations were identified in the *ALG2* gene of the index patient (Supp. Table S5). The first induces a frameshift through a single-base deletion (c.1040delG). The effect of the second mutation is unclear, since the point mutation, c.393G>T, results in an amino acid exchange (p.K131N), but also seems to alter the stability of the *ALG2* transcript [Thiel et al., 2003].

Group 3: Luminally-Acting Mannosyltransferases

In the ER lumen, three mannosyltransferases catalyze the stepwise addition of the last four Man units, ending with the formation of dolichol-PP-$GlcNAc_2Man_9$. Those three enzymes are hydrophobic proteins with multiple TM domains and use dolichol-P-Man as donor substrate. All of them have been related to a form of CDG.

Mannosyltransferase 6 (ALG3-CDG, CDG-Id)

The clinical presentation was comparable in most of the 11 ALG3-CDG patients and could be summarized as moderate with mainly neurological symptoms. Accordingly, the patients present with a failure to thrive, psychomotor retardation, epilepsy, and microcephaly. Facial dysmorphism, hypotonia, and visual impairment are also observed in the majority of the cases [Stibler et al., 1995; Denecke et al., 2005; Schollen et al., 2005; Sun et al., 2005a; Kranz et al., 2007c; Rimella-Le-Huu et al., in press]. However, a particular feature of this form of CDG constitutes deformations of hands and feet [Denecke et al., 2005; Schollen et al., 2005; Sun et al., 2005a; Kranz et al., 2007c; Rimella-Le-Huu et al., in press], which are not commonly seen in CDG.

After being flipped into the ER lumen, the dolichol-PP-$GlcNAc_2Man_5$ intermediate is elongated for one Man unit by the *ALG3*-encoded α-1,3 mannosyltransferase 6. It is predicted to span the ER membrane seven times within its 438-amino acid sequence (Fig. 7). The asparagines at positions p.N83 and p.N253 represent potential N-glycosylation sites. The TM domains and the loops protruding to the ER lumen display the highest level of conservation among eukaryotes.

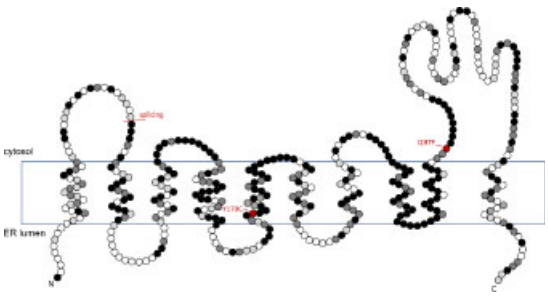


Figure 4. Schematic model of DPAGT1. Mutations and the conservation of the DPAGT1 amino acids among the human (NP_001373.2), mouse (NP_031901.2), zebra fish (NP_001082880.1), fruit fly (NP_609608.1), nematode (NP_507859.2), and budding yeast (NP_009802.1) proteins were mapped as in Figure 2. The membrane topology prediction of the DPAGT1 enzyme was performed running the TMpred (www.ch.embnet.org/software/TMPRED_form.html), the TMHMM (www.cbs.dtu.dk/services/TMHMM-2.0) [Krogh et al., 2001], the DAS (www.sbc.su.se/~miklos/DAS) [Cserzo et al., 1997], and the SOSUI (http://bp.nuap.nagoya-u.ac.jp/sosui/sosui_submit.html) [Hirokawa et al., 1998] algorithms.

Figure 5. Schematic model of mannosyltransferase 1 (ALG1). Mutations and the conservation of the ALG1 amino acids among the human (NP_061982.3), mouse (NP_663337.2), zebra fish (NP_956161.1), fruit fly (NP_650662.1), nematode (AAC77507.2), and budding yeast (NP_009668.1) proteins were mapped as in Figure 2. Membrane topology prediction of the mannosyltransferase 1 was performed as for the DPAGT1 enzyme in Figure 4.

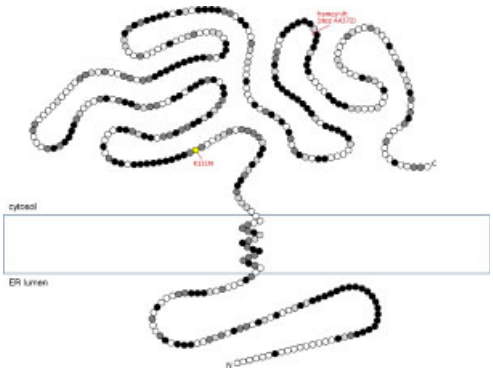


Figure 6. Schematic model of mannosyltransferase 2 (ALG2). Mutations and the conservation of the ALG2 amino acids among the human (NP_149078.1), mouse (NP_064382.3), zebra fish (NP_001098406.1), fruit fly (NP_647772.1), nematode (NP_495010.2), and budding yeast (NP_011450.1) proteins were mapped as in Figure 2. Membrane topology prediction of the mannosyltransferase 2 was performed as for the DPAGT1 enzyme in Figure 4.

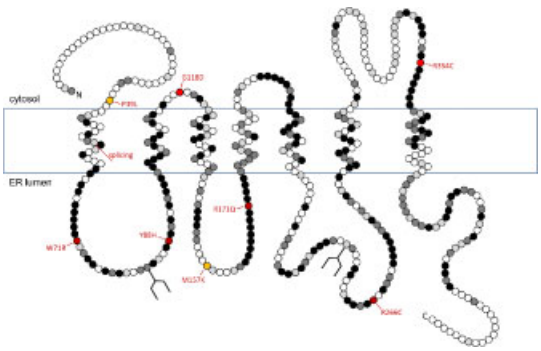


Figure 7. Schematic model of mannosyltransferase 6 (ALG3). Mutations and the conservation of the ALG3 amino acids among the human (NP_005778.1), mouse (NP_666051.2), zebra fish (NP_001018532.1), fruit fly (NP_523829.2), nematode (NP_496950.2), and budding yeast (NP_009471.1) proteins were mapped as in Figure 2. Membrane topology prediction of the mannosyltransferase 6 was performed as for the DPAGT1 enzyme in Figure 4. Two potential N-glycosylation sites at position p.N83 and p.N253 are shown schematically.

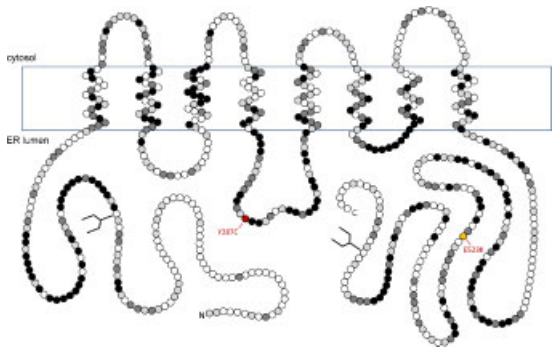


Figure 8. Schematic model of mannosyltransferase 7-9 (ALG9). Mutations and the conservation of the ALG9 amino acids among the human (NP_001071158.1), mouse (NP_598742.1), zebra fish (CAN88585.1), fruit fly (NP_651353.1), nematode (NP_496282.2), and budding yeast (NP_014180.1) proteins were mapped as in Figure 2. Membrane topology prediction of the mannosyltransferase 7-9 was performed as for the DPAGT1 enzyme in Figure 4. Two potential N-glycosylation sites at position p.N77 and p.N593 are shown schematically.

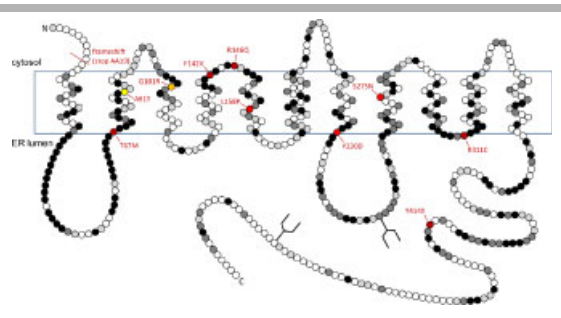


Figure 9. Schematic model of mannosyltransferase 8 (ALG12). Mutations and the conservation of the ALG12 amino acids among the human (NP_077010.1), mouse (EDL04396.1), zebra fish (NP_001092219.1), fruit fly (NP_649939.1), nematode (NP_505071.1), and budding yeast (NP_014427.1) proteins were mapped as in Figure 2. Membrane topology prediction of the mannosyltransferase 8 was performed as for the DPAGT1 enzyme in Figure 4. Two potential N-glycosylation sites at position p.N250 and p.N463 are shown schematically.

The 11 ALG3-CDG cases account for eight point mutations and a single splicing defect (Supp. Table S6) [Stibler et al., 1995; Körner et al., 1999; Schollen et al., 2002, 2005; Denecke et al., 2004, 2005; Sun et al., 2005a; Kranz et al., 2007c; Rimella-Le-Huu et al., in press]. Five of these missense mutations lead to substitutions of highly conserved amino residues within the luminally oriented loops (Fig. 7). Considering their degree of conservation and their orientation with respect to the ER membrane, these mutated amino acids are likely to be involved in the formation of the active center.

Mannosyltransferase 7-9 (ALG9-CDG, CDG-II)

Only three cases of ALG9-CDG have been characterized to date [Frank et al., 2004; Weinstein et al., 2005; Vleugels et al., 2009]. The corresponding patients presented with typical CDG symptoms such as developmental delay, psychomotor retardation, hypotonia, seizures, hepatomegaly, microcephaly, and pericardial effusion. Gastrointestinal problems [Vleugels et al., 2009] and bronchial asthma [Frank et al., 2004] were reported in one case. This form of CDG exhibits no unique feature and can be classified as moderate, although the three cases described to date do not allow a conclusion to be drawn on the clinical picture.

