Review

# Molecular basis of carbohydrate-deficient glycoprotein syndromes type I with normal phosphomannomutase activity

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## Abstract

Carbohydrate deficient glycoprotein syndromes (CDGS) are inherited disorders in glycosylation. Isoelectric focusing of serum transferrin is used as a biochemical indicator of CDGS; however, this technique cannot diagnose the molecular defect. Even though phosphomannomutase (PMM) deficiency accounts for the great majority of known CDGS cases (CDGS type Ia), newly discovered cases have significantly different clinical presentations than the PMM-deficient patients. These differences arise from other defects affecting the biosynthesis of *N*-linked oligosaccharides in the endoplasmic reticulum and in the Golgi compartment. The most notable is the loss of phosphomannose isomerase (PMI) (CDGS type Ib). It causes severe hypoglycemia, protein-losing enteropathy, vomiting, diarrhea, and congenital hepatic fibrosis. In contrast to PMM-deficiency, there is no developmental delay nor neuropathy. Most symptoms in the PMI-deficient patients can be successfully treated with dietary mannose supplements. Another defect is the lack of glucosylation of the lipid-linked oligosaccharide precursor. The clinical features of this form of CDGS are milder, but similar to, PMM-deficient patients. Yeast genetic and biochemical techniques were critical in unraveling these disorders since many of the defective genes were known in yeast and corresponding mutants were available for complementation. Yeast strains carrying mutations in the homologous genes are likely to provide conclusive identification of the primary defects in novel CDGS types that affect the synthesis and transfer of precursor oligosaccharides. © 1999 Elsevier Science B.V. All rights reserved.

*Keywords:* Carbohydrate deficient glycoprotein syndrome; Mannose; Therapy; Phosphomannose isomerase; α-Glucosyltransferase; Metabolic disorder

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## 1. Introduction

Carbohydrate deficient glycoprotein syndrome (CDGS) is a generic name for a group of inherited disorders with primary defects in glycoconjugate biosynthesis [1–3]. The disorders are usually detected by alterations in proteins with *N*-linked oligosaccharides, but other glycosylation pathways may also be compromised [4]. This review highlights newly discovered types of CDGS, their clinical manifestations, effective therapy, and implications for other diseases. Much of this progress was possible because several of the genes had already been identified in the yeast *Saccharomyces cerevisiae*, and strains were available for complementation analysis.

## 2. Features of the most common form of CDGS

CDGS patients have altered protein *N*-glycosylation [1,5–9]. Many of the known patients are defective in the enzyme phosphomannomutase (PMM) that converts Man-6-P into Man-1-P as shown in Fig. 1. The human enzyme has about 57% identity to the yeast enzyme and is required for glycoprotein synthesis. In both yeast and higher organisms, Man-1-P is then converted into GDP-Man, which is used to synthesize all mannose-containing glycoconjugates. Thus, defects in PMM are predicted to reduce the amount of *N*-linked oligosaccharides, glycophospholipid anchors, *O*-mannosylated proteins [10–13], and proteins with the newly identified *C*-mannosylation [14–16]. The effects of PMM deficiency have only been examined so far in relation to *N*-linked glycosylation, where they result in hypoglycosylation of many glycoproteins. Serum glycoproteins are the most frequently studied since they are readily available.

Although PMM-deficient patients share many symptoms, these can vary among individual patients. Not all of the symptoms are always present in each individual. Typically, severe mental and psychomotor retardation, characteristic dysmorphic features, retinitis pigmentosa, gastrointestinal tract problems leading to a failure to thrive and liver pathology are displayed. Coagulation problems may arise due to deficiencies in serum glycoproteins, including antithrombin III (ATIII) and protein C. Some of the patients must be tube-fed, and all have limited mobility. Mortality is about 20% within the first 2 years. This disorder, called CDGS type 1a, and its clinical, biochemical and genetic aspects are presented in [2] of this volume. It is the best studied of all known varieties of CDGS, since it was first described in 1980 and the primary defect was identified in 1995.

## 3. Serum transferrin IEF: an important tool for identifying glycosylation disorders

If patients present with an aggregate of the symptoms described above, CDGS can be biochemically confirmed using an isoelectric focusing (IEF) analysis of serum transferrin [17–21]. As mentioned above, transferrin is only one of many glycoproteins altered in CDGS.

Transferrin is currently the most widely used biochemical indicator of CDGS. It has two *N*-glycosylation sites which are usually occupied by disialylated biantennery chains forming tetrasialo transferrin. If entire *N*-linked chains are missing or, if correctly added *N*-linked chains are not processed correctly
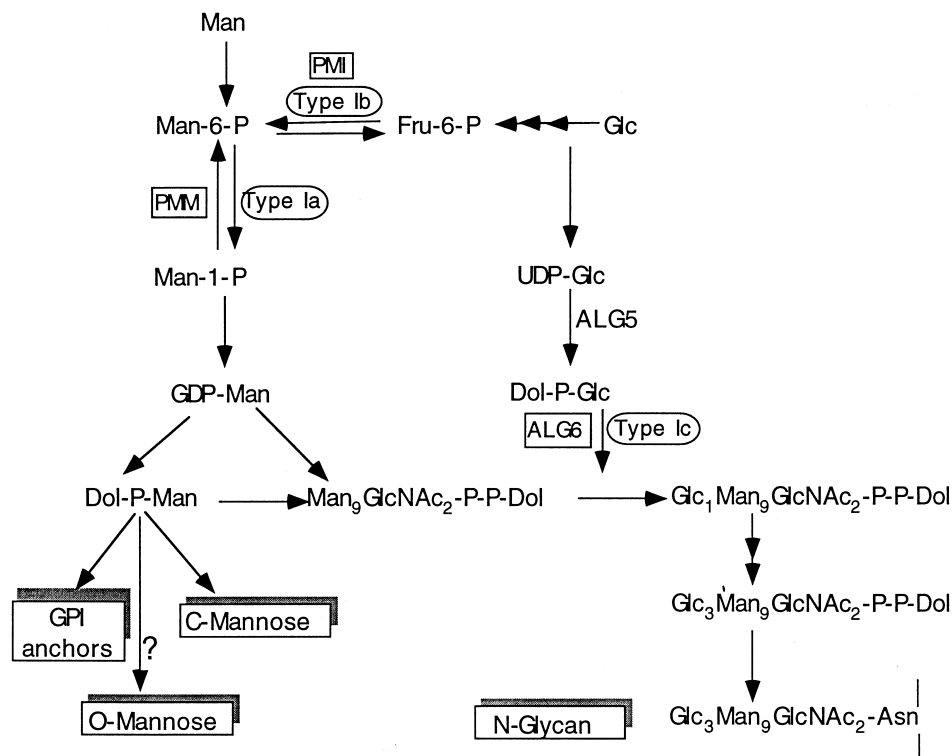
Fig. 1. Simplified pathway of lipid-linked oligosaccharide precursor synthesis showing known defects. The biosynthesis of Dol-P-P-oligosaccharide precursor is altered in several different varieties of CDGS, Types Ia, Ib, and Ic. The known defects are indicated by the boxes. The most common identified defect is a loss of the enzyme phosphomannomutase (PMM) which normally catalyzes the formation of Man-1-P from Man-6-P. PMM-deficiency results in severe mental and psychomotor retardation (type Ia). Another defect is the loss of phosphomannose isomerase (PMI), resulting in a decrease of the formation of Man-6-P from Fru-6-P. However, Man-6-P can also be generated directly from Man using hexokinase. Patients lacking PMI can have severe hypoglycemia, congenital hepatic fibrosis, coagulopathy, severe diarrhea and vomiting (type Ib). Surprisingly, these patients do not show psychomotor retardation or peripheral neuropathy. Dietary mannose treatments can significantly improve the clinical condition of PMI-deficient patients, but thus far have not shown improvement in PMM-deficient patients. A new type of CDGS, called type Ic, is a clinically milder version of PMM deficiency. It is caused by the lack of formation of $Glc_1Man_9GlcNAc_2$-P-P-Dol. This results from a defect in the α1,3-glucosyltransferase that adds the first Glc residue to the lipid-bound oligosaccharide chain. In yeast, the *ALG6* gene product is responsible for carrying out this reaction. Yeast mutants have also been identified for both PMM and PMI, and were important for identifying the human deficiencies. The highly conserved early steps in *N*-linked oligosaccharide biosynthesis are likely to make yeast a critical tool for unraveling other human glycosylation defects. Defects in PMM and PMI probably also affect the synthesis of GPI anchors, *C*-mannosylated and *O*-mannosylated proteins. α1,3-Glucosyltransferase deficiency is specific for the *N*-linked pathway.

