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QUARTERLY

Review

Carbohydrate-deficient glycoprotein syndromes

Jerzy Kościelak

Department of Biochemistry, Institute of Hematology and Blood Transfusion, Warszawa, Poland

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Carbohydrate-deficient glycoprotein syndromes are rare, multisystemic diseases, typically with major nervous system impairment, that are caused by hypo- and unglycosylation of N-linked glycoproteins. Hence, a biochemical evidence of this abnormality, like hypoglycosylation of serum transferrin is essential for diagnosis. Clinically and biochemically, six types of the disease have been delineated. Three of them are caused by deficiencies of the enzymes that are required for a proper glycosylation of lipid – (dolichol) linked oligosaccharide (phosphomannomutase or phosphomannose isomerase or alpha-glycosyltransferase), and one results from a deficiency of Golgi resident *N*-acetylglucosaminyltransferase II. In addition one variant of the disease has been reported as due to a defective biosynthesis of dolichol iself. The diseases are heritable but genetics has been established for only two types. Therapy, based on administration of mannose to patients is currently under investigation. It benefits patients with deficiency of phosphomannose isomerase. Taking into account the complexity of N-linked glycosylation of proteins more of the disease variants is expected to be found.

Carbohydrate-deficient glycoprotein syndromes (CDGS) are a group of multisystem diseases with a worldwide occurrence. Incidence has been recently estimated at 1/80 000 of live births [1]. Thus, in Poland, a country of almost 40 million inhabitants, there should be about five hundred patients with CDGS. In fact fewer patients have been reported in the

✉Corresponding address: Prof. Dr. Jerzy Kościelak, Institute of Hematology and Blood Transfusion, Chocimska 5, 00-957 Warszawa, Poland; Tel/fax: (48 22) 848 9515; Fax: (48 22) 848 8970; e-mail: kosci@atos.warman.com.pl

Abbreviations: CDGS, carbohydrate-deficient glycoprotein syndrome(s); CHO, Chinese hamster ovary; DolPP, dolichyldiphosphate; ER, endoplasmic reticulum; GnII, *N*-acetylglucosaminyltransferase II; LLO, lipid-linked oligosaccharide; PMI, phosphomannose isomerase; PMM, phosphomannomutase.

whole world and in Poland only several are known. Hence, the disease is not properly diagnosed.

Clinical symptoms and diagnosis of CDGS develop from the neonatal period with failure to thrive, clumsiness, sometimes growth retardation, liver insufficiency, and cardiac symptoms [2]. There is abnormal distribution of fat especially in the buttocks. Gradually psychomotor retardation, neuropathy, axial hypotonia, coagulation abnormalities, and dysfunction of many organs including liver become evident. The activity of lysosomal enzymes in blood serum is often increased [3]. Mortality is high in the first five years of life and amounts to about 30%. Later on patients seem to adapt to the disease and the mortality drops. With extrovert disposition and a low IQ in the range of 50–60%, the patients seem to be quite content. Another feature of the disease is dysmorphism that apart from the abnormal distribution of fat manifests in the face and skeleton. Ears are low set and large, bridge of the nose is high, lower lip sometimes everted, and lower jaw prominent [2, 4, 5]. Eyes are almond shaped, and strabismus is common. Thorax is broad and short, with nipples often inverted while legs are thin and long.

The disease is caused by carbohydrate deficiency of N-linked glycoproteins manifested by their hypoglycosylation and unglycosylation. The former term describes a situation when the non-reducing termini of glycoprotein glycans contain fewer than the normal number of glycosyl residues whereas the latter – a situation when entire glycans are missing. Thus, an indispensable element of the diagnosis is some evidence for a deficiency of carbohydrates in glycoproteins. This is usually obtained through analysis of serum transferrin [6] that contains two N-linked glycans. These glycans are of the complex type and are present in blood serum as bi- (see Fig. 1), tri- and tetraantennary glycoforms [7, 8]. Each antenna is terminated with a sialic acid residue. The major glycoform of normal serum transferrin contains two biantennary glycans, with four sialic acid residues and only low amounts of trisialo and pentasialo forms. In CDGS, the major transferrin species contains only two sialic acid residues. Other glycoforms may also be present, sometimes with minor amounts of those devoid of sialic acid. To determine the glycosylation status of transferrin, blood serum is subjected to electrofocusing followed by immunostaining of transferrin bands with anti-transferrin antibody. It should be remembered, though, that hypoglycosylation of transferrin may occur also in alcoholics [9, 10]. The disease was first described in 1980 [11]. At present 6 types of the disease are known. The classification takes into account both clinical and biochemical abnormalities. Type Ia is a severe disease with most symptoms present in affected patients [2, 12, 13]. This type is also most common and accounts for about 80% of all reported cases [3]. Type Ib manifest itself as a protein losing enteropathy with coagulation abnormalities and hypoglycemia but without neurological symptoms [14–16]. Types II–IV were reported only in 2 patients each. In type II there is a severe nervous system impairment but peripheral neuropathy is missing [17]. Type III clinically resembles type Ia but psychomotor retardation, although pronounced, is stationary, polyneuropathy is absent