The two identified mutations (Supp. Table S7) occur homozygously in the *ALG9* gene, which codes for a 611–amino acid α-1,2 mannosyltransferase. The enzyme, which is predicted to include eight TM domains and two N-glycosylation sites, catalyzes the addition of the seventh and the ninth Man of the dolichol-linked oligosaccharide. The mutations p.Y287C and p.E523K lie within conserved loops protruding into the ER lumen (Fig. 8) [Frank et al., 2004; Weinstein et al., 2005; Vleugels et al., 2009].

Mannosyltransferase 8 (ALG12-CDG, CDG-Ig)

Six of the eight characterized ALG12-CDG patients present a similar set of clinical features, including facial dysmorphism, psychomotor retardation, developmental delay, hypotonia, and decreased coagulation factors. Prominent also are respiratory impairment and feeding problems, and the absence of seizures, gastrointestinal, and hepatic symptoms [Chantret et al., 2002; Grubenmann et al., 2002; Thiel et al., 2002; Di Rocco et al., 2005; Eklund et al., 2005a]. The low levels of serum immunoglobulin G define a possible indicator for mannosyltransferase 8 deficiency. The overall moderate severity of ALG12-CDG was also supported by the observation that some patients were actually able to walk and showed speech involvement [Eklund et al., 2005a]. However, two ALG12-CDG siblings demonstrate a much more severe disease [Kranz et al., 2007a]. In addition to the symptoms previously mentioned, these two patients presented with skeletal dysplasia, generalized edema and audiovisual impairment. Both patients died within the first two years of life.

The α-1,6 mannosyltransferase 8, encoded by the human *ALG12* gene, transfers the eighth Man from dolichol-P-Man to the dolichol-linked oligosaccharide. The 488–amino acid protein is predicted to include 11 TM domains (Fig. 9). The enzyme is conserved among eukaryotes, whereas TM domains and the predicted loops oriented to the ER display the highest level of conservation. Two asparagines at positions p.N250 and p.N463 represent consensus sequences for N-glycosylation.

To date, eight ALG12-CDG cases have been described encompassing 11 mutations, of which nine are missense, one nonsense, and one is a single-base deletion causing a frameshift (Supp. Table S8) [Chantret et al., 2002; Grubenmann et al., 2002; Thiel et al., 2002; Di Rocco et al., 2005; Eklund et al., 2005a; Kranz et al., 2007a]. Noteworthy, nearly all mutations are mapped to TM domains or to their borders and most of these mutations lead to substitution of highly conserved amino acids (Fig. 9). The siblings, which presented with the more severe clinical features, were compound heterozygous for the p.G101R and the p.R146Q mutations [Kranz et al., 2007a]. While the first mutation is so far unique, the second was already discovered in another case of ALG12-CDG in combination with the p.T67M mutation [Grubenmann et al., 2002]. According to the severe clinical outcome, the p.G101 and the p.R146 residues seem to be essential for ALG12 functionality. This assumption is supported by finding that the glycine at position p.G101 is mainly conserved among eukaryotes, while the arginine at position p.R146 is even strictly conserved in all eukaryotic model organisms examined (Fig. 9).

Figure 10. Schematic model of glucosyltransferase 1 (ALG6). Mutations and the conservation of the ALG6 amino acids among the human (NP_037471.2), mouse (NP_001074733.1), fruit fly (NP_609393.1), nematode (NP_495685.1), and budding yeast (NP_014644.1) proteins were mapped as in Figure 2. The protein sequence of *Tetraodon nigroviridis* (CAG11585.1) was used instead of the zebra fish protein, since only a truncated *D. rerio* isoform could be retrieved from the genome database. Membrane topology prediction of the glucosyltransferase 1 was performed as for the DPAGT1 enzyme in Figure 4. A potential N-glycosylation site at position p.N59 is shown schematically.

Group 4: Luminally-Acting Glucosyltransferases

The biosynthesis of the dolichol-linked oligosaccharide is completed by the successive addition of three Glc units (Fig. 1). This task is achieved by three ER membrane glucosyltransferases, encoded by the *ALG6*, the *ALG8* and the *ALG10* gene, which utilize dolichol-P-Glc as donor substrate. While defects in the first two enzymes have been associated to CDG, no mutation in the *ALG10* gene has so far been identified.

Glucosyltransferase 1 (ALG6-CDG, CDG-Ic)

The deficiency of ALG6 glucosyltransferase is the second most frequent form of CDG after PMM2-CDG with 36 cases registered so far and representing 20 distinct mutations [Imbach et al., 1999; Grunewald et al., 2000; Hanefeld et al., 2000; Imbach et al., 2000a; Westphal et al., 2000a,b, 2003; de Lonlay et al., 2001; Schollen et al., 2002; Newell et al., 2003; Sun et al., 2005b; Vuillaumier-Barrot, 2005; Eklund et al., 2006]. The clinical presentation could be described as mild to moderately severe, with psychomotor retardation, developmental delay, seizures, hypotonia, coagulopathy, feeding problems, and visual impairment. The occurrence of strong dysmorphic features, gastrointestinal problems, or protein-losing enteropathy is rather rare.

The ALG6 α-1,3 glucosyltransferase is 507 amino acids long and is predicted to span the ER membrane 11 times (Fig. 10). The 20 *ALG6* mutations represent nine missense, one nonsense, four splicing, and five deletion mutations (Supp. Table S9). In one case, a portion of the chromosome 1 including the *ALG6* gene is deleted as a de novo event [Eklund et al., 2006]. Two of the identified point mutations (p.Y131H and p.F304S) are assumed to be single nucleotide polymorphisms [Vuillaumier-Barrot et al., 2001; Westphal et al., 2003]. While these single nucleotide polymorphisms do not appear to be pathogenic by themselves, they may lead to reduced N-glycosylation when combined to other mutations along the pathway of dolichol-linked oligosaccharide assembly [Westphal et al., 2002]. Surprisingly, 3 out of the 5 deletion mutations lead to an in-frame removal of isoleucine at position p.I299 [Hanefeld et al., 2000; Westphal et al., 2000b; Sun et al., 2005b]. The deletion of three consecutive base triplets in three independent patients determines a deletion hotspot. The amino acids surrounding p.I299 are encoded to some extend by the DNA repeat

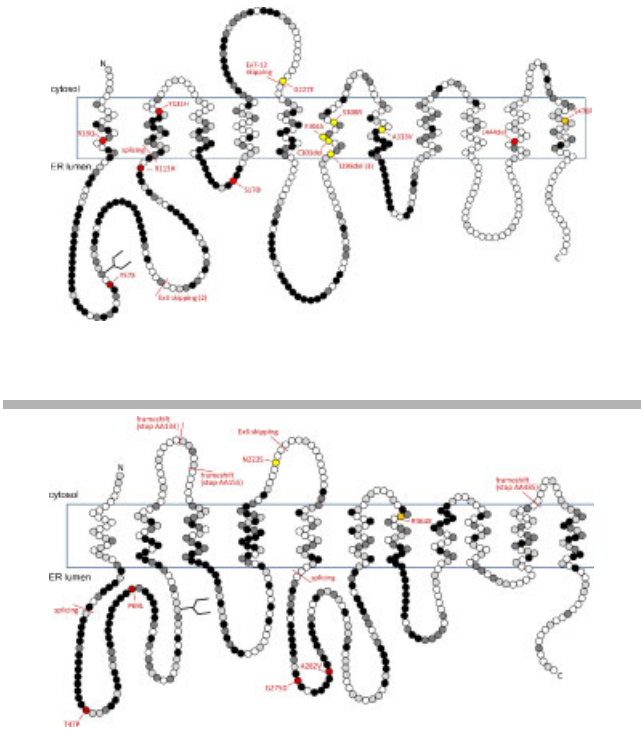


Figure 11. Schematic model of glucosyltransferase 2 (ALG8). Mutations and the conservation of the ALG8 amino acids among the human (NP_076984.2), mouse (NP_950200.2), zebra fish (NP_001017647.1), fruit fly (NP_572355.1), nematode (NP_001021940.1), and budding yeast (NP_014710.1) proteins were mapped as in Figure 2. Membrane topology prediction of the glucosyltransferase 2 was performed as for the DPAGT1 enzyme in Figure 4. A potential N-glycosylation site at position p.N96 is shown schematically.

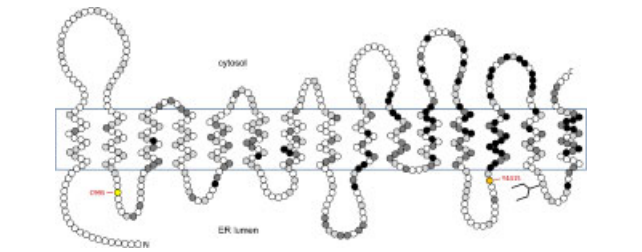


Figure 12. Schematic model of dolichol kinase (DOLK). Mutations and the conservation of the DOLK amino acids among the human (NP_055723.1), mouse (NP_808316.1), zebra fish (NP_001103954.1), fruit fly (NP_611139.1), nematode (NP_001022925.1), and budding yeast (NP_013726.1) proteins were mapped as in Figure 2. The *C. elegans* dolichol kinase is much shorter than the other eukaryotic orthologs, leading to an overall reduced conservation grade within the N-terminal part. Membrane topology prediction of the dolichol kinase was performed as for the DPAGT1 enzyme in Figure 4. A potential N-glycosylation site at position p.N500 is shown schematically.

Figure 15. Schematic model of RFT1. Mutations and the conservation of the RFT1 amino acids among the human (NP_443091.1), mouse (NP_808483.2), zebra fish (XP_688354.3), fruit fly (NP_572246.1), nematode (NP_001023610.1), and budding yeast (NP_009533.1) proteins were mapped as in Figure 2. Membrane topology prediction of the RFT1 protein was performed as for the DPAGT1 enzyme in Figure 4. A potential N-glycosylation site at position p.N227 is shown schematically.