[22–24], undersialylated forms with fewer sialic acids will appear. This produces carbohydrate-deficient transferrins (CDT) (Fig. 2). CDT is also seen in uncontrolled fructosemia, galactosemia or following heavy alcohol consumption [25–29]. These latter IEF alterations are temporary and appear to normalize within a few weeks of beginning proper treatment. Since a small number of transferrin molecules have triantennary oligosaccharides due to increased branching, more highly sialylated forms, e.g. penta- and hexasialo, are also seen. These forms increase temporarily during acute phase reactions and pregnancy [21].

Traditionally, a specific IEF pattern has defined the two main types (1 and 2) of CDGS. The PMM-deficient patients have been designated to have a 'type 1' transferrin pattern characterized by the prevalence of 4, 2 or no sialic acid residues on transferrin, whereas transferrin species carrying 3 or 1 sialic acid residues are underrepresented in these patients. This pattern is due to the complete absence of *N*-linked oliogosaccharide chains on one or both

of the Asn sites of transferrin and is explained by incomplete glycosylation of the protein in the endoplasmic reticulum [22,23,30]. Based on studies in the model system *S. cerevisiae* [31], deficiencies in many different biosynthetic steps can potentially generate a similar or identical transferrin IEF pattern. Patients who do not have a PMM deficiency, such as those described below, also have a 'type 1' IEF pattern. Carbohydrate deficient transferrins (CDT) indicate a glycosylation abnormality, but not the primary defect. This complicates the use of transferrins to type patients clinically. On the other hand, if only undersialylated transferrin (as detected by IEF) is used to define altered glycosylation, then CDGS could potentially include patients without the classical symptoms and presentations seen in PMM-deficient CDGS patients. A biochemical IEF pattern definition of CDGS allows eventual assignment of specific defective genes to the disease.

## 4. Another clinical variant of CDGS

A 6-year-old boy with normal PMM activity was diagnosed with CDGS since his transferrin IEF pattern was identical to that of PMM-deficient CDGS patients. Surprisingly, this patient's clinical profile was incompatible with known CDGS patients [32]. He did not have the typical neurologic, dysmorphic or abnormal skeletal features seen in PMM-deficient children. He showed failure to thrive, recurrent and severe hypoglycemia, evidence of thrombotic lesions, along with hepatic and gastrointestinal problems, such as severe vomiting and diarrhea. His persistently low antithrombin III level prompted transferrin IEF analysis, in spite of the atypical clinical presentation.

The patient's clinical history differed from PMM-deficient CDGS children, who are recognizable at or soon after birth. This boy appeared normal at birth, but at about 1 year of age he began having gastrointestinal problems such as vomiting, diarrhea, protein-losing enteropathy and partial villus atrophy in the small intestine. This led to a possible diagnosis of celiac disease, which is an allergic-like condition caused by consuming gluten-containing products, such as wheat. Strict dietary control gave marginal improvement, but much less than that expected for
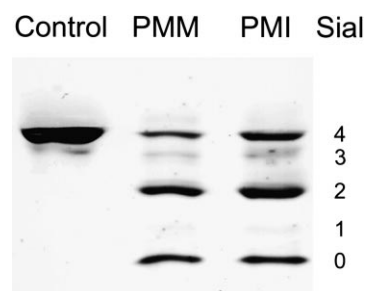


Fig. 2. Comparison of transferrin IEF in control and CDGS patients with different defects. Serum from control (left lane), PMM-deficient (middle lane) and PMI-deficient (right lane) patients was analyzed for transferrin isoelectric focusing pattern. In control, the major band is tetrasialo transferrin having two *N*-linked chains each with two sialic acids. The patterns of the two patients are identical even though they are caused by different defects and have dramatically different clinical symptoms. The patients have residual tetrasialo transferrin, but the absence of one or both *N*-linked chains causes the appearance of disialo- (middle band) and asialo- (lowest band) transferrin. The number of sialic acids per molecule is shown on the right side.

celiac patients. The intestinal biopsy showed evidence of microthrombic lesions suggesting a dangerous procoagulant state. Electron photomicrographs of enterocytes showed that the ER was distended with large amounts of unidentified precipitated proteins filling the lumen. Since he was hypoalbuminemic, he received periodic transfusions of albumin. Low ATIII (10–30% of normal) and the evidence of thromboses prompted anti-coagulant therapy to reduce the risk of clot formation. This may have caused several bouts of serious intestinal bleeding. One life-threatening bleeding episode led to compassionate use of oral mannose therapy, based on previous work showing that exogenous mannose corrected underglycosylation of PMM-deficient fibroblasts [33].

This patient has now been on oral mannose therapy (0.1–0.15 g/kg) 3–5 times per day for 2 years. The hypoglycemia, hypoalbuminemia, protein-losing enteropathy, ATIII deficiency, and chronic symptoms permanently reversed within several months of starting mannose (Fig. 3). His underglycosylated transferrin and $\alpha_1$-antitrypsin IEF patterns essentially returned to normal by 11 months (Fig. 4). Thus, mannose appeared to reverse all the symptoms, but the metabolic basis was not identified until the end of the first year of therapy.