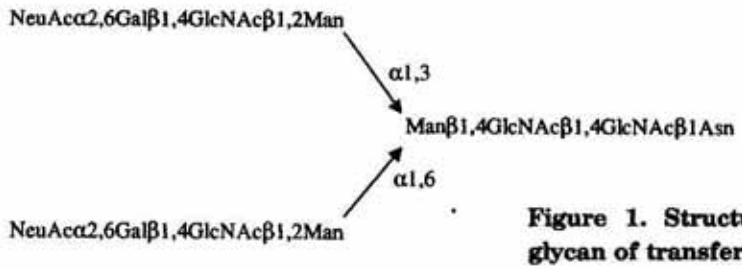

Figure 1. Structure of a biantennary glycan of transferrin.

and both retinal pigmentary degeneration and cerebellar hypoplasia are present [18]. In Type IV there are no neurological symptoms, dysmorphism is minor, liver function is not affected but epilepsy is present [19]. Type V is differentiated from Type Ia mainly on the basis of biochemical abnormalities [20, 21]. The latter have been identified also in Types Ib [14–16] and II [22, 23].

BIOCHEMICAL ABNORMALITIES IN CDGS

The biosynthesis of N-linked glycans is a complex process that, counting only glycosyltransferases and glycosidases, involves over 30 different enzymes. It starts in the endoplasmic reticulum (ER) where an oligosaccharide containing 14 sugars (2GlcNAc, 9Man, and 3Glc residues, see Fig. 2) is assembled on dolichyldiphosphate (DolPP) [24–27]. The biosynthesis takes place initially at the cytosolic side, and later on the lumenal side of ER to form the so called lipid-linked oligosaccharide (LLO). The last step in the biosynthesis is the addition of 3 glucose residues. Thereafter the whole oligosaccharide is transferred to a nascent protein by the enzyme oligosaccharyltransferase [28]. Next, two glucose residues are removed and the glycoprotein with only a single Glc residue binds to calnexin, a lectin like molecule with chaperone function [29, 30]. The bound glycoprotein attains its final conformation; if the conformation is incorrect the glycoproteins is degraded. This process has been aptly named the quality control. The last glucose residue is removed, the glycoprotein freed from the complex with calnexin, packed into transport vesicles and sent to Golgi apparatus where the biosynthesis is completed. En route the glycan may be trimmed by one or more mannose residues to form oligomannosidic or hybrid types of N-glycans, respectively [31, 32]. Mannose residues of oligomannosidic type glycans may acquire lysosomal targeting signals, i.e. phosphate groups at position 6, through the sequential action of GlcNAc-phosphotransferase and phosphodiesterase. Otherwise more mannose residues are cleaved, partly in ER and partly in the Golgi, to form complex type N-glycans. GlcNAc transferases I and II that initiate antennae on the third and sixth arms of the trimannosyl core, respectively, are essential in this process. The antennae are elongated by galactosyl, *N*-acetylglucosaminyl, sometimes *N*-acetylgalactosaminyl, and sialic acid or fucose residues to form complex type glycans. Apart from transferases, glycosylation (as well as sulfation and phosphorylation) reactions in the Golgi require specific transporters essential for the translocation of respective donors of reactive groups to the cisternae lumen [33].

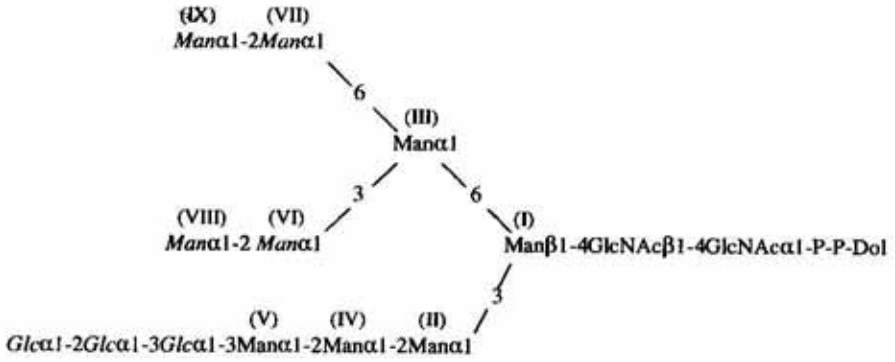

Figure 2. Structure of lipid linked oligosaccharide.