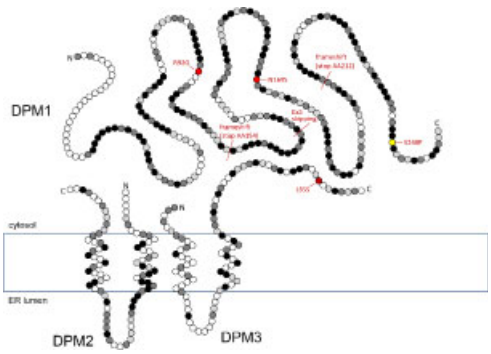


Figure 13. Schematic model of dolichol-P-Man synthase (DPM1/2/3). Mutations and the conservation of the DPM1 amino acids among the human (NP_003850.1), mouse (NP_034202.1), zebra fish (NP_001003596.1), fruit fly (NP_609980.1), nematode (NP_499931.2), and budding yeast (NP_015509.1) proteins were mapped as in Figure 2. Conservation of the DPM2 amino acids among the human (NP_003854.1), mouse (NP_034203.1) and zebra fish (NP_001116318.1) proteins and of the DPM3 amino acids among the human (NP_714963.1), mouse (NP_081043.1), zebra fish (NP_957103.2), fruit fly (NP_001034051.1), and nematode (NP_502366.1) proteins and the DPM3 mutation were also mapped as in Figure 2. Given that the *S. cerevisiae* dolichol-P-Man synthase is a monomeric enzyme [Maeda and Kinoshita, 2008], the DPM2 (NP_595676.1) and DPM3 (NP_596640.1) protein sequences from *Schizosaccharomyces pombe* were used for the particular alignments. Additionally, the aberrant DPM2 sequences of *D. melanogaster* and *C. elegans* were displaced by the DPM2 proteins of *Drosophila ananassae* (EDV40477.1) and *Dictyostelium discoideum* (XP_644349.1), respectively. Membrane topology prediction of the dolichol-P-Man synthase subunits 2 and 3 was performed as for the DPAGT1 enzyme in Figure 4. The organization of the entire complex was adapted from Maeda and Kinoshita [2008].

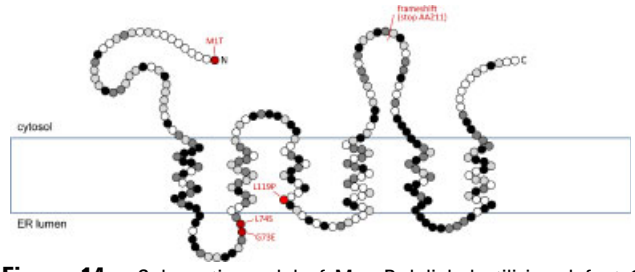
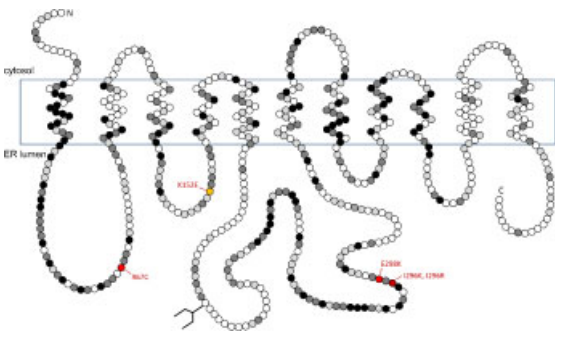


Figure 14. Schematic model of Man-P-dolichol utilizing defect 1 (MPDU1). Mutations and the conservation of the MPDU1 amino acids among the human (NP_004861.2), mouse (NP_036030.2), zebra fish (NP_001002130.1), fruit fly (NP_608889.1), and nematode (NP_505155.1) proteins were mapped as in Figure 2. Since MPDU1 is neither present in *S. cerevisiae* nor in *S. pombe*, the orthologous protein of *Aspergillus niger* (XP_001401680.1) was used for the alignment. Membrane topology prediction of the Man-P-dolichol utilizing defect 1 protein was performed as for the DPAGT1 enzyme in Figure 4.



c.382TAATAAT, which might facilitate deletions. The majority of the mutations affect amino acids positioned within the 11 TM domains (Fig. 10). This fact suggests that TM domains might not only play an important role in defining the protein structure, but also in catalysis, presumably through the binding to dolichol-linked substrates. Two missense mutations (p.R113H and p.S170I) change strictly conserved amino residues mapping to ER luminal loops, which might constitute part of the active site (Fig. 10).

Glucosyltransferase 2 (ALG8-CDG, CDG-Ih)

Whereas ALG6 deficiency yields a rather mild form of CDG, mutations in the *ALG8* glucosyltransferase gene lead to a severe form of the disease. Five of nine ALG8-deficient patients died within the first months of life [Schollen et al., 2004; Eklund et al., 2005b; Stölting et al., in press; Vesela et al., in press]. These cases were accompanied by multiple symptoms such as strong dysmorphic features, hypotonia, gastrointestinal disorders, hepatomegaly, coagulopathy, edema, cardiorespiratory problems, protein-losing enteropathy, and ascites. Remarkably, neurologic involvement was rather minimal in most of the patients [Chantret et al., 2003; Schollen et al., 2004; Eklund et al., 2005b; Vesela et al., in press]. However, a pair of siblings displayed a much milder form of ALG8 deficiency. Besides dysmorphic features, they presented with hypotonia, ataxia, and mental retardation [Stölting et al., in press].

The ALG8 α-1,3 glucosyltransferase is predicted to be an 11 TM domain ER protein with a size of 526 amino acids (Fig. 11). To date, 12 mutations have been described (Supp. Table S10) [Chantret et al., 2003; Schollen et al., 2004; Eklund et al., 2005b; Stölting et al., in press; Vesela et al., in press]. The p.N222S mutation is likely to represent a single nucleotide polymorphism, since the healthy father of a patient is homozygous for this mutation [Schollen et al., 2004]. The other 11 mutations segregate into four missense mutations, one nonsense mutation, three splicing defects, and three frameshift mutations. The missense mutations p.T47P, p.P69L, p.G275D, and p.A282V locate to ER luminal domains and lead to substitutions of strictly conserved amino acids (Fig. 11), thereby probably disrupting the enzymatic center. The residual mutations, leading to truncated forms of the ALG8 protein, decrease the catalytic ability of the affected enzyme drastically. This might, at least partly, explain the severe progression of ALG8-CDG.

Group 5: Proteins Affecting Dolichol-Linked Carbohydrates

In addition to the cytosolic PMM2 and PMI enzymes and the ER glycosyltransferases, a handful of proteins are also involved in the biosynthesis of the dolichol-linked oligosaccharide required for N-glycosylation. Some of these proteins have an established activity, whereas others represent essential components without clearly assigned functions. By phosphorylating dolichol, the dolichol kinase enzyme enables the transfer of GlcNAc-P to dolichol-P, thereby initiating the biosynthesis of dolichol-linked oligosaccharide (Fig. 1) [Shridas and Waechter, 2006]. The products of the *DPM* genes form a trimeric complex that catalyses the synthesis of dolichol-P-Man [Maeda and Kinoshita, 2008]. In contrast to the previous enzymes, the function of the MPDU1 and RFT1 proteins can only be predicted. MPDU1 and its hamster ortholog Lec35 have been proposed to be involved in the utilization of the sugar donor substrates dolichol-P-Man and -Glc [Anand et al., 2001; Schenk et al., 2001]. The RFT1 protein has been demonstrated to be involved in the translocation of the dolichol-linked $GlcNAc_2Man_5$ intermediate into the ER lumen [Helenius et al., 2002]. Although the proteins included in this group have diverse biological functions, they all yield to a CDG phenotype in case of mutation.

Dolichol kinase (DOLK-CDG, CDG-Im)

Four cases of DOLK-CDG have been reported to date [Kranz et al., 2007b]. The loss of dolichol kinase affects the biosynthesis of dolichol-linked oligosaccharides; therefore, it is also classified as a form of CDG. All four reported patients died in early childhood [Kranz et al., 2007b]. This very severe clinical phenotype is marked by hypotonia, skin disorders, and the loss of hair. Individual patients also presented with cardiomyopathy, seizures, hypoglycemia, microcephaly, and visual impairment [Kranz et al., 2007b].

The mainly hydrophobic 538 amino acids of the dolichol kinase are predicted to form a 15 TM domain protein, which might be glycosylated at asparagine p.N500 (Fig. 12). This prediction differs slightly with an earlier model, which assigned 13 membrane-spanning domains [Shridas and Waechter, 2006]. Nevertheless, the N- and the C-termini, as well as the strongly conserved putative CTP-binding pocket (p.S459–p.E474) respect the experimentally proven orientation of the human enzyme regarding the ER membrane (Fig. 12) [Shridas and Waechter, 2006]. The level of sequence conservation of the N-terminal region is relatively low, because the *Caenorhabditis elegans* dolichol kinase, with its 281 amino acids, is much shorter than the average size of dolichol kinases in other organisms. This short enzyme overlaps mainly within the C-terminal part, containing the putative CTP-binding domain (Fig. 12). One of the two homozygously occurring point mutations converts a mainly conserved tyrosine (p.Y441S) in the C-terminal part of the kinase (Supp. Table S11). The other mutation leads to the alteration of a nonconserved cysteine at position p.C99 to a serine, this time in the N-terminal part of the enzyme (Fig. 12). The severe outcome of two trivial point mutations indicates that accurate dolichol kinase function is essential for viability. This statement might not only be due to a defect of N-glycosylation since other functions are assigned to dolichol and dolichol-P [Swiezewska and Danikiewicz, 2005].

