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Review

Congenital disorders of glycosylation. Part I. Defects of protein N-glycosylation

Bogdan Cylwik✉, Marcin Naklicki, Lech Chrostek and Ewa Gruszewska

Department of Biochemical Diagnostics, Medical University of Bialystok, Białystok, Poland

Glycosylation is the most common chemical process of protein modification and occurs in every living cell. Disturbances of this process may be either congenital or acquired. Congenital disorders of glycosylation (CDG) are a rapidly growing disease family, with about 50 disorders reported since its first clinical description in 1980. Most of the human diseases have been discovered recently. CDG result from defects in the synthesis of the N- and O-glycans moiety of glycoproteins, and in the attachment to the polypeptide chain of proteins. These defects have been found in the activation, presentation, and transport of sugar precursors, in the enzymes responsible for glycosylation, and in proteins that control the traffic of component. There are two main types of protein glycosylation: N-glycosylation and O-glycosylation. Most diseases are due to defects in the N-glycosylation pathway. For the sake of convenience, CDG were divided into 2 types, type I and II. CDG can affect nearly all organs and systems. The considerable variability of clinical features makes it difficult to recognize patients with CDG. Diagnosis can be made on the basis of abnormal glycosylation display. In this paper, an overview of CDG with a new nomenclature limited to the group of protein N-glycosylation disorders, clinical phenotype and diagnostic approach, have been presented. The location, reasons for defects, and the number of cases have been also described. This publication aims to draw attention to the possibility of occurrence of CDG in each multisystem disorder with an unknown origin.

Key words: N-glycosylation, genetic defects, diagnostics

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INTRODUCTION

Glycosylation is the most common modification of chemical proteins that takes place in every human cell (Freeze & Schachter, 2009). This process involves a covalent attachment of one or several carbohydrate chains to the amide group of the asparagine (N-glycoprotein), or rarely to the hydroxyl group of serine or threonine (O-glycoprotein) of the protein polypeptide chain (Freeze & Schachter, 2009). The N-glycosylation pathway in the cytoplasm, endoplasmic reticulum (ER), and Golgi apparatus has four main steps: biosynthesis of lipid-linked oligosaccharide (LLO), *en block* transfer of carbohydrate chain from dolichol phosphate to the nascent polypeptide chain of protein, remodeling of protein-bound N-glycan in the ER (basic glycosylation), and further modification of N-glycan in Golgi (final glycosylation) (Freeze & Schachter, 2009). The full N-glycosylation pathway is a site-specific process consisting of at least 40 steps, and depends on a number of enzymes (especially glycosyltransferases) and some transport proteins. The biosynthesis of all N-glycans occurs in a uniform manner. A complete absence of N-glycans is lethal. The carbohydrate component (N- or O-glycan) of glycoproteins constitutes at least 20% (sometimes only 1%), and in some cases reaches even up to 80% of the molecular weight. The glycans usually consist of hexoses and their derivatives (most often mannose, galactose, N-acetylglucosoamine, N-acetylgalactosoamine) and various sialic acids (Freeze & Schachter, 2009). Sialic acids are typically located at the terminal end of glycan structures. In plasma glycoproteins, sialic acids constitute from 3 to 7% of the glycoprotein content, however in breast milk or mucoses and epithelia about 25%. In turn, the glycoproteins of leukocyte membrane can contain up to 50% of sialic acids (Sillanaukee *et al*., 1999). Most of the blood plasma, tissue fluids, mucus, and membrane proteins are glycosylated.

Correct glycosylation is essential for obtaining and supporting a normal biological activity of proteins, and its impairment leads to the synthesis of glycoproteins with reduced or lost function. Glycosylation is the primary cause of microheterogeneity in proteins. It means that a particular glycoprotein may exist in many molecular variants that differ in the structure of carbohydrates (glycoforms). In healthy people, glycoproteins have a normal protein isoform pattern that may change in a characteristic manner in many diseases. Generally, disturbances of glycosylation may be either congenital or acquired. Congenital disorders of glycosylation were originally called carbohydrate-deficient glycoprotein syndromes (CDGS) (Niehues *et al*., 1998; Carchon *et al*., 1999; Korner *et al*., 1999) and after year 2000 were renamed on congenital disorders of glycosylation (CDG) (Participants, 2000). They result from genetic defects leading to the deficiency or loss of enzyme activity involved in glycan synthesis and processing, or to deficiency of specific transporters (Freeze & Schachter, 2009). Genetic defects cause severe multiorgan and multisystem disorders that are manifested as early as in the first months of life. About 20% of patients do not live past their fifth year of life. Broad clinical features involve many system organs, but specifically relate to the development of certain regions of the brain and functions of the gastrointestinal, hepatic,