Carbohydrate residue typed in "italic" are derived from either DolPMan or DolPGlc. Glucosylation may take place after addition of mannose residue number V or IX [23].

Each of these major steps is prone to malfunction due to mutations and indeed many of them malfunction in different types of CDGS. Defects in transporters of nucleotide sugars have not yet been described, although Chinese hamster ovary (CHO) cells with mutated

transporters are known [33]. Oligosaccharyltransferase in CDGS appears to be normal yet the enzyme activity has been determined in only a few patients [34]. The same applies to GlcNAc-1-P transferase, the first enzyme in LLO biosynthesis [35]. Most cases of CDGS result from a deficient supply of mannose for the assembly of LLO. This mannose is supplied by guanosinediphospho-mannose (GDP-Man) and dolichylphosphomannose (Dol-P-Man). In CDGS type Ia and Ib the supply of "activated" mannose is insufficient because of aberrant mannose metabolism (see Fig. 3). The key mannose donor is GDP-Man. This compound donates the first 5 mannose residues to LLO, and is also a precursor of GDP-fucose [24–27]. The remaining 4 mannose residues are supplied to LLO by Dol-P-Man which itself is synthesized from Dol-P and GDP-Man. Thus, directly or indirectly all mannose residues in LLO are derived from GDP-Man. Dol-P-Man donates

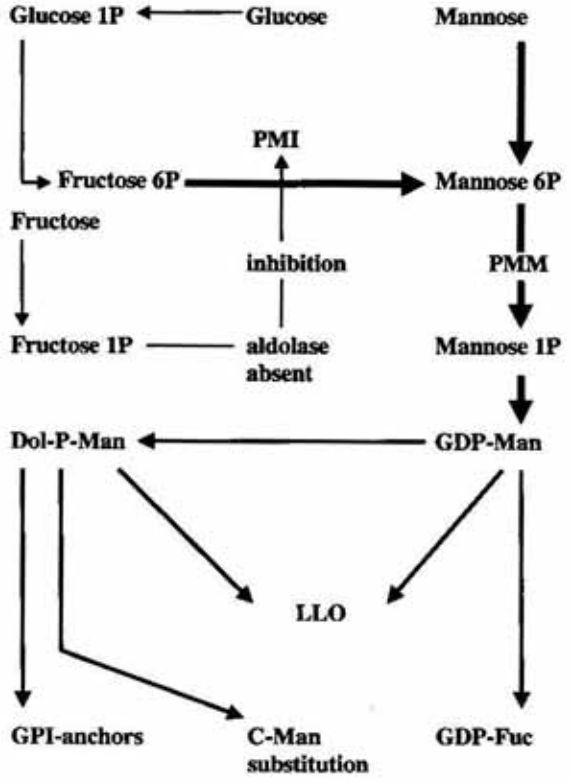

Figure 3. Scheme of biosynthesis of mannose phosphates and mannose donors used in N-glycosylation of proteins.

mannose residues also to enzymes synthesizing GPI-anchors [36] and a mysterious C-mannosyltransferase that links mannose residue to C2 of indole ring of tryptophan number 7 of human ribonuclease II through a carbon–carbon linkage [37–40]. Ribonuclease II has identical primary structure as the neurotoxin of eosinophil granules. GDP-Man is synthesized from mannose-1-phosphate (Man-1-P) while the latter from mannose-6-phosphate (Man-6-P) by the action of phosphomannomutase (PMM) [10, 11] (Fig. 3). Man-6-P may arise either directly from mannose [41] or from fructose-6-phosphate (Fru-6-P) by phosphomannose isomerase (PMI) [42]. In CDGS Ia and CDGS Ib the activities of PMM [11, 12, 41–44] and PMI [13, 14], respectively, are low resulting in a diminished level of GDP-Man. An inspection of metabolic pathways allows a conclusion that PMM deficiency should produce a more severe form of the disease because this enzyme is the sole supplier of Man-1-P for GDP-Man. PMI on the other hand supplies only a part of the needed Man-6-P. The other part is provided by direct phosphorylation of mannose [41]. Finally, the reason of CDGS-like symptoms in hereditary fructose intolerance [45] has been explained by the fact that fructose-1-phosphate (Fru-1-P) that accumulates in this disease [46] inhibits PMI [47]. The accumulation of Fru-1-P is due to a deficiency of aldolase II that under normal conditions splits the excess of Fru-1-P to dihydroxyacetone phosphate and glyceraldehyde [46]. There is evidence that a deficient supply of mannose in CDGS Ia results in the assembly of a curtailed form of LLO with fewer than normal (on the average, 5) [43] mannose residues, transfer of the curtailed LLO-derived glycans to nascent proteins [43, 48, 49], and an overload of the quality control mechanism [50]. Overloading of the quality control is manifested among others by the dilatation of ER canaliculi, clearly seen in electron microscopy.