Dolichol-P-Man synthase (DPM1-CDG, CDG-Ie and DPM3-CDG, CDG-Io)

Recurrent seizures, hypotonia, developmental delay, dysmorphic features, microcephaly, visual impairment, and in some cases ataxia and coagulopathy are the most prominent symptoms found in DPM1-CDG patients [Imbach et al., 2000b; Kim et al., 2000; Garcia-Silva et al., 2004; Dancourt et al., 2006]. The individual cases exhibit certain clinical variations, although all are in the range of moderate to severe. The index DPM3-CDG patient described recently [Lefeber et al., 2009] presented with a very mild phenotype. Except for a mild myopathy, a dilated cardiomyopathy, moderate muscular dystrophy, and a single stroke-like episode, the adult patient is able to lead a virtually normal life.

In humans, dolichol-P-Man synthase is an oligomeric enzyme complex assembled by the *DPM1*, *DPM2*, and *DPM3* gene products. So far, the 260-amino acid-long catalytic subunit DPM1 and the tethering polypeptide DPM3 have been described as causes of CDG. DPM2 and DPM3, both ER membrane proteins with two TM domains each and a length of 84 and 92 amino acids, respectively, are required to target the cytosolic DPM1 protein to the ER membrane. While DPM3 interacts directly with DPM1, DPM2 stabilizes the complex by binding to DPM3 (Fig. 13) [Maeda and Kinoshita, 2008].

To date, eight DPM1-CDG patients have been described, representing six distinct mutations (Supp. Table S12) [Imbach et al., 2000b; Kim et al., 2000; Garcia-Silva et al., 2004; Vuillaumier-Barrot, 2005; Dancourt et al., 2006]. Three of the mutations are deletions or splice defects and lead all to a frameshift and hence to truncated forms of the enzyme. The three point mutations convert single amino acids of various conservation levels at different sites of the DPM1 protein (Fig. 13). In *DPM3*, the homozygous point mutation p.L85S converts a strictly conserved leucine residue within the terminal coiled-coil domain, which is required for tethering the catalytic DPM1 subunit to the ER membrane (Supp. Table S13) [Lefeber et al., 2009]. Notably, mutations in the DPM complex lead not only to a disorder of N-glycosylation, but O-mannosylation and glycosylphosphatidylinositol-anchor formation are also impaired, since dolichol-P-Man is also utilized as a donor substrate for these posttranslational modifications [Maeda and Kinoshita, 2008].

Man-P-dolichol utilizing defect 1 (MPDU1-CDG, CDG-If)

The clinical outcome of the four described MPDU1-CDG patients is variable. While one patient died in early childhood due to a seizure-induced apnea, the others present with mild to moderately severe phenotypes. Shared symptoms are psychomotor retardation, seizures, hypotonia, gastrointestinal problems, visual impairment, and, as an atypical hallmark, skin disorders [Kranz et al., 2001; Schenk et al., 2001].

The human *MPDU1* gene, whose product is orthologous to the Chinese hamster Lec35 protein, encodes a 247–amino acid ER membrane protein that is predicted to form six TM domains (Fig. 14). Despite its important role in mammalian N-glycosylation, no Lec35 ortholog could be identified in the yeast *S. cerevisiae*. The Man-P-dolichol utilizing defect 1 protein has been proposed to be involved in the lateral distribution of dolichol-P-Man and dolichol-P-Glc within the ER membrane [Schenk et al., 2001]. It is therefore important for the availability of these sugar substrates to the ER mannosyl- and glucosyltransferases. Accordingly, MPDU1 deficiency leads to the accumulation of dolichol-PP-$GlcNAc_2Man_5$ and dolichol-PP-$GlcNAc_2Man_9$ intermediates [Kranz et al., 2001; Schenk et al., 2001]. The four cases of MPDU1-CDG characterized to date revealed five mutations (Supp. Table S14). Four of them are common missense mutations and the fifth is the deletion of a single nucleotide (c.511delC), which leads to a frameshift [Kranz et al., 2001; Schenk et al., 2001]. The mutation of the start methionine in one of the patient's allele switches the translation start to the next methionine in a different frame and thus leads to the loss of the MPDU1 protein [Schenk et al., 2001]. The other point mutations affect highly conserved amino acids throughout the protein (Fig. 14).

RFT1 (RFT1-CDG, CDG-In)

Mutations in the *RFT1* gene have only been described recently. They lead to the accumulation of dolichol-PP-$GlcNAc_2Man_5$ [Haeuptle et al., 2008; Vleugels et al., in press]. The exact function of the RFT1 protein is currently intensively discussed. It is definitely involved in the translocation of the dolichol-linked oligosaccharide $GlcNAc_2Man_5$ into the ER lumen, but it has still to be demonstrated whether RFT1 is the flippase itself [Helenius et al., 2002; Frank et al., 2008]. At any rate, mutations in the *RFT1* gene lead to a disorder of N-glycosylation termed RFT1-CDG [Haeuptle et al., 2008]. At least 2 out of the 6 patients died in childhood, suggesting a severe phenotype. Common clinical features include developmental delay, hypotonia, seizures, feeding problems, dysmorphic features, and sensorineural deafness, which could be taken as a characteristic feature. In addition, some patients suffered from coagulopathy, visual impairment, and respiratory problems [Haeuptle et al., 2008; Vleugels et al., in press].

The human RFT1 protein with its 541 amino acids spans the ER membrane 11 times according to prediction algorithms (Fig. 15). The asparagine at position p.N227 might be N-glycosylated. All five identified point mutations convert strongly conserved amino acids (Supp. Table S15) [Haeuptle et al., 2008; Vleugels et al., in press]. Interestingly, one patient is heterozygous for two different point mutations affecting the same nucleotide (c.887T>A/G) and consequently modifying the same amino acid differently (p.I296K/R). All mutated residues are localized to the first three ER luminally-oriented loops of the RFT1 protein. These loops display, together with the TM domains, an overall high conservation among species and might therefore be important for RFT1 functionality (Fig. 15).

Discussion

The comparison of nearly 1,000 cases of deficient dolichol-linked oligosaccharide biosynthesis (Table 1) conveys interesting facts regarding the clinical severity of the disease in relation to the position of the genetic defect along the biosynthetic pathway. The biosynthesis of dolichol-linked oligosaccharides is sequential [Kornfeld and Kornfeld, 1985] (Fig. 1), meaning that a block along the pathway will result in the accumulation of an incomplete oligosaccharide. The oligosaccharyltransferase (OST) complex preferentially transfers the complete oligosaccharide to asparagine residues on acceptor proteins, but incomplete oligosaccharides can to some extent be recognized by OST and transferred to proteins, yet at a low efficiency [Körner et al., 1999; Cipollo et al., 2001]. It is expected that this efficiency decreases with the degree of incompleteness of the dolichol-linked oligosaccharide. Accordingly, deficiency of ALG3 and ALG9 should be more severe than deficiency of ALG6 and ALG8. Furthermore, deficiency of cytosolically-active enzymes like ALG1 and ALG2 should be even more severe, because the accumulating dolichol-linked oligosaccharides remain unavailable to the luminally-oriented OST complex. This gradation in the level of N-glycosylation output based on the position of the genetic defect is clearly visible in yeast glycosylation mutants [Huffaker and Robbins, 1982; Kukuruzinska and Robbins, 1987; Jackson et al., 1993; Stagljar et al., 1994; Burda and Aebi, 1998]. However, such a position effect is not clear-cut when examining human CDG cases. This model would predict that the terminal glucosylation defects seen in ALG6 and ALG8 deficiency would be milder than the mannosylation defects seen in ALG3, ALG9, and ALG12 deficiency, for example. However, the association of ALG8 deficiency with a very severe disease [Schollen et al., 2004] indicates that even dolichol-linked oligosaccharides lacking the terminal two Glc residues account for a profound disorder of N-glycosylation. The severity of ALG8-CDG suggests that protein underglycosylation may not be the only defect underlying the disease. Since the terminal Glc residues of N-glycans are involved in the quality control of glycoprotein folding [Ellgaard and Helenius, 2003], it is possible that glucosylated dolichol-linked oligosaccharides participate in the regulation of this process, too, such as through the regulation of glucosidase-II activity [Deprez et al., 2005]. Consequently, alteration of glycoprotein folding might also account for the severity of ALG8-CDG.

The absence of ALG10 defects among CDG cases described to date is surprising. The ALG10 enzyme catalyzes the addition of the third Glc [Burda and Aebi, 1998] of the dolichol-linked oligosaccharide (Fig. 1). Assuming that all defects of dolichol-linked oligosaccharide biosynthesis lead to a disorder of N-glycosylation, ALG10 defects would be expected to present with clinical features typical of CDG. Hence, it is puzzling that no case of ALG10 deficiency has yet been documented. On the other hand, this absence of ALG10 deficiency may be explained if the defect indeed does not significantly impair the process of N-glycosylation, thereby remaining clinically undetectable. At the other side of the scale, it could be that *ALG10* is essential for embryonic development and that even a minor decrease of ALG10 activity may not be compatible with life. To further address this question, it is important to continue screening for new forms of CDG.

The identification of additional mutations and gene defects, especially in the glycosylation genes that have not yet been associated to CDG (Fig. 1), will provide additional evidence on the relationship between the extent of N-glycosylation, the position along the biosynthetic pathway, and the severity of the clinical picture. Interestingly, a similar absence of position effect is also observed in the forms of congenital muscular dystrophies that are caused by defects of O-mannosylation. In fact, the clinical severity of the disorders is related to the nature of the mutation rather than by the position of the mutated gene along the O-mannosylation pathway [Godfrey et al., 2007].