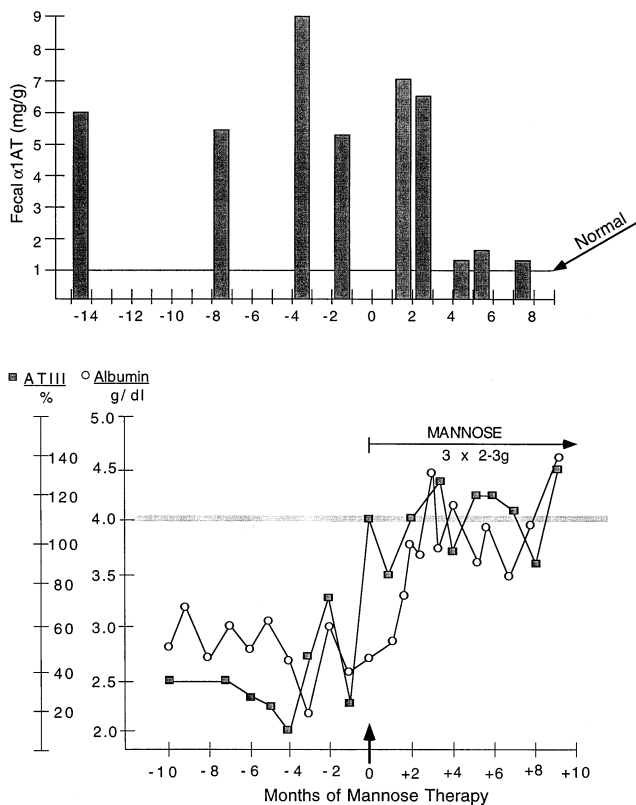Direct assay of fibroblast and leukocyte lysates

Fig. 3. Protein levels in a CDGS type 1b patient before and after mannose therapy. Prior to starting mannose therapy, the patient suffered from reduced levels of ATIII and albumin resulting from protein-losing enteropathy (fecal $\alpha$1-antitrypsin), and received periodic albumin and immunoglobulin transfusions. Anticoagulant therapy (Marcumar) was included to reduce the risk of thromboses. At time '0', mannose was given orally 5 times a day at 100–150 mg/kg. Soon after beginning mannose therapy, protein-losing enteropathy was reversed (top panel), and the ATIII and albumin concentrations returned to normal levels (horizontal line) (bottom panel).

[32] showed that this patient has an 85–95% deficiency in phosphomannose isomerase (PMI) (fructose-6-P $\leftrightarrow$ mannose-6-P). PMI activity was also assayed using intact cells by following the fate of exogenous [2-$^3$H]mannose. When [2-$^3$H]mannose enters the cell, it is phosphorylated to Man-6-P which has only two fates. It can be incorporated into glycoconjugates and their precursors via the PMM reaction, or it can be converted into fructose-6-P via PMI. In the latter reaction, the label is permanently lost as $^3$HOH. By comparing the relative incorporation of label into glycoconjugates including dolichyl-pyrophosphate-linked oligosaccharides to that into $^3$HOH, the PMI activity in the mutant cell can be

measured relative to control cells (Fig. 5). This patient had about 16% residual PMI activity using this assay, which has the advantage of being independent of the imposed artificial conditions required in the in vitro assay. Leukocytes from the mother, father and an unaffected sib had heterozygous levels (34–65%) of PMI activity when measured by the in vitro assay.

Human PMI had been cloned previously [34] based on its homology to yeast. Loss of PMI is lethal in yeast unless the cells are supplied with both mannose and glucose, showing that glucose is the major if not the exclusive source for mannose [35]. Analysis of the PMI from the parents and patient indicted that the paternal allele carried a mutation at amino acid 219 that converted an R$\rightarrow$Q. This R is con-
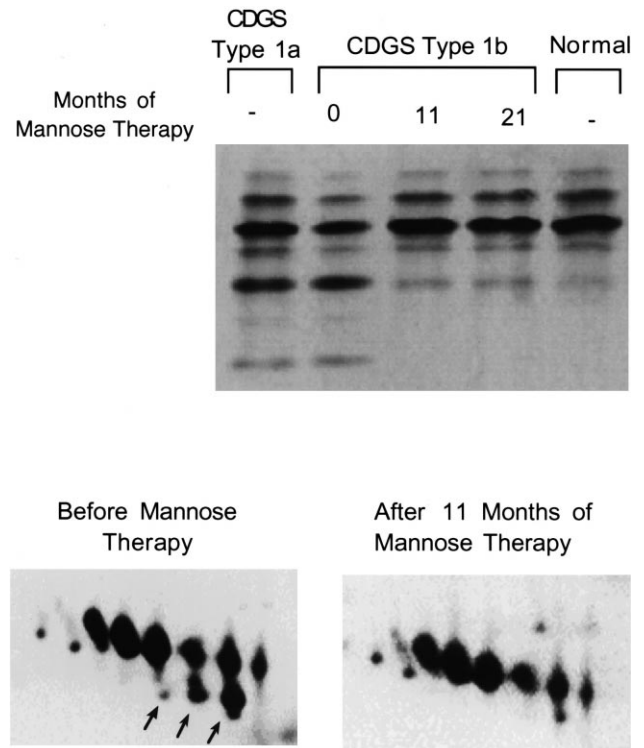


Fig. 4. Effects of mannose therapy on the glycosylation of transferrin and $\alpha$1-antitrypsin in PMI-deficient patient. The top panel shows serum transferrin IEF analysis of a PMM-deficient CDGS patient (lane 1) and the PMI-deficient patient before mannose therapy (lane 2) and then 11 (lane 3) and 21 (lane 4) months after starting mannose therapy. Normal control (lane 5). In the two bottom panels, $\alpha$1-antitrypsin was analyzed by two-dimensional gel electrophoresis before mannose therapy began and then after 11 months of mannose therapy. Undersialylated glycoforms (arrows) disappeared or were considerably reduced after mannose therapy. (From Ref. [32], used with permission from J. Clin. Invest.)
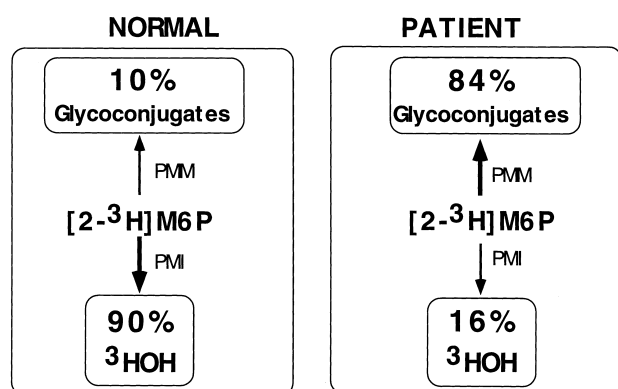
## NORMAL