Structures of hypoglycosylated glycoproteins of the complex type may be, at least in

theory, corrected by trimming of mannose residues that occurs during the processing of N-glycoproteins, and even after the oligomannosidic type glycoproteins reach the plasma membrane [51]. On the other hand, there is no mechanism available for enriching the oligomannosidic glycans with additional mannose residues. Thus, they should remain mannose-deficient and hence also mannose-6-phosphate deficient. A low amount of the mannose-6-phosphate, lysosomal targeting signal, on the enzymes may result in their deranged transport to lysosomes and seepage to blood.

A recently described type V of CDGS is due to defective glucosylation of LLO [20, 21]. Since glucose residues in LLO are required for an efficient transfer of LLO to protein the result is much the same, as in hypo and unglycosylation of N-linked glycoproteins. There is, however, a difference: whereas in Type Ia and Ib a curtailed oligosaccharide is transferred to the protein, in CDGS Type V the size of the oligosaccharide is normal, but it is transferred at a reduced rate. Thus, unglycosylation probably prevails in CDGS type V. A similar metabolic defect as in CDGS type V has been recently described in the MI8-5 mutant of CHO [52]. In Japan yet another metabolic defect in CDGS was reported that is due to a deficiency of the enzyme saturase which reduces the alpha isoprenoid unit of dolichol (Fig. 4) [53]. With the alpha unit unreduced the dolichol is not a good substrate for GlcNAc-1-P transferase and LLO is synthesized at a lower rate resulting again in unglycosylation and hypoglycosylation of N-linked glycoproteins. It should be noted, however, that PMM activity in fibroblasts of these patients was low.

Enzyme deficiency in CDGS Type II involves *N*-acetylglucosaminyl transferase II (GnII) that initiates a branch on the 6th arm of the trimannosyl core of N-linked glycoproteins [22, 23]. Hence, biantennary glycoproteins substituted on 3rd and 6th arms of the core cannot be synthesized [6, 21]. Previously a deficiency of GnII was claimed to be the cause of a variant of congenital dyserythropoietic anemia type II [54]. The gene for the latter disease has, however, no relation to the GnII gene and is located on a different chromosome [55, 56].

GENETICS

CDGS type Ia and II are transmitted in an autosomal recessive manner. The *PMM2* gene that encodes the disease associated PMM was localized to chromosome 16p13 [56, 57] and a number of largely missense mutations characterized [57–61]. Interestingly, another gene for *PMM*, designated *PMM1*, a homologue of yeast *PMM*, was found in humans [62–64]. The gene was localized to chromosome 22q13 [62] but had no relation to CDGS. The *PMM1* and *PMM2* genes share 60% homology in the coding sequence and their exon/intron boundaries are conserved [65]. A third PMM related gene is a pseudogene showing 88% homology with *PMM2* and located on chromosome 18p. Unexpectedly, several base substitutions in

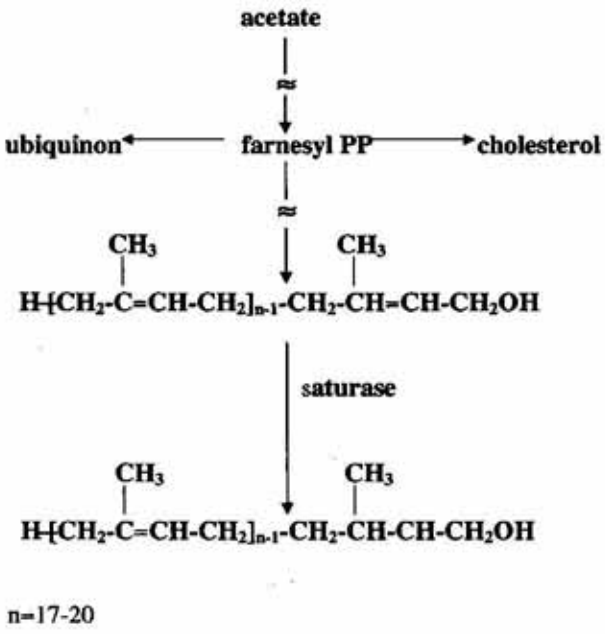

Figure 4. A simplified scheme of dolichol biosynthesis.