Clinical Relevance

The analysis of mutations identified to date shows that 150 out of the total 203 genetic alterations (Table 1) are missense mutations, whereas 112, i.e., 75%, of these mutations affect highly conserved amino acid residues among eukaryotic proteins. Since conserved amino acids are usually parts of functional motifs [Kinch and Grishin, 2002], it can be assumed that most CDG mutations alter the functions of the affected proteins. Accordingly, mutations of lowly conserved amino acids would yield minor enzymatic deficiencies that may be accompanied by mild symptoms or even remain asymptomatic. Along this line, it can be predicted that proteins showing the highest degree of sequence conservation among eukaryotes are likely to be more often associated with a disease phenotype. In case of dolichol-linked oligosaccharide biosynthesis, the PMM2 protein is by far the most conserved protein of the pathway (Fig. 2). Supporting this fact, half of the known mutations (103/203) affect the *PMM2* gene (Table 1). However, other proteins of the pathway also show a high degree of sequence conservation among eukaryotes, as for example the MPI and DPAGT1 proteins (Figs. 3 and 4). Yet only three mutations have been found in the *DPAGT1* gene so far, indicating that sequence conservation alone does not account for the incidence of a genetic disease.

By examining the occurrence of *PMM2* mutations, Schollen et al. [2000b] noticed that the p.R141H mutation is very prevalent in the European population with a carrier frequency of about 1 out of 70. Since this mutation is never found at the homozygous state in CDG patients [Matthijs et al., 1998] due to its inactivating properties on PMM2 activity, it can only be assumed that a selection pressure accounts for the maintenance of this mutation in human populations. However, the nature of this selection pressure is unknown and one can only speculate as to whether a reduction of N-glycosylation is related to reducing the susceptibility to pathogens binding N-glycans as receptors. Such arguments are beyond the scope of this review, but they certainly provide exciting points of reflection when discussing the biological and evolutionary relevance of glycosylation disorders.

Future Prospects

The analysis of mutations in genes involved in dolichol-linked oligosaccharide biosynthesis shows that they mainly affect conserved amino acid residues, thereby impairing protein function. Since a complete loss of protein function is usually not compatible with life, as seen in mice lacking PMM2, MPI, and DPAGT1 activity [Marek et al., 1999; DeRossi et al., 2006; Thiel et al., 2006], the mutations encountered in CDG certainly enable a significant level of N-glycosylation output. At the threshold of normal N-glycosylation, the transferrin IEF test may be not sensitive enough to detect N-glycosylation defects caused by mild mutations. The study of these mutations may represent the next challenge in CDG research, since mild disorders of glycosylation are often associated to mild neurological presentations such as slight mental retardation [Giurgea et al., 2005]. The development of new sensitive tests will certainly contribute to determine the true incidence of CDG and to better understand the physiological impact of N-glycosylation.

Acknowledgments

We thank Eric G. Berger for critically reading the manuscript, and Gert Matthijs and Patricie Paesold-Burda for the communication of unpublished mutations. This work was supported by the Körber Foundation and the Swiss National Science Foundation grant 31003A-116039.

References

Aebi M, Helenius A, Schenk B, Barone R, Fiumara A, Berger EG, Hennet T, Imbach T, Stutz A, Bjursell C, Uller A, Wahlstrom JG, Briones P, Cardo E, Clayton P, Winchester B, Cormier-Dalre V, de Lonlay P, Cuer M, Dupre T, Seta N, de Koning T, Dorland L, de Loos F, Kupers L, Fabritz L, Hasilik M, Marquardt T, Niehues R, Freeze H, Grunewald S, Heykants L, Jaeken J, Matthijs G, Schollen E, Keir G, Kjaergaard S, Schwartz M, Skovby F, Klein A, Roussel P, Körner C, Lubke T, Thiel C, Von Figura K, Koscielak J, Krasnewich D, Lehle L, Peters V, Raab M, Saether O, Schachter H, Van Schaftingen E, Verbert A, Vilaseca MA, Wevers R, Yamashita K. 1999. Carbohydrate-deficient glycoprotein syndromes become congenital disorders of glycosylation: an updated nomenclature for CDG. First International Workshop on CDGS. Glycoconj J 16:669–671.

Anand M, Rush JS, Ray S, Doucey MA, Weik J, Ware FE, Hofsteenge J, Waechter CJ, Lehrman MA. 2001. Requirement of the Lec35 gene for all known classes of monosaccharide-P-dolichol-dependent glycosyltransferase reactions in mammals. Mol Biol Cell 12:487–501.

Babovic-Vuksanovic D, Patterson MC, Schwenk WF, O'Brien JF, Vockley J, Freeze HH, Mehta DP, Michels VV. 1999. Severe hypoglycemia as a presenting symptom of carbohydrate-deficient glycoprotein syndrome. J Pediatr 135:775–781.

Briones P, Vilaseca MA, Schollen E, Ferrer I, Maties M, Busquets C, Artuch R, Gort L, Marco M, van Schaftingen E, Matthijs G, Jaeken J, Chabas A. 2002. Biochemical and molecular studies in 26 Spanish patients with congenital disorder of glycosylation type Ia. J Inherit Metab Dis 25:635–646.

Burda P, Aebi M. 1998. The ALG10 locus of Saccharomyces cerevisiae encodes the alpha-1,2 glucosyltransferase of the endoplasmic reticulum: the terminal glucose of the lipid-linked oligosaccharide is required for efficient N-linked glycosylation. Glycobiology 8:455–462.

Callewaert N, Schollen E, Vanhecke A, Jaeken J, Matthijs G, Contreras R. 2003. Increased fucosylation and reduced branching of serum glycoprotein N-glycans in all known subtypes of congenital disorder of glycosylation I. Glycobiology 13:367–375.

Chantret I, Dupre T, Delenda C, Bucher S, Dancourt J, Barnier A, Charollais A, Heron D, Bader-Meunier B, Danos O, Seta N, Durand G, Oriol R, Codogno P, Moore SE. 2002. Congenital disorders of glycosylation type Ig is defined by a deficiency in dolichyl-P-mannose:$Man_7GlcNAc_2$-PP-dolichyl mannosyltransferase. J Biol Chem 277:25815–25822.

Chantret I, Dancourt J, Dupre T, Delenda C, Bucher S, Vuillaumier-Barrot S, Ogier de Baulny H, Peletan C, Danos O, Seta N, Durand G, Oriol R, Codogno P, Moore SE. 2003. A deficiency in dolichyl-P-glucose:$Glc1Man_9GlcNAc_2$-PP-dolichyl alpha3-glucosyltransferase defines a new subtype of congenital disorders of glycosylation. J Biol Chem 278:9962–9971.

Cipollo JF, Trimble RB, Chi JH, Yan Q, Dean N. 2001. The yeast ALG11 gene specifies addition of the terminal alpha 1,2-Man to the $Man_5GlcNAc_2$-PP-dolichol N-glycosylation intermediate formed on the cytosolic side of the endoplasmic reticulum. J Biol Chem 276:21828–21840.

Cleasby A, Wonacott A, Skarzynski T, Hubbard RE, Davies GJ, Proudfoot AE, Bernard AR, Payton MA, Wells TN. 1996. The X-ray crystal structure of phosphomannose isomerase from *Candida albicans* at 1.7 angstrom resolution. Nat Struct Biol 3:470–479.

Coman D, Klingberg S, Morris D, McGill J, Mercer H. 2005. Congenital disorder of glycosylation type Ia in a 6-year-old girl with a mild intellectual phenotype: two novel PMM2 mutations. J Inherit Metab Dis 28:1189–1190.

Cserzo M, Wallin E, Simon I, von Heijne G, Elofsson A. 1997. Prediction of transmembrane alpha-helices in prokaryotic membrane proteins: the dense alignment surface method. Protein Eng 10:673–676.

Dancourt J, Vuillaumier-Barrot S, de Baulny HO, Sfaello I, Barnier A, le Bizec C, Dupre T, Durand G, Seta N, Moore SE. 2006. A new intronic mutation in the DPM1 gene is associated with a milder form of CDG Ie in two French siblings. Pediatr Res 59:835–839.

de Koning TJ, Toet M, Dorland L, de Vries LS, van den Berg IE, Duran M, Poll-The BT. 1998. Recurrent nonimmune hydrops fetalis associated with carbohydrate-deficient glycoprotein syndrome. J Inherit Metab Dis 21:681–682.

de Lonlay P, Cuer M, Vuillaumier-Barrot S, Beaune G, Castelnau P, Kretz M, Durand G, Saudubray JM, Seta N. 1999. Hyperinsulinemic hypoglycemia as a presenting sign in phosphomannose isomerase deficiency: a new manifestation of carbohydrate-deficient glycoprotein syndrome treatable with mannose. J Pediatr 135:379–383.

de Lonlay P, Seta N, Barrot S, Chabrol B, Drouin V, Gabriel BM, Journel H, Kretz M, Laurent J, Le Merrer M, Leroy A, Pedespan D, Sarda P, Villeneuve N, Schmitz J, van Schaftingen E, Matthijs G, Jaeken J, Korner C, Munnich A, Saudubray JM, Cormier-Daire V. 2001. A broad spectrum of clinical presentations in congenital disorders of glycosylation I: a series of 26 cases. J Med Genet 38:14–19.

Denecke J, Kranz C, Kemming D, Koch HG, Marquardt T. 2004. An activated 5′ cryptic splice site in the human ALG3 gene generates a premature termination codon insensitive to nonsense-mediated mRNA decay in a new case of congenital disorder of glycosylation type Id (CDG-Id). Hum Mutat 23:477–486.

Denecke J, Kranz C, von Kleist-Retzow J, Bosse K, Herkenrath P, Debus O, Harms E, Marquardt T. 2005. Congenital disorder of glycosylation type Id: clinical phenotype, molecular analysis, prenatal diagnosis, and glycosylation of fetal proteins. Pediatr Res 58:248–253.