✉e-mail: cylwikb@umb.edu.pl

Abbreviations: CDG, congenital disorders of glycosylation; Dol-P, dolichol phosphate; Dol-P-Glc, dolichol-P-glucose; Dol-P-Man, dolichol-P-mannose; ER, endoplasmic reticulum; Fru-6-P, fructose-6-phosphate; GDP-Man, guanosine diphosphate-mannose; IEF, isoelectrofocusing; LLO, lipid-linked oligosaccharide; Man-1-P, mannose-1-phosphate; Man-6-P, mannose-6-phosphate; OST, oligosaccharyltransferase complex; PMI, phosphomannose isomerase; PMM2, phosphomannomutase II; Tf, transferrin; TIEF, transferrin isoelectrofocusing

visual, and immune systems (Freeze & Schachter, 2009). More than 30 years have passed (Jaeken *et al*., 1980) since the first clinical description of the defect of protein glycosylation by Jaeken et al. (in 1980). Since that time 45 CDG have been identified (Theodore & Morava, 2011). Congenital disorders of glycosylation are a rapidly growing disease family (in years 2003-2010 the number rose from 12 to 45), and knowing that about 250 genes are considered to be involved in glycosylation, it is believed that many diseases will be identified in the future (Jaeken, 2010; Jaeken, 2011). In 2009, a novel nomenclature for all types of CDG was proposed, which combines the name of the defective gene followed by a common „-CDG" ending (e.g. PMM2-CDG instead CDG Ia) (Jaeken *et al*., 2009).

In this paper, an overview of congenital disorders of N-glycosylation pathway with a novel nomenclature, classification, location, and reasons for defects, clinical phenotype, and diagnostic approach, have been presented. The paper aims to increase access to scientific information about genetic diseases, especially in cases with an unknown origin, and to facilitate their diagnosis.

NOVEL CLASSIFICATION OF CONGENITAL DISORDERS OF GLYCOSYLATION

In 2009, a novel classification of CDG was proposed, according to the type of glycosylation defect that distinguishes 4 categories of disorders:

- Defects of protein N-glycosylation (17 diseases)
- Defects of protein O-glycosylation (8 diseases)
- Defects of lipid glycosylation and of glycosylphosphatidylinositol (GPI) anchor glycosylation (3 diseases)
- Defects in multiple glycosylation pathways and in other pathways (17 diseases) (Jaeken, 2011).

Due to the fact that the most frequently identified types of CDG are associated with disrupted or defective N-glycosylation pathway, this article concerns only this category of diseases.

DIVISION AND CHARACTERIZATION OF CDG RELATED TO THE IMPAIRED PATHWAY OF N-GLYCOSYLATION

For the sake of convenience, CDG defects related to the impaired pathway of N-glycosylation (17 diseases are known) are divided into two types, I and II:

CDG type I involves disrupted synthesis of the lipid linked oligosaccharide precursor (LLO) and its transfer to polypeptide chain of protein:

— 15 subtypes (according to the new nomenclature): PMM2-CDG (CDG-Ia), PMI-CDG (CDG-Ib), ALG6-CDG (CDG-Ic), ALG3-CDG (CDG-Id), ALG12-CDG (CDG-Ig), ALG8-CDG (CDG-Ih), ALG2-CDG (CDG-Ii), DPAGT1-CDG (CDG-Ij), ALG1-CDG (CDG-Ik), ALG9-CDG (CDG-Il), RFT1-CDG (CDG-ln), ALG11-CDG (CDG-Ip), DDOST-CDG (CDG-Ir): TUSC3-CDG, MAGT1-CDG)

CDG type II involves malfunctioning trimming/processing of the protein-bound oligosaccharide chain:

— 2 subtypes: MGAT2-CDG (CDG-IIa), GCS1-CDG (CDG-IIb).