PMM2 that are associated with the disease are also present at the corresponding positions in the pseudogene [65].

One missense mutation was characterized in the *PMI* gene of a patient with CDGS type Ib [14]. The mutated gene was transmitted from patient's father while the mother was a healthy homozygote. Surprisingly, though both the father and the patient were obviously heterozygotes only the latter had lowered PMI activity in fibroblasts and leukocytes. In the cells of the father the activity of PMI was normal.

The gene for GnII is present as a single copy on chromosome 14 [66]. Two different missense mutations in only two affected families known were characterized [67]. Relatives of one patient were either heterozygotes or normal homozygotes with all heterozygotes showing a significant reduction of GnII activity in mononuclear cells.

TREATMENT

It has been established that the aberrant synthesis of N-linked glycoproteins in CDGS fibroblasts in culture may be corrected by the addition of mannose at 1 mM concentration to the medium [68]. It was also found that oral mannose reaches blood and is not toxic [69]. Contrary to previous assumptions, most of the mannose used for glycoprotein synthesis is taken up directly through glucose tolerant, mannose specific transporters [41]. Several such transporters have been identified [70–73]. The contribution of glucose-derived, PMI mediated mannose amounts about 25% [41].

Unfortunately, the treatment with mannose does not benefit patients with type Ia of CDGS yet it affects but not normalizes transferrin isofocusing profile in blood serum [74, 75]. Mannose therapy is effective in type Ib of the disease [14]. Patients improved clinically after only weeks of treatment while the glycosylation pattern of N-linked glycoproteins normalized only after 11 months. A greater efficacy of mannose in the treatment of CDGS type Ib is presumably a consequence of only partially blocked formation of Man-6-P (Fig. 3).

CONCLUSIONS

Carbohydrates in glycoproteins are known to perform a large number of diverse functions such as stabilization of protein structure, protection from proteolytic degradation, conferring proper folding, providing recognition sites and signals for lectins, adhesins, targeting processes, for whole cells, and even pathogenic microorganisms [76, 77]. Thus, carbohydrate chains may affect biological activities of the protein moiety of glycoproteins. This multitude of functions of carbohydrates in glycoproteins explains the variability of clinical symptoms in CDGS. In many instances symptoms may be easily ascribed to a biochemical lesion: for example coagulation abnormalities involving mostly factor XI, proteins C, S and antithrombin III [78] may be explained by their glycoprotein nature. Likewise, increased spreading and reduced proliferation of CDGS fibroblats are probably due to a deficiency of decorin, a small proteoglycan that contains one O-linked glycosaminoglycan chain and three N-glycans [79, 80]. It has been already pointed out that the seepage of lysosomal enzymes to blood, as observed in patients with CDGS, may be due to a deficiency of Man-6-P targeting signal on lysosomal enzymes.

In spite of a general understanding of the pathogenesis of CDGS many fine points need to be elucidated. For example, what is the specific link between neurological symptoms and CDGS? Is it due only to hypoglycosylation or unglycosylation of N-linked glycoproteins of the nervous system? Or maybe also O-mannosyl-linked glycoproteins [81–83] are involved? Why neuropathy is missing in CDGS type II? Why not all N-linked glycoproteins in

CDGS seem to be carbohydrate deficient [84] What is the biochemical basis of hypoglycosylation in CDGS? Is it due to the fact that many glycosyltranferases themselves are glycoproteins and hence may exhibit impaired activity? Future studies will certainly provide answers to these questions. Taking into account the complexity of the biosynthesis of N-linked glycans we may also expect more subtypes of CDGS to be discovered [85].

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Note added in proof

Two more defects in CDGS i.e. of GDP-Fuc transporter and Dol-P-Man synthase have been recently described (quoted after Freeze *et al.*, 1999, Glycoconjugate Journal 16, p. S41 (Abstracts of XV International Symposium on Glycoconjugates, Tokio, Japan, August 22-27). Number of patients with CDGS type II has increased to 5 (H. Schachter, personal communication).