Deprez P, Gautschi M, Helenius A. 2005. More than one glycan is needed for ER glucosidase II to allow entry of glycoproteins into the calnexin/calreticulin cycle. Mol Cell 19:183–195.

DeRossi C, Bode L, Eklund EA, Zhang F, Davis JA, Westphal V, Wang L, Borowsky AD, Freeze HH. 2006. Ablation of mouse phosphomannose isomerase (Mpi) causes mannose 6-phosphate accumulation, toxicity, and embryonic lethality. J Biol Chem 281:5916–5927.

Di Rocco M, Hennet T, Grubenmann CE, Pagliardini S, Allegri AE, Frank CG, Aebi M, Vignola S, Jaeken J. 2005. Congenital disorder of glycosylation (CDG) Ig: report on a patient and review of the literature. J Inherit Metab Dis 28: 1162–1164.

Eklund EA, Newell JW, Sun L, Seo NS, Alper G, Willert J, Freeze HH. 2005a. Molecular and clinical description of the first US patients with congenital disorder of glycosylation Ig. Mol Genet Metab 84:25–31.

Eklund EA, Sun L, Westphal V, Northrop JL, Freeze HH, Scaglia F. 2005b. Congenital disorder of glycosylation (CDG)-Ih patient with a severe hepato-intestinal phenotype and evolving central nervous system pathology. J Pediatr 147: 847–850.

Eklund EA, Sun L, Yang SP, Pasion RM, Thorland EC, Freeze HH. 2006. Congenital disorder of glycosylation Ic due to a de novo deletion and an hALG-6 mutation. Biochem Biophys Res Commun 339:755–760.

Ellgaard L, Helenius A. 2003. Quality control in the endoplasmic reticulum. Nat Rev Mol Cell Biol 4:181–191.

Foulquier F, Vasile E, Schollen E, Callewaert N, Raemaekers T, Quelhas D, Jaeken J, Mills P, Winchester B, Krieger M, Annaert W, Matthijs G. 2006. Conserved oligomeric Golgi complex subunit 1 deficiency reveals a previously uncharacterized congenital disorder of glycosylation type II. Proc Natl Acad Sci USA 103:3764–3769.

Foulquier F, Ungar D, Reynders E, Zeevaert R, Mills P, Garcia-Silva MT, Briones P, Winchester B, Morelle W, Krieger M, Annaert W, Matthijs G. 2007. A new inborn error of glycosylation due to a Cog8 deficiency reveals a critical role for the Cog1-Cog8 interaction in COG complex formation. Hum Mol Genet 16:717–730.

Frank CG, Grubenmann CE, Eyaid W, Berger EG, Aebi M, Hennet T. 2004. Identification and functional analysis of a defect in the human ALG9 gene: definition of congenital disorder of glycosylation type IL. Am J Hum Genet 75:146–150.

Frank CG, Sanyal S, Rush JS, Waechter CJ, Menon AK. 2008. Does Rft1 flip an N-glycan lipid precursor? Nature 454:E3–E4; discussion E4–E5.

Freeze HH. 2006. Genetic defects in the human glycome. Nat Rev Genet 7:537–551.

Garcia-Silva MT, Matthijs G, Schollen E, Cabrera JC, Sanchez del Pozo J, Marti Herreros M, Simon R, Maties M, Martin Hernandez E, Hennet T, Briones P. 2004. Congenital disorder of glycosylation (CDG) type Ie. A new patient. J Inherit Metab Dis 27:591–600.

Giurgea I, Michel A, Le Merrer M, Seta N, de Lonlay P. 2005. Underdiagnosis of mild congenital disorders of glycosylation type Ia. Pediatr Neurol 32: 121–123.

Godfrey C, Clement E, Mein R, Brockington M, Smith J, Talim B, Straub V, Robb S, Quinlivan R, Feng L, Jimenez-Mallebrera C, Mercuri E, Manzur AY, Kinali M, Torelli S, Brown SC, Sewry CA, Bushby K, Topaloglu H, North K, Abbs S, Muntoni F. 2007. Refining genotype phenotype correlations in muscular dystrophies with defective glycosylation of dystroglycan. Brain 130(Pt 10): 2725–2735.

Grubenmann CE, Frank CG, Kjaergaard S, Berger EG, Aebi M, Hennet T. 2002. ALG12 mannosyltransferase defect in congenital disorder of glycosylation type lg. Hum Mol Genet 11:2331–2339.

Grubenmann CE, Frank CG, Hulsmeier AJ, Schollen E, Matthijs G, Mayatepek E, Berger EG, Aebi M, Hennet T. 2004. Deficiency of the first mannosylation step in the N-glycosylation pathway causes congenital disorder of glycosylation type Ik. Hum Mol Genet 13:535–542.

Grunewald S, Imbach T, Huijben K, Rubio-Gozalbo ME, Verrips A, de Klerk JB, Stroink H, de Rijk-van Andel JF, Van Hove JL, Wendel U, Matthijs G, Hennet T, Jaeken J, Wevers RA. 2000. Clinical and biochemical characteristics of congenital disorder of glycosylation type Ic, the first recognized endoplasmic reticulum defect in N-glycan synthesis. Ann Neurol 47:776–781.

Grunewald S, Schollen E, Van Schaftingen E, Jaeken J, Matthijs G. 2001. High residual activity of PMM2 in patients' fibroblasts: possible pitfall in the diagnosis of CDG-Ia (phosphomannomutase deficiency). Am J Hum Genet 68:347–354.

Haeuptle MA, Pujol FM, Neupert C, Winchester B, Kastaniotis AJ, Aebi M, Hennet T. 2008. Human RFT1 deficiency leads to a disorder of N-linked glycosylation. Am J Hum Genet 82:600–606.

Hanefeld F, Korner C, Holzbach-Eberle U, von Figura K. 2000. Congenital disorder of glycosylation-Ic: case report and genetic defect. Neuropediatrics 31:60–62.

Helenius J, Ng DT, Marolda CL, Walter P, Valvano MA, Aebi M. 2002. Translocation of lipid-linked oligosaccharides across the ER membrane requires Rft1 protein. Nature 415:447–450.

Hirokawa T, Boon-Chieng S, Mitaku S. 1998. SOSUI: classification and secondary structure prediction system for membrane proteins. Bioinformatics 14:378–379.

Huffaker TC, Robbins PW. 1982. Temperature-sensitive yeast mutants deficient in asparagine-linked glycosylation. J Biol Chem 257:3203–3210.

Imbach T, Burda P, Kuhnert P, Wevers RA, Aebi M, Berger EG, Hennet T. 1999. A mutation in the human ortholog of the *Saccharomyces cerevisiae* ALG6 gene causes carbohydrate-deficient glycoprotein syndrome type-Ic. Proc Natl Acad Sci USA 96:6982–6987.

Imbach T, Grunewald S, Schenk B, Burda P, Schollen E, Wevers RA, Jaeken J, de Klerk JB, Berger EG, Matthijs G, Aebi M, Hennet T. 2000a. Multi-allelic origin of congenital disorder of glycosylation (CDG)-Ic. Hum Genet 106: 538–545.

Imbach T, Schenk B, Schollen E, Burda P, Stutz A, Grunewald S, Bailie NM, King MD, Jaeken J, Matthijs G, Berger EG, Aebi M, Hennet T. 2000b. Deficiency of dolichol-phosphate-mannose synthase-1 causes congenital disorder of glycosylation type Ie. J Clin Invest 105:233–239.

Jackson BJ, Kukuruzinska MA, Robbins P. 1993. Biosynthesis of asparagine-linked oligosaccharides in *Saccharomyces cerevisiae*: the alg2 mutation. Glycobiology 3:357–364.

Jaeken J, Matthijs G, Saudubray JM, Dionisi-Vici C, Bertini E, de Lonlay P, Henri H, Carchon H, Schollen E, Van Schaftingen E. 1998. Phosphomannose isomerase deficiency: a carbohydrate-deficient glycoprotein syndrome with hepatic-intestinal presentation. Am J Hum Genet 62:1535–1539.

Jaeken J, Hennet T, Freeze HH, Matthijs G. 2008. On the nomenclature of congenital disorders of glycosylation (CDG). J Inherit Metab Dis 31:669–672.

Kim S, Westphal V, Srikrishna G, Mehta DP, Peterson S, Filiano J, Karnes PS, Patterson MC, Freeze HH. 2000. Dolichol phosphate mannose synthase (DPM1) mutations define congenital disorder of glycosylation Ie (CDG-Ie). J Clin Invest 105:191–198.

Kinch LN, Grishin NV. 2002. Evolution of protein structures and functions. Curr Opin Struct Biol 12:400–408.

Körner C, Knauer R, Stephani U, Marquardt T, Lehle L, von Figura K. 1999. Carbohydrate deficient glycoprotein syndrome type IV: deficiency of dolichyl-P-Man:Man(5)GlcNAc(2)-PP-dolichyl mannosyltransferase. EMBO J 18:6816–6822.

Kornfeld R, Kornfeld S. 1985. Assembly of asparagine-linked oligosaccharides. Annu Rev Biochem 54:631–664.

Kranz C, Denecke J, Lehrman MA, Ray S, Kienz P, Kreissel G, Sagi D, Peter-Katalinic J, Freeze HH, Schmid T, Jackowski-Dohrmann S, Harms E, Marquardt T. 2001. A mutation in the human MPDU1 gene causes congenital disorder of glycosylation type If (CDG-If). J Clin Invest 108:1613–1619.

Kranz C, Denecke J, Lehle L, Sohlbach K, Jeske S, Meinhardt F, Rossi R, Gudowius S, Marquardt T. 2004. Congenital disorder of glycosylation type Ik (CDG-Ik): a defect of mannosyltransferase I. Am J Hum Genet 74:545–551.