$$10\% \text{ Glycoconjugates}$$

$\uparrow$ PMM

$$[2\text{-}^3H]M6P$$

$\downarrow$ PMI

$$90\% \quad ^3HOH$$

## PATIENT

$$84\% \text{ Glycoconjugates}$$

$\uparrow$ PMM

$$[2\text{-}^3H]M6P$$

$\downarrow$ PMI

$$16\% \quad ^3HOH$$

Fig. 5. Schematic view of [$^3$H]mannose metabolism in PMI-deficient patient. When [2-$^3$H]mannose enters the cell, it is phosphorylated to [2-$^3$H]Man-6-P, which can be acted on by two different enzymes, PMM or PMI. Reaction with PMM leads to glycoconjugate synthesis. Reaction with PMI leads to the irreversible loss of $^3$HOH and further metabolism of non-labeled Fru-6-P. Under these conditions, about 90% of the [2-$^3$H]Man is converted to $^3$HOH and 10% is found in glycoconjugates. In PMI-deficient fibroblasts, the proportion found in glycoconjugates increases to 84% and $^3$HOH is reduced to about 16%. This assay provides an accurate in vivo measure of PMI activity within the cells.

served in *Candida albicans*, *Caenorhabditis elegans* and mouse PMI, but it does not appear to be located in the active site of the enzyme. Active PMI is thought to be a monomer and it is not known to require binding to other proteins for catalytic activity. Expression of this abnormal allele in COS cells showed 0–10% of normal activity compared to control. The maternal allele showed no mutation in the coding region. This allele was greatly under-represented in RT-PCR clones derived from the patient. This suggested that the maternal allele was inefficiently transcribed or unstable. The maternal allele probably accounted for very little active enzyme in the patient. In line with this hypothesis, the mother's leukocytes had only 34% of normal PMI activity. Both parents are asymptomatic.

Since this patient has the same transferrin pattern as PMM-deficient patients who are defined as type 1a, PMI deficiency has been designated type 1b.

## 5. Additional patients with PMI deficiency

Currently there are eight additional patients who have been identified with PMI deficiency. In one study [36], Jaeken et al. found two PMI deficient patients with a similar clinical presentation. The first patient had two mutations changing amino acid residues conserved in *S. cerevisiae* and *C. albicans* PMI: S102L and M138T. The Glu-137 position is probably involved in binding $Zn^{2+}$, which is required for enzymatic activity. Unfortunately, this patient died at age 4 years, prior to diagnosis and did not have an opportunity for mannose therapy. Another patient had very low PMI activity and an abnormal transferrin IEF pattern during the first several months of life. He had severe vomiting and a spectrum of symptoms that included generalized hypotonia, megaloblastic anemia, liver dysfunction, low ATIII, and diffuse hypodensity of white matter. No mutations were reported for his PMI. At age 4 months, he began a diet containing solid food such as vegetables, fruit and meat. He subsequently stopped vomiting, showed improved weight gain and muscle tone. The biochemical parameters gradually improved and became normal by 10 months of age. Transferrin IEF normalized. He is now healthy with normal psychomotor development. This dramatic reversal was tentatively attributed to changing to a diet containing increased amounts of available mannose. Very little is known about the availability of mannose in the human diet, but some foods contain indigestible galactomannans which have β-linked mannose residues that are probably not easily hydrolyzed in the digestive system [37–40]. However, it is difficult to know how much of an 'indigestible' carbohydrate would be sufficient to provide mannose for PMI-deficient patients.

In another study, three teenage sibs of one consanguineous family had only ∼10–20% of normal PMI activity [41]. They had milder symptoms than the patient described above, and did not have demonstrated protein-losing enteropathy. They had vomiting and diarrhea that resulted in dehydration and hospitalizations about once a year. The onset began at 10 months of age in two of the patients and at 2 months of age in the other. Albumin was always low. A liver biopsy was consistent with congenital hepatic fibrosis. The hepatic symptoms were variable and ranged from mildly enlarged liver to liver failure. Mental and motor development were completely normal. The asymptomatic parents had heterozygous PMI levels. Although the specific defects have not

been localized within the *PMI1* gene, it is clear that the same PMI deficiency can generate a range of intestinal and hepatic symptoms of variable severity. The results of mannose therapy have not yet been published.

Another PMI-deficient patient was a 2-month-old child with hypoglycemia, vomiting, hepatomegaly, elevated transaminases, diarrhea, low ATIII and Factor XI, and hypoalbuminemia [42]. Hypoglycemia was reported to result from hyperinsulinemia. He developed a thrombosis from a central venous catheter used for delivery of nutrients. Mannose therapy ($4 \times 0.17$ g/kg b.wt.) led to clinical improvement. Sequencing of the cDNA revealed that the patient was a compound heterozygote. The mutant PMI alleles were not expressed, so the deleterious effects of these mutations on enzyme activity are not proven. The beneficial effects of mannose therapy support the lack of sufficient PMI activity.

A study on another PMI-deficient patient shows significant improvement with mannose therapy [43]. In this case, a 2-year-old girl with ∼5–9% of normal PMI activity showed protein-losing enteropathy, low serum protein, generalized edema, severe hypoglycemia, and elevated liver transaminases. She developed a thrombosis, had reduced coagulation factors, ATIII, and protein S. After 4 months of mannose therapy, anemia, hypoalbuminemia and hypoglycemia resolved. Elevated liver-derived transaminases have decreased, but have not become normal. The mutations have not been determined. A 14-year-old patient with protein-losing enteropathy and hypoglycemia has been treated with $4 \times 0.1$ g mannose/kg b.wt. for 5 months. Both clinical features improved (CDG Family Network, unpublished, HTTP:// www.cdgs.com/).

Since mannose supplementation will probably be a life-long therapy for PMI-deficient patients, side effects have to be carefully monitored. The only side effect that can be directly attributed to mannose is a slight rise in glycated hemoglobin (HbA1c) in the first patient described above. Mannose is about five times as active in non-enzymatic glycation as an equivalent concentration of glucose [44]. The level of HbA1c decreased when the mannose dosage was reduced. There is a practical limit to the amount of ingested mannose that patients can be given at any one time since concentrations greater than about 0.2

g/kg b.wt. cause osmotic diarrhea in most people [45,46].

Since the diagnosis and treatment of potentially fatal PMI-deficiency is straightforward, it seems very worthwhile to consider that previously diagnosed cases of idiopathic congenital hepatic fibrosis, hypoglycemia, coagulopathies, and failure to thrive should be considered as potential PMI deficiencies. Two case reports in the literature are likely to fall under this umbrella [47,48] and in those cases, 4 of the 5 patients from two families died from multiple thromboses.