PMI-CDG (CDG-Ib): deficiency of phosphomannose isomerase (EC 5.3.1.8)

Phosphomannose isomerase occurs in the cytoplasm and catalyzes the conversion of fructose-6-phosphate (Fru-6-P) to the mannose-6-phosphate (Man-6-P), which is converted to the mannose-1-phosphate (Man-1-P). Man-1-P serves as a substrate in the nucleotide sugar GDP-Man, which is crucial for proper N-glycosylation. Man-1-P serves as a donor substrate for the formation of dolichol-P-mannose (Dol-P-Man) and the initial $Man_5GlcNAc_2$-P-Dol structure (Fig. 1a) (Freeze & Schachter, 2009). Mannose is one of the basic monosaccharides involved in the synthesis of fourteen-sugar lipid-linked oligosaccharide (LLO) precursor ($Glc_3Man_9GlcNAc_2$-PP-Dol). PMI-CDG results from mutations in the *PMI* gene that leads to the deficiency of phosphomannose isomerase (Table 1) (Niehues *et al*., 1998). Reduced phosphomannose isomerase activity decreases the Man-6-P pool, what causes the glycosylated proteins to be devoid of entire sugar chains and the Asn-X-Ser or Asn-X-Thr N-glycosylation sequons of polypeptide chain to be unoccupied.

The clinical phenotype of PMI-CDG was first described in 1986 in 4 children whose parents came from Canada (Pelletier *et al*., 1986). Until now, 20 patients affected by this defect are known (Niehues *et al*., 1998). In contrast to PMM2-CDG, these patients do not have psychomotor retardation, but mainly hepatic-intestinal symptoms (severe vomiting, diarrhea, gastrointestinal bleeding, and protein-losing enteropathy) are present (Jaeken, 2010; Freeze & Westphal, 2001). Coagulation disturbances and hypoglycemia have been found in some patients (Freeze & Westphal, 2001; Jaeken, 2010). It is the only one of all CDG disorders that can be treated effectively with oral mannose supplementation (Marquardt & Denecke, 2003; de Lonlay & Seta, 2009). The effectiveness of mannose therapy is possible due to the fact that these patients have a normal activity of hexokinase, which provides an alternative pathway (phosphorylation) for the synthesis of mannose-6-phosphate (Man-6-P) from mannose (Freeze & Schachter, 2009). Serum transferrin isoform profile showed a pattern consistent with CDG type I (the increase of asialo- and disialo, and the decrease of tetrasialotransferrin) (Jaeken, 2010). The diagnosis of this defect is confirmed by a significant deficiency of phosphomannose isomerase in leukocytes or fibroblasts (with activity decreased to approximately 7% of the values in healthy subjects) (Niehues *et al*., 1998). Laboratory studies indicate a decrease in serum glucose (with an inadequate increase of insulin levels), cholesterol and protein concentrations, elevated transaminases, tubular proteinuria and a low level of antithrombin III (Niehues *et al*., 1998).

PMM2-CDG (CDG-Ia): deficiency of phosphomannomutase II (EC 5.4.2.8)

Phosphomannomutase II occurs in the cytoplasm and catalyzes the conversion of mannose-6-phosphate (Man-6-P) to the mannose-1-phosphate (Man-1-P), which is a precursor required for the synthesis of GDP-mannose (GDP-Man) and dolichol-P-mannose (Dol-P-Man) (Fig. 1a) (Carchon *et al*., 1999). Both donors are substrates for the mannosyltransferases involved in the synthesis of lipid-linked oligosaccharide (LLO) precursor. The level of these donors is decreased in CDG patients. PMM2-CDG results from mutations in the *PMM2* gene, which codes for a phosphomannomutase (tab.1). Over 90 mutations of this gene have been identified, with R141H and F119L being the most commonly identified, particularly in Northern Europe. In Denmark, about 1 in 60 people, and about 1 in 79 Dutch/Flemish people are carriers of one copy of the R141H mutation. The F119L mutation probably originates near Denmark,