Kranz C, Basinger AA, Gucsavas-Calikoglu M, Sun L, Powell CM, Henderson FW, Aylsworth AS, Freeze HH. 2007a. Expanding spectrum of congenital disorder of glycosylation Ig (CDG-Ig): sibs with a unique skeletal dysplasia, hypogammaglobulinemia, cardiomyopathy, genital malformations, and early lethality. Am J Med Genet A 143A:1371–1378.

Kranz C, Jungeblut C, Denecke J, Erlekotte A, Sohlbach C, Debus V, Kehl HG, Harms E, Reith A, Reichel S, Grobe H, Hammersen G, Schwarzer U, Marquardt T. 2007b. A defect in dolichol phosphate biosynthesis causes a new inherited disorder with death in early infancy. Am J Hum Genet 80: 433–440.

Kranz C, Sun L, Eklund EA, Krasnewich D, Casey JR, Freeze HH. 2007c. CDG-Id in two siblings with partially different phenotypes. Am J Med Genet A 143A: 1414–1420.

Krogh A, Larsson B, von Heijne G, Sonnhammer EL. 2001. Predicting transmembrane protein topology with a hidden Markov model: application to complete genomes. J Mol Biol 305:567–580.

Kukuruzinska MA, Robbins PW. 1987. Protein glycosylation in yeast: transcript heterogeneity of the ALG7 gene. Proc Natl Acad Sci USA 84:2145–2149.

Le Bizec C, Vuillaumier-Barrot S, Barnier A, Dupre T, Durand G, Seta N. 2005. A new insight into PMM2 mutations in the French population. Hum Mutat 25:504–505.

Lefeber DJ, Schonberger J, Morava E, Guillard M, Huyben KM, Verrijp K, Grafakou O, Evangeliou A, Preijers FW, Manta P, Yildiz J, Grunewald S, Spilioti M, van den Elzen C, Klein D, Hess D, Ashida H, Hofsteenge J, Maeda Y, van den Heuvel L, Lammens M, Lehle L, Wevers RA. 2009. Deficiency of Dol-P-Man synthase subunit DPM3 bridges the congenital disorders of glycosylation with the dystroglycanopathies. Am J Hum Genet 85:76–86.

Leroy JG. 2006. Congenital disorders of N-glycosylation including diseases associated with O- as well as N-glycosylation defects. Pediatr Res 60:643–656.

Lubke T, Marquardt T, Etzioni A, Hartmann E, von Figura K, Korner C. 2001. Complementation cloning identifies CDG-IIc, a new type of congenital disorders of glycosylation, as a GDP-fucose transporter deficiency. Nat Genet 28:73–76.

Maeda Y, Kinoshita T. 2008. Dolichol-phosphate mannose synthase: structure, function and regulation. Biochim Biophys Acta 1780:861–868.

Marek KW, Vijay IK, Marth JD. 1999. A recessive deletion in the GlcNAc-1-phosphotransferase gene results in peri-implantation embryonic lethality. Glycobiology 9:1263–1271.

Martinez-Duncker I, Dupre T, Piller V, Piller F, Candelier JJ, Trichet C, Tchernia G, Oriol R, Mollicone R. 2005. Genetic complementation reveals a novel human congenital disorder of glycosylation of type II, due to inactivation of the Golgi CMP-sialic acid transporter. Blood 105:2671–2676.

Matthijs G, Schollen E, Pardon E, Veiga-Da-Cunha M, Jaeken J, Cassiman JJ, Van Schaftingen E. 1997. Mutations in PMM2, a phosphomannomutase gene on chromosome 16p13, in carbohydrate-deficient glycoprotein type I syndrome (Jaeken syndrome). Nat Genet 16:88–92.

Matthijs G, Schollen E, Van Schaftingen E, Cassiman JJ, Jaeken J. 1998. Lack of homozygotes for the most frequent disease allele in carbohydrate-deficient glycoprotein syndrome type 1A. Am J Hum Genet 62:542–550.

Matthijs G, Schollen E, Heykants L, Grunewald S. 1999. Phosphomannomutase deficiency: the molecular basis of the classical Jaeken syndrome (CDGS type Ia). Mol Genet Metab 68:220–226.

Matthijs G, Schollen E, Bjursell C, Erlandson A, Freeze H, Imtiaz F, Kjaergaard S, Martinsson T, Schwartz M, Seta N, Vuillaumier-Barrot S, Westphal V, Winchester B. 2000. Mutations in PMM2 that cause congenital disorders of glycosylation, type Ia (CDG-Ia). Hum Mutat 16:386–394.

Matthijs G. 2005. Research network: EUROGLYCANET: a European network focused on congenital disorders of glycosylation. Eur J Hum Genet 13:395–397.

Mizugishi K, Yamanaka K, Kuwajima K, Yuasa I, Shigemoto K, Kondo I. 1999. Missense mutations in the phosphomannomutase 2 gene of two Japanese siblings with carbohydrate-deficient glycoprotein syndrome type I. Brain Dev 21:223–228.

Newell JW, Seo NS, Enns GM, McCraken M, Mantovani JF, Freeze HH. 2003. Congenital disorder of glycosylation Ic in patients of Indian origin. Mol Genet Metab 79:221–228.

Niehues R, Hasilik M, Alton G, Körner C, Schiebe-Sukumar M, Koch HG, Zimmer KP, Wu R, Harms E, Reiter K, von Figura K, Freeze HH, Harms HK, Marquardt T. 1998. Carbohydrate-deficient glycoprotein syndrome type Ib. Phosphomannose isomerase deficiency and mannose therapy. J Clin Invest 101:1414–1420.

Ono H, Sakura N, Yamashita K, Yuasa I, Ohno K. 2003. Novel nonsense mutation (R194X) in the PMM2 gene in a Japanese patient with congenital disorder of glycosylation type Ia. Brain Dev 25:525–528.

Panneerselvam K, Freeze HH. 1996. Mannose corrects altered N-glycosylation in carbohydrate-deficient glycoprotein syndrome fibroblasts. J Clin Invest 97: 1478–1487.

Penel-Capelle D, Dobbelaere D, Jaeken J, Klein A, Cartigny M, Weill J. 2003. Congenital disorder of glycosylation Ib (CDG-Ib) without gastrointestinal symptoms. J Inherit Metab Dis 26:83–85.

Perez-Duenas B, Garcia-Cazorla A, Pineda M, Poo P, Campistol J, Cusi V, Schollen E, Matthijs G, Grunewald S, Briones P, Perez-Cerda C, Artuch R, Vilaseca MA. 2009. Long-term evolution of eight Spanish patients with CDG type Ia: typical and atypical manifestations. Eur J Paediatr Neurol 13:444–451.

Pirard M, Matthijs G, Heykants L, Schollen E, Grunewald S, Jaeken J, van Schaftingen E. 1999. Effect of mutations found in carbohydrate-deficient glycoprotein syndrome type IA on the activity of phosphomannomutase 2. FEBS Lett 452:319–322.

Rimella-Le-Huu A, Henry H, Kern I, Hanquinet S, Roulet-Perez E, Newman CJ, Superti-Furga A, Bonafe L, Ballhausen D. Congenital disorder of glycosylation type Id (CDG Id): phenotypic, biochemical and molecular characterization of a new patient. J Inherit Metab Dis (in press). [http://dx.doi.org/10.1007/s10545-008-0959-x]

Schenk B, Imbach T, Frank CG, Grubenmann CE, Raymond GV, Hurvitz H, Korn-Lubetzki I, Revel-Vik S, Raas-Rotschild A, Luder AS, Jaeken J, Berger EG, Matthijs G, Hennet T, Aebi M. 2001. MPDU1 mutations underlie a novel human congenital disorder of glycosylation, designated type If. J Clin Invest 108:1687–1695.

Schollen E, Dorland L, de Koning TJ, Van Diggelen OP, Huijmans JG, Marquardt T, Babovic-Vuksanovic D, Patterson M, Imtiaz F, Winchester B, Adamowicz M, Pronicka E, Freeze H, Matthijs G. 2000a. Genomic organization of the human phosphomannose isomerase (MPI) gene and mutation analysis in patients with congenital disorders of glycosylation type Ib (CDG-Ib). Hum Mutat 16:247–252.

Schollen E, Kjaergaard S, Legius E, Schwartz M, Matthijs G. 2000b. Lack of Hardy-Weinberg equilibrium for the most prevalent PMM2 mutation in CDG-Ia (congenital disorders of glycosylation type Ia). Eur J Hum Genet 8:367–371.

Schollen E, Martens K, Geuzens E, Matthijs G. 2002. DHPLC analysis as a platform for molecular diagnosis of congenital disorders of glycosylation (CDG). Eur J Hum Genet 10:643–648.

Schollen E, Frank CG, Keldermans L, Reyntjens R, Grubenmann CE, Clayton PT, Winchester BG, Smeitink J, Wevers RA, Aebi M, Hennet T, Matthijs G. 2004. Clinical and molecular features of three patients with congenital disorders of glycosylation type Ih (CDG-Ih) (ALG8 deficiency). J Med Genet 41:550–556.

Schollen E, Grunewald S, Keldermans L, Albrecht B, Korner C, Matthijs G. 2005. CDG-Id caused by homozygosity for an ALG3 mutation due to segmental maternal isodisomy UPD3(q21.3-qter). Eur J Med Genet 48:153–158.

Schollen E, Keldermans L, Foulquier F, Briones P, Chabas A, Sanchez-Valverde F, Adamowicz M, Pronicka E, Wevers R, Matthijs G. 2007. Characterization of two unusual truncating PMM2 mutations in two CDG-Ia patients. Mol Genet Metab 90:408–413.

Schwarz M, Thiel C, Lubbehusen J, Dorland B, de Koning T, von Figura K, Lehle L, Körner C. 2004. Deficiency of GDP-Man:GlcNAc2-PP-dolichol mannosyltransferase causes congenital disorder of glycosylation type Ik. Am J Hum Genet 74:472–481.