## 6. A novel CDGS type I caused by a deficiency in glucosylation of the lipid-linked oligosaccharides

A novel type of CDGS was recently reported based on the analysis of four related patients with CDGS type I serum sialotransferrin pattern, presumably resulting from the absence of entire *N*-linked chains. These patients resembled the PMM-deficient patients, but they showed considerably milder presentations. All of these patients had normal PMM activity [49]. A detailed analysis of patient derived fibroblasts revealed that glycoprotein biosynthesis was drastically reduced in these cells. Inspection of the biosynthesis of the dolichylpyrophosphate-linked oligosaccharide $Glc_3Man_9GlcNAc_2$, the substrate for the *N*-linked glycosylation, showed an impaired glucosylation of the oligosaccharide and resulted in the accumulation of dolichylpyrophosphate-linked $Man_9GlcNAc_2$. It is well known that glucosylation of the lipid-linked oligosaccharide is important for efficient transfer of the oligosaccharide to protein by the oligosaccharyltransferase [50]. The impaired glucosylation of the lipid-linked oligosaccharide therefore explained the hypoglycosylation of glycoproteins observed in these patients. However, it was noted that glucosylation of the oligosaccharide was not totally absent in patient fibroblasts because small, but significant, amounts of fully assembled $Glc_3Man_9GlcNAc_2$ were observed. This altered glucosylation of the lipid-linked oligosaccharide can be caused by a deficiency of the enzyme adding the first α1,3-linked glucosyl residue to the $Man_9GlcNAc_2$ oligosaccharide. This enzyme, dolichyl-P-Glc:$Man_9GlcNAc_2$-PP-dolichol glucosyltransferase, transfers a

glucosyl residue from dolichylphosphoglucose to $Man_9GlcNAc_2$-PP-dolichol. Alternatively, impaired dolichylphosphoglucose synthase activity can also lead to the accumulation of dolichylpyrophosphate-linked $Man_9GlcNAc_2$. To address this issue directly, the cDNA encoding dolichylphosphoglucose synthase and dolichyl-P-Glc:$Man_9GlcNAc_2$-PP-dolichol glucosyltransferase from healthy individuals and patient fibroblasts were isolated. The identification of the cDNAs was based on the sequence of the corresponding yeast loci, *ALG5* [51] and *ALG6* [52], respectively. No mutation in the patient ALG5 cDNA was found, however, a mutation changing an alanine residue at position 333 to a valine residue was detected in the patient *ALG6* cDNA. This seemingly minor change had important consequences for enzymatic activity, since expression of normal human cDNA complements a deletion of the *ALG6* locus in yeast. However, expression of the mutant cDNA was not able to complement the deletion in the *ALG6* locus. This finding provided: (a) the proof that the isolated cDNA encoded dolichyl-P-Glc:$Man_9GlcNAc_2$-PP-dolichol glucosyltransferase activity; and (b) the functional link between the mutation found in these patients and the impaired glucosylation of the oligosaccharide. Genotypic analysis showed that all four related patients were homozygous for the mutation in question. The parents of two of these patients were heterozygous [69].

Recently, a 7-year-old girl with mental and psychomotor retardation and coagulation problems was reported [53]. Serum transferrin IEF also revealed the same pattern as seen in the above patients, but with normal PMM and PMI activities. A detailed analysis of the lipid-bound oligosaccharides showed the accumulation of $Man_9GlcNAc_2$-PP-dolichol in fibroblasts from this patient. In addition, a strongly reduced activity of the dolichyl-P-Glc:$Man_9GlcNAc_2$-PP-dolichol glucosyltransferase was measured in fibroblast derived extracts. These results suggest that also in this patient, a mutant *ALG6* allele might be responsible for the disease.

It is important to note that this novel type of CDGS is distinct from CDGS type Ia and Ib. It is only expected to affect *N*-linked protein glycosylation, whereas a deficiency in PMM and PMI also alters the biosynthesis of GPI anchors as well as *O*- and *C*-mannosylation in the ER. It will be interesting to see whether specific clinical manifestations can be attributed to the different CDGS types.

Von Figura and co-workers named this case CDGS type V; however, we suggest that it be called type Ic. We propose a more simplified nomenclature for CDGS that is still based on transferrin IEF, but differentiates the absence of entire chains from defects in subsequent oligosaccharide processing. Type I includes defects in the synthesis of precursors and the addition of chains in the ER by oligosaccharyltransferase, whereas type II represents defects altering the processing of the *N*-linked oligosaccharide after its transfer to protein by the oligosaccharyltransferase. This would accommodate the known ways of creating deficiencies in glycosylation and would allow a preliminary classification of novel cases based on biochemical analysis (e.g. isoelectrofocusing of serum transferrin). A precise classification of a given syndrome (e.g. CDGS type Ic) is only possible when the defect is identified at a molecular level. The pleiotropic effect of mutations altering the pathway of *N*-linked glycosylation requires such a stringent classification, but we are aware that it does not simplify the clinician's task of dealing with the highly variable clinical presentations [7].

*N*-Linked protein glycosylation in the ER is a complex pathway and the analysis of CDGS type I has revealed a deficiency in this process as the primary cause for the disease. Biochemical analysis of the pathway proved to be difficult because it directly involves very diverse biological macromolecules such as lipids, oligosaccharides and proteins. Yeast molecular genetics allowed significant progress in the analysis and characterization of the process (for a review, see [31]) and brought to light a high degree of conservation of the pathway in eukaryotic cells. As shown for the analysis of CDGS type Ic, this evolutionary conservation makes it possible to apply the yeast system as a tool to identify directly the primary cause of different types of CDGS.

## 7. Are there other variations of CDGS?

About 25–30% of all diagnosed CDGS patients with an altered transferrin IEF pattern have normal PMM activity and therefore must have other defects. PMM-deficient patients are clinically the best char-

acterized of the group; they represent the diagnostic standard. However, the clinical variations among the non-PMM deficient patients are considerably broader than seen within the PMM-deficient cases. Some non-PMM deficient patients currently under investigation were detected by keen-eyed clinicians who suspected glycosylation abnormalities even though the patients did not present the typical symptoms of PMM deficiency.

Considering the large number of steps involved in *N*-glycosylation, there are many possible lesions. Up to 0.5–1% of the transcribed human genes may be devoted to the production or recognition of glycosylated molecules [54]. Not all such defects might produce symptoms, of course. On the other hand, it could mean that glycosylation-based disorders are considerably more common than is currently appreciated, as suggested in recent editorials [55,56] and reviews [4]. Reports of several non-PMM cases with the typical underglycosylation pattern seen for type 1 cases have appeared [57,58]. In one study [57], both patients had serious respiratory problems, protein-losing enteropathy and thrombocytopenia. Both died soon after birth. The other report shows only that the defect is not in PMM, but displayed an altered transferrin IEF and unusual oligosaccharide structures. This raises an important issue. The IEF reveals only altered sialylation, it does not reveal the structure of the oligosaccharide chain. Knowing the structure may be of considerable benefit in trying to assess the primary defect, but it might also mislead. Some glycosyltransferases, such as sialyltransferase, depend on glycosylation for full activity [59]. Decreases in sialylation may be secondary to misglycosylation caused by another defect in an earlier portion of the pathway.

It is important to point out that not all glycosylation defects can be detected with serum transferrin IEF, since transferrin has only a portion of known types of *N*-linked chains. For instance, leukocyte adhesion deficiency II [60,61] is literally a CDGS since misglycosylated proteins are produced due to a lesion in a general aspect of fucosylation. However, most serum glycoproteins, including transferrin, are not fucosylated and would not indicate altered fucosylation. Other diagnostic tests that do not rely on transferrin need to be developed to screen for other forms of altered glycosylation.

## 8. Future directions

Additional defects in patients who show the same transferrin IEF pattern as PMM-, PMI-, and glucosyltransferase-deficient patients continue to be found. Work is in progress using the yeast system to analyze such patients in much the same way it was used for pin-pointing the α-glucosyltransferase deficiency. Since humans and yeast share nearly all of the steps for generating a dolichol-linked precursor and transferring it to protein, it is difficult to imagine a more complementary system for tracking down these defects [31]. Biosynthesis of both the carbohydrate chain and the lipid carrier would be amenable to analysis in yeast, and defects in either pathway would be expected to compromise glycosylation and lead to other forms of CDGS [62–65].

Currently, PMM-deficiency is the most common CDGS defect known; only a handful of patients define the other varieties. However, in contrast to PMM-deficient patients who have been recognized for nearly 20 years, the defects described here have only been recognized for a very short time. The populations of these new patients actually may be greater than PMM-deficient patients. In addition, some glycosylation defects may have already been described in the medical literature, but were not recognized as such. They may have been viewed as idiopathic 'variants' or 'types' of well-known disorders. A good example is congenital hepatic fibrosis [66] where at least some cases in the literature can now be recognized as likely PMI deficiencies [47,48]. Clinicians acutely aware of the importance of glycosylation will be vital for identifying new patients and for linking diseases to altered glycosylation. Some of these disorders may be treatable by simple dietary therapy, but our paltry understanding of direct use of monosaccharide therapy for glycosylation diseases requires further development [67,68].

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## References

[1] D. Krasnewich, W.A. Gahl, Carbohydrate-deficient glycoprotein syndrome, Adv. Pediatr. 44 (1997) 109–140.

[2] H. Carchon, E. Van Schaftingen, G. Matthijs, J. Jaeken, Carbohydrate-deficient glycoprotein syndrome type 1A (phosphomannomutase-deficiency), Biochim. Biophys. Acta 1455 (1999) 155–165.

[3] H. Schachter, J. Jaeken, Carbohydrate-deficient glycoprotein syndrome type II, Biochim. Biophys. Acta 1455 (1999) 179–192.

[4] H.H. Freeze, Disorders in protein glycosylation and potential therapy: tip of an iceberg?, J. Pediatr. 133 (1998) 593–600.

[5] H. Schachter, J. Tan, M. Sarkar, B. Yip, S. Chen, J. Dunn, J. Jaeken, Defective glycosyltransferases are not good for your health, Adv. Exp. Med. Biol. 435 (1998) 9–27.

[6] G. McDowell, W.A. Gahl, Inherited disorders of glycoprotein synthesis: cell biological insights, Proc. Soc. Exp. Biol. Med. 215 (1997) 145–157.

[7] J. Jaeken, H. Carchon, H. Stibler, The carbohydrate-deficient glycoprotein syndromes: pre-Golgi and Golgi disorders?, Glycobiology 3 (1993) 423–428.

[8] J. Jaeken, H. Stibler, B. Hagberg, The carbohydrate-deficient glycoprotein syndrome. A new inherited multisystemic disease with severe nervous system involvement, Acta Paediatr. Scand. Suppl. 375 (1991) 1–71.

[9] B.A. Hagberg, G. Blennow, B. Kristiansson, H. Stibler, Carbohydrate-deficient glycoprotein syndromes: peculiar group of new disorders, Pediatr. Neurol. 9 (1993) 255–262.

[10] C.T. Yuen, W. Chai, R.W. Loveless, A.M. Lawson, R.U. Margolis, T. Feizi, Brain contains HNK-1 immunoreactive O-glycans of the sulfoglucuronyl lactosamine series that terminate in 2-linked or 2,6-linked hexose (mannose), J. Biol. Chem. 272 (1997) 8924–8931.

[11] A. Chiba, K. Matsumura, H. Yamada, T. Inazu, T. Shimizu, S. Kusunoki, I. Kanazawa, A. Kobata, T. Endo, Structures of sialylated O-linked oligosaccharides of bovine peripheral nerve alpha-dystroglycan. The role of a novel O-mannosyltype oligosaccharide in the binding of alpha-dystroglycan with laminin, J. Biol. Chem. 272 (1997) 2156–2162.

[12] R. Kornfeld, S. Kornfeld, Assembly of asparagine-linked oligosaccharides, Annu. Rev. Biochem. 54 (1985) 631–634.

[13] R.N. Cole, G.W. Hart, Glycosyl-phosphatidylinositol anchors: structure, biosynthesis and function, in: J. Montreuil et al. (Eds.), Glycoproteins, Elsevier, Amsterdam, pp. 69–88.

[14] M.A. Doucey, D. Hess, R. Cacan, J. Hofsteenge, Protein C-mannosylation is enzyme-catalysed and uses dolichylphosphate-mannose as a precursor, Mol. Biol. Cell 9 (1998) 291–300.

[15] J. Krieg, S. Hartmann, A. Vicentini, W. Glasner, D. Hess, J. Hofsteenge, Recognition signal for C-mannosylation of TRP-7 in RNase 2 consists of sequence TRP-X-X-TRP, Mol. Biol. Cell 9 (1998) 301–309.

[16] J. Krieg, W. Glasner, A. Vicentini, M.A. Doucey, A. Loffler, D. Hess, J. Hofsteenge, C-Mannosylation of human RNase 2 is an intracellular process performed by a variety of cultured cells, J. Biol. Chem. 272 (1997) 26687–26692.

[17] H. Stibler, J. Jaeken, Carbohydrate deficient serum transferrin in a new systemic hereditary syndrome, Arch. Dis. Child. 65 (1990) 107–111.

[18] H. Stibler, Carbohydrate-deficient transferrin in serum: a new marker of potentially harmful alcohol consumption reviewed, Clin. Chem. 37 (1991) 2029–2037.