Shridas P, Waechter CJ. 2006. Human dolichol kinase, a polytopic endoplasmic reticulum membrane protein with a cytoplasmically oriented CTP-binding site. J Biol Chem 281:31696–31704.

Silvaggi NR, Zhang C, Lu Z, Dai J, Dunaway-Mariano D, Allen KN. 2006. The X-ray crystal structures of human alpha-phosphomannomutase 1 reveal the structural basis of congenital disorder of glycosylation type 1a. J Biol Chem 281: 14918–14926.

Stagljar I, te Heesen S, Aebi M. 1994. New phenotype of mutations deficient in glucosylation of the lipid-linked oligosaccharide: cloning of the ALG8 locus. Proc Natl Acad Sci USA 91:5977–5981.

Stibler H, Stephani U, Kutsch U. 1995. Carbohydrate-deficient glycoprotein syndrome—a fourth subtype. Neuropediatrics 26:235–237.

Stölting T, Omran H, Erlekotte A, Denecke J, Reunert J, Marquardt T. Novel ALG8 mutations expand the clinical spectrum of congenital disorder of glycosylation type Ih. Mol Genet Metab (in press). [http://dx.doi.org/10.1016/j.ymgme.2009.06.010]

Sun L, Eklund EA, Chung WK, Wang C, Cohen J, Freeze HH. 2005a. Congenital disorder of glycosylation id presenting with hyperinsulinemic hypoglycemia and islet cell hyperplasia. J Clin Endocrinol Metab 90:4371–4375.

Sun L, Eklund EA, Van Hove JL, Freeze HH, Thomas JA. 2005b. Clinical and molecular characterization of the first adult congenital disorder of glycosylation (CDG) type Ic patient. Am J Med Genet A 137:22–26.

Swiezewska E, Danikiewicz W. 2005. Polyisoprenoids: structure, biosynthesis and function. Prog Lipid Res 44:235–258.

Tayebi N, Andrews DQ, Park JK, Orvisky E, McReynolds J, Sidransky E, Krasnewich DM. 2002. A deletion-insertion mutation in the phosphomannomutase 2 gene in an African American patient with congenital disorders of glycosylation-Ia. Am J Med Genet 108:241–246.

Thiel C, Schwarz M, Hasilik M, Grieben U, Hanefeld F, Lehle L, von Figura K, Korner C. 2002. Deficiency of dolichyl-P-Man:$Man_7GlcNAc_2$-PP-dolichyl mannosyltransferase causes congenital disorder of glycosylation type Ig. Biochem J 367(Pt 1):195–201.

Thiel C, Schwarz M, Peng J, Grzmil M, Hasilik M, Braulke T, Kohlschutter A, von Figura K, Lehle L, Korner C. 2003. A new type of congenital disorders of glycosylation (CDG-Ii) provides new insights into the early steps of dolichol-linked oligosaccharide biosynthesis. J Biol Chem 278:22498–22505.

Thiel C, Lubke T, Matthijs G, von Figura K, Korner C. 2006. Targeted disruption of the mouse phosphomannomutase 2 gene causes early embryonic lethality. Mol Cell Biol 26:5615–5620.

Thompson JD, Higgins DG, Gibson TJ. 1994. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Res 22:4673–4680.

Thong MK, Fietz M, Nicholls C, Lee MH, Asma O. Congenital disorder of glycosylation type Ia in a Malaysian family: clinical outcome and description of a novel PMM2 mutation. J Inherit Metab Dis (in press). [http://dx.doi.org/10.1007/s10545-009-1031-1]

Topaz O, Shurman DL, Bergman R, Indelman M, Ratajczak P, Mizrachi M, Khamaysi Z, Behar D, Petronius D, Friedman V, Zelikovic I, Raimer S, Metzker A, Richard G, Sprecher E. 2004. Mutations in GALNT3, encoding a protein involved in O-linked glycosylation, cause familial tumoral calcinosis. Nat Genet 36:579–581.

Truin G, Guillard M, Lefeber DJ, Sykut-Cegielska J, Adamowicz M, Hoppenreijs E, Sengers RC, Wevers RA, Morava E. 2008. Pericardial and abdominal fluid accumulation in congenital disorder of glycosylation type Ia. Mol Genet Metab 94:481–484.

van Eijk HG, van Noort WL, Dubelaar ML, van der Heul C. 1983. The microheterogeneity of human transferrins in biological fluids. Clin Chim Acta 132:167–171.

Vega AI, Perez-Cerda C, Desviat LR, Matthijs G, Ugarte M, Perez B. 2009. Functional analysis of three splicing mutations identified in the PMM2 gene: toward a new therapy for congenital disorder of glycosylation type Ia. Hum Mutat 30:795–803.

Vermeer S, Kremer HP, Leijten QH, Scheffer H, Matthijs G, Wevers RA, Knoers NA, Morava E, Lefeber DJ. 2007. Cerebellar ataxia and congenital disorder of glycosylation Ia (CDG-Ia) with normal routine CDG screening. J Neurol 254:1356–1358.

Vesela K, Honzik T, Hansikova H, Haeuptle MA, Semberova J, Stranak Z, Hennet T, Zeman J. A new case of ALG8 deficiency (CDG-Ih). J Inherit Metab Dis (in press). [http://dx.doi.org/10.1007/s10545-009-1203-z]

Vleugels W, Keldermans L, Jaeken J, Butters TD, Michalski JC, Matthijs G, Foulquier F. 2009. Quality control of glycoproteins bearing truncated glycans in an ALG9-defective (CDG-IL) patient. Glycobiology 19:910–917.

Vleugels W, Haeuptle MA, Ng BG, Michalski JC, Battini R, Dionisi-Vici C, Ludman MD, Jaeken J, Foulquier F, Freeze H, Matthijs G, Hennet T. RFT1 deficiency in three novel CDG patients. Hum Mutat (in press). [http://dx.doi.org/10.1002/humu.21085]

Vuillaumier-Barrot S, Le Bizec C, Durand G, Seta N. 2001. The T911C (F304S) substitution in the human ALG6 gene is a common polymorphism and not a causal mutation of CDG-Ic. J Hum Genet 46:547–548.

Vuillaumier-Barrot S, Le Bizec C, de Lonlay P, Barnier A, Mitchell G, Pelletier V, Prevost C, Saudubray JM, Durand G, Seta N. 2002. Protein losing enteropathy-hepatic fibrosis syndrome in Saguenay-Lac St-Jean, Quebec is a congenital disorder of glycosylation type Ib. J Med Genet 39:849–851.

Vuillaumier-Barrot S. 2005. [Molecular diagnosis of congenital disorders of glycosylation]. Ann Biol Clin (Paris) 63:135–143. [French]

Vuillaumier-Barrot S, Le Bizec C, De Lonlay P, Madinier-Chappat N, Barnier A, Dupre T, Durand G, Seta N. 2006. PMM2 intronic branch-site mutations in CDG-Ia. Mol Genet Metab 87:337–340.

Weinstein M, Schollen E, Matthijs G, Neupert C, Hennet T, Grubenmann CE, Frank CG, Aebi M, Clarke JT, Griffiths A, Seargeant L, Poplawski N. 2005. CDG-IL: an infant with a novel mutation in the ALG9 gene and additional phenotypic features. Am J Med Genet A 136:194–197.

Westphal V, Murch S, Kim S, Srikrishna G, Winchester B, Day R, Freeze HH. 2000a. Reduced heparan sulfate accumulation in enterocytes contributes to protein-losing enteropathy in a congenital disorder of glycosylation. Am J Pathol 157:1917–1925.

Westphal V, Schottstadt C, Marquardt T, Freeze HH. 2000b. Analysis of multiple mutations in the hALG6 gene in a patient with congenital disorder of glycosylation Ic. Mol Genet Metab 70:219–223.

Westphal V, Enns GM, McCracken MF, Freeze HH. 2001a. Functional analysis of novel mutations in a congenital disorder of glycosylation Ia patient with mixed Asian ancestry. Mol Genet Metab 73:71–76.

Westphal V, Kjaergaard S, Davis JA, Peterson SM, Skovby F, Freeze HH. 2001b. Genetic and metabolic analysis of the first adult with congenital disorder of glycosylation type Ib: long-term outcome and effects of mannose supplementation. Mol Genet Metab 73:77–85.

Westphal V, Kjaergaard S, Schollen E, Martens K, Grunewald S, Schwartz M, Matthijs G, Freeze HH. 2002. A frequent mild mutation in ALG6 may exacerbate the clinical severity of patients with congenital disorder of glycosylation Ia (CDG-Ia) caused by phosphomannomutase deficiency. Hum Mol Genet 11:599–604.

Westphal V, Xiao M, Kwok PY, Freeze HH. 2003. Identification of a frequent variant in ALG6, the cause of congenital disorder of glycosylation-Ic. Hum Mutat 22:420–421.

Wu X, Rush JS, Karaoglu D, Krasnewich D, Lubinsky MS, Waechter CJ, Gilmore R, Freeze HH. 2003. Deficiency of UDP-GlcNAc:dolichol phosphate N-acetylglucosamine-1 phosphate transferase (DPAGT1) causes a novel congenital disorder of glycosylation type Ij. Hum Mutat 22:144–150.

Wu X, Steet RA, Bohorov O, Bakker J, Newell J, Krieger M, Spaapen L, Kornfeld S, Freeze HH. 2004. Mutation of the COG complex subunit gene COG7 causes a lethal congenital disorder. Nat Med 10:518–523.

Wurm D, Hansgen A, Kim YJ, Lindinger A, Baghai A, Gortner L. 2007. Early fatal course in siblings with CDG-Ia (caused by two novel mutations in the PMM2 gene): clinical, molecular and autopsy findings. Eur J Pediatr 166:377–378.