[19] J. Jaeken, H. Stibler, in: L. Wetterberg (Ed.), Genetics of Neuropsychiatric Diseases, Wenner-Green International Symposium Series, 1989, pp. 69–80.

[20] K. Lof, T. Koivula, K. Seppa, T. Fukunaga, P. Sillanaukee, Semi-automatic method for determination of different isoforms of carbohydrate-deficient transferrin, Clin. Chim. Acta 217 (1993) 175–186.

[21] G. De Jong, R. Feelders, W.L. van Noort, H.G. Van Eijk, Transferrin microheterogeneity as a probe in normal and disease states, Glycoconj. J. 12 (1995) 219–226.

[22] K. Yamashita, T. Ohkura, H. Ideo, K. Ohno, M. Kanai, Electrospray ionization-mass spectrometric analysis of serum transferrin isoforms in patients with carbohydrate-deficient glycoprotein syndrome, J. Biochem. 114 (1993) 766–769.

[23] K. Yamashita, H. Ideo, T. Ohkura, K. Fukushima, I. Yuasa, K. Ohno, K. Takeshita, Sugar chains of serum transferrin from patients with carbohydrate deficient glycoprotein syndrome. Evidence of asparagine-N-linked oligosaccharide transfer deficiency, J. Biol. Chem. 268 (1993) 5783–5789.

[24] B. Coddeville, H. Carchon, J. Jaeken, G. Briand, G. Spik, Determination of glycan structures and molecular masses of the glycovariants of serum transferrin from a patient with carbohydrate deficient glycoprotein syndrome type II, Glycoconj. J. 15 (1998) 265–273.

[25] H. Stibler, U. von Dobeln, B. Kristiansson, C. Guthenberg, Carbohydrate-deficient transferrin in galactosaemia, Acta Paediatr. 86 (1997) 1377–1378.

[26] J. Jaeken, M. Pirard, M. Adamowicz, E. Pronicka, E. Van Schaftingen, Inhibition of phosphomannose isomerase by fructose 1-phosphate: an explanation for defective N-glycosylation in hereditary fructose intolerance, Pediatr. Res. 40 (1996) 764–766.

[27] M. Adamowicz, E. Pronicka, Carbohydrate deficient glycoprotein syndrome-like transferrin isoelectric focusing pattern in untreated fructosaemia [letter], Eur. J. Pediatr. 155 (1996) 347–348.

[28] J. Charlwood, P. Clayton, G. Keir, N. Mian, B. Winchester, Defective galactosylation of serum transferrin in galactosemia, Glycobiology 8 (1998) 351–357.

[29] E. Landberg, P. Pahlsson, A. Lundblad, A. Arnetorp, J.O. Jeppsson, Carbohydrate composition of serum transferrin

isoforms from patients with high alcohol consumption, Biochem. Biophys. Res. Commun. 210 (1995) 267–274.

[30] Y. Wada, A. Nishikawa, N. Okamoto, K. Inui, H. Tsukamoto, S. Okada, N. Taniguchi, Structure of serum transferrin in carbohydrate-deficient glycoprotein syndrome, Biochem. Biophys. Res. Commun. 189 (1992) 832–836.

[31] P. Burda, M. Aebi, The dolichol pathway of N-linked glycosylation, Biochim. Biophys. Acta 1426 (1999) 239–257.

[32] R. Niehues, M. Hasilik, G. Alton, C. Korner, M. Schiebe-Sukumar, H.G. Koch, K.P. Zimmer, R. Wu, E. Harms, K. Reiter, K. Von Figura, H.H. Freeze, H.K. Harms, T. Marquardt, Carbohydrate-deficient glycoprotein syndrome type Ib. Phosphomannose isomerase deficiency and mannose therapy, J. Clin. Invest. 101 (1998) 1414–1420.

[33] K. Panneerselvam, H.H. Freeze, Mannose corrects altered N-glycosylation in carbohydrate-deficient glycoprotein syndrome fibroblasts, J. Clin. Invest. 97 (1996) 1478–1487.

[34] A.E. Proudfoot, G. Turcatti, T.N. Wells, M.A. Payton, D.J. Smith, Purification, cDNA cloning and heterologous expression of human phosphomannose isomerase, Eur. J. Biochem. 219 (1994) 415–423.

[35] D.J. Smith, A. Proudfoot, L. Friedli, L.S. Klig, G. Paravicini, M.A. Payton, PMI40, an intron-containing gene required for early steps in yeast mannosylation, Mol. Cell. Biol. 12 (1992) 2924–2930.

[36] J. Jaeken, G. Matthijs, J.M. Saudubray, C. Dionisivici, E. Bertini, P. Delonlay, H. Henri, H. Carchon, E. Schollen, E. Vanschaftingen, Phosphomannose isomerase deficiency-A carbohydrate-deficient glycoprotein syndrome with hepatic-intestinal presentation, Am. J. Hum. Genet. 62 (1998) 1535–1539.

[37] S.E. Davis, B.A. Lewis, in: A. Jeanes, J. Hodge (Eds.), Physiological effects of food carbohydrates, American Chemical Society, Washington, DC, 1975, pp. 296–311.

[38] B. Seetharam, A.N. Radhakrishnan, Intestinal-glycosidases: α-mannosidase and β-galactosidase from monkey small intestine, Indian J. Biochem. Biophys. 9 (1972) 59–63.

[39] J.S.G. Reid, in: J. Bewley (Ed.), Biochemistry of Storage Carbohydrates in Green Plants, Academic Press, London, 1985, pp. 265–288.

[40] J.D. Bewley, J.S. Reid, in: Biochemistry of Storage Carbohydrates in Green Plants, Academic Press, London, 1985, pp. 289–304.

[41] T.J. de Koning, L. Dorland, O.P. van Diggelen, A.M.C. Boonman, G.J. de Jong, W.L. van Noort, J. De Schryver, M. Duran, I.E.T. van den Berg, G.J. Gerwig, R. Berger, B.T. Poll-The, A novel disorder of N-glycosylation due to phosphomannose isomerase deficiency, Biochem. Biophys. Res. Commun. 245 (1998) 38–42.

[42] P. De Lonlay, M. Cuer, S. Barrot, P. Castelnau, G. Touati, G. Durand, J.M. Saudubray, N. Seta, Hyperinsulinemic hypoglycemia as presenting symptom in phosphomannose isomerase deficiency: a new presentation of carbohydrate-deficient glycoprotein syndrome treatable by mannose, J. Inherit. Metab. Dis., 21 (1998) 96 (Abstr).

[43] D. Babovica, M.C. Patterson, W.F. Schwenk, J.F. O'Brien, J. Vockley, H.H. Freeze, D.P. Mehta, V.V. Michels, Severe hypoglycemia as a presenting symptom of carbohydrate-deficient glycoprotein syndrome, J. Pediatr., in press.

[44] H.F. Bunn, P.J. Higgins, Reaction of monosaccharides with proteins: possible evolutionary significance, Science 213 (1981) 222–224.

[45] G. Alton, S. Kjaergaard, J.R. Etchison, F. Skovby, H.H. Freeze, Oral ingestion of mannose elevates blood mannose levels: a first step toward a potential therapy for carbohydrate-deficient glycoprotein syndrome type I, Biochem. Mol. Med. 60 (1997) 127–133.

[46] F.C. Wood, G.F. Cahill, Mannose utilization in man, J. Clin. Invest. 42 (1963) 1300–1312.

[47] V.A. Pelletier, N. Galéano, P. Brochu, C.L. Morin, A.M. Weber, C.C. Roy, Secretory diarrhea with protein-losing enteropathy, enterocolitis cystica superfilialis, intestinal lymphangiectasia, and congenital hepatic fibrosis: a new syndrome, J. Pediatr. 108 (1986) 61–65.

[48] P.S. Pedersen, I. Tygstrup, Congenital hepatic fibrosis combined with protein-losing enteropathy and recurrent thrombosis, Acta Paediatr. Scand. 69 (1998) 571–574.

[49] P. Burda, L. Borsig, J. de Rijk-van Andel, R. Wevers, J. Jaeken, H. Carchon, E.G. Berger, M. Aebi, A novel carbohydrate-deficient glycoprotein syndrome characterized by a deficiency in glucosylation of the dolichol-linked oligosaccharide, J. Clin. Invest. 102 (1998) 647–652.

[50] M.J. Spiro, R.G. Spiro, V.D. Bhoyroo, Glycosylation of proteins by oligosaccharide-lipids. Studies on a thyroid enzyme involved in oligosaccharide transfer and the role of glucose in this reaction, J. Biol. Chem. 254 (1979) 7668–7674.

[51] S. Heesen, L. Lehle, A. Weissmann, M. Aebi, Isolation of the ALG5 locus encoding the UDP-glucose:dolichyl-phosphate glucosyltransferase from Saccharomyces cerevisiae, Eur. J. Biochem. 224 (1994) 71–79.

[52] G. Reiss, S. te Heesen, J. Zimmerman, P.W. Robbins, M. Aebi, Isolation of the ALG6 locus of Saccharomyces cerevisiae required for glucosylation in the N-linked glycosylation pathway, Glycobiology 6 (1996) 493–498.

[53] C. Körner, R. Knauer, U. Holzbach, F. Hanefeld, L. Lehle, K. Von Figura, Carbohydrate-deficient glycoprotein syndrome type V: deficiency of dolichyl-P-Glc:Man₉GlcNAc₂-PP-dolichyl glucosyltransferase, Proc. Natl. Acad. Sci. USA 95 (1998) 13200–13205.

[54] A. Varki, J. Marth, Oligosaccharides in vertebrate development, Dev. Biol. 6 (1995) 127–138.

[55] W.A. Gahl, Carbohydrate-deficient glycoprotein syndrome: hidden treasures, J. Lab. Clin. Med. 129 (1997) 394–395.

[56] S. Kornfeld, Diseases of abnormal protein glycosylation – an emerging area, J. Clin. Invest. 101 (1998) 1293–1295.

[57] M.J. Acarregui, T.N. George, W.J. Rhead, Carbohydrate-deficient glycoprotein syndrome type 1 with profound thrombocytopenia and normal phosphomannomutase and phosphomannose isomerase activities, J. Pediatr. 133 (1998) 697–700.

[58] J. Charlwood, P. Clayton, A. Johnson, G. Keir, N. Mian, B.

Winchester, A case of the carbohydrate-deficient glycopro-
tein syndrome type 1 (CDGS type 1) with normal phospho-
mannomutase activity, J. Inherit. Metab. Dis. 20 (1997) 817–
826.

[59] D.G. Fast, J.C. Jamieson, G. McCaffrey, The role of the
carboydrate chains of Gal beta-1,4-GlcNAc alpha 2,6-sialyl-
transferase for enzyme activity, Biochim. Biophys. Acta 1202
(1993) 325–330.

[60] A. Karsan, C.J. Cornejo, R.K. Winn, B.R. Schwartz, W.
Way, N. Lannir, R. Gershoni-Baruch, A. Etzioni, H.D.
Ochs, J.M. Harlan, Leukocyte adhesion deficiency type II
is a generalized defect of de novo GDP-fucose biosynthesis.
Endothelial cell fucosylation is not required for neutrophil
rolling on human nonlymphoid endothelium, J. Clin. Invest.
101 (1998) 2438–2445.

[61] J.B. Lowe, Selectin ligands, leukocyte trafficking, and fuco-
syltransferase genes, Kidney Int. 51 (1997) 1418–1426.

[62] S.S. Krag, The importance of being dolichol, Biochem. Bio-
phys. Res. Commun. 243 (1998) 1–5.

[63] C.W. Hall, K.R. McLachlan, S.S. Krag, A.R. Robbins, Re-
duced utilization of $Man_5GlcNAc_2$-P-P-Lipid in Lec9 mu-
tant of Chinese hamster ovary cells – analysis of the steps
in oligosaccharide-lipid assembly, J. Cell Biochem. 67 (1997)
201–215.

[64] C.W. Hall, A.R. Robbins, S.S. Krag, Synthesis of dolichol in
a polyprenol reductase mutant is restored by elevation of cis-
prenyl transferase activity, Arch. Biochem. Biophys. 343
(1997) 19–26.

[65] T. Ohkura, K. Fukushima, A. Kurisaki, H. Sagami, K.
Ogura, K. Ohno, S. Hara-Kuge, K. Yamashita, A partial
deficiency of dehydrodolichol reduction is a cause of carbo-
hydrate-deficient glycoprotein syndrome type I, J. Biol.
Chem. 272 (1997) 6868–6875.

[66] H.H. Freeze, New diagnosis and treatment of congenital
hepatic fibrosis, J. Pediatr. Gastroent. Nutr. 29 (1999) 104–
106.

[67] H.H. Freeze, Human glycosylation disorders and sugar sup-
plement therapy, Biochem. Biophys. Res. Commun., in
press.

[68] A. Martin, C. Rambal, V. Berger, S. Perier, P. Louisot,
Availability of specific sugars for glycoconjugate biosynthesis
– a need for further investigations in man, Biochimie 80
(1998) 75–86.

[69] T. Imbach, P. Burda, P. Kuhner, R.A. Wevers, M. Aebi,
E.G. Berger, T. Hennet, A mutation in the human ortholog
of the *Saccharomyces cerevisiae ALG6* gene causes carbohy-
drate-deficient glycoprotein syndrome type-Ic, Proc. Natl.
Acad. Sci. USA 96 (1999) 6982–6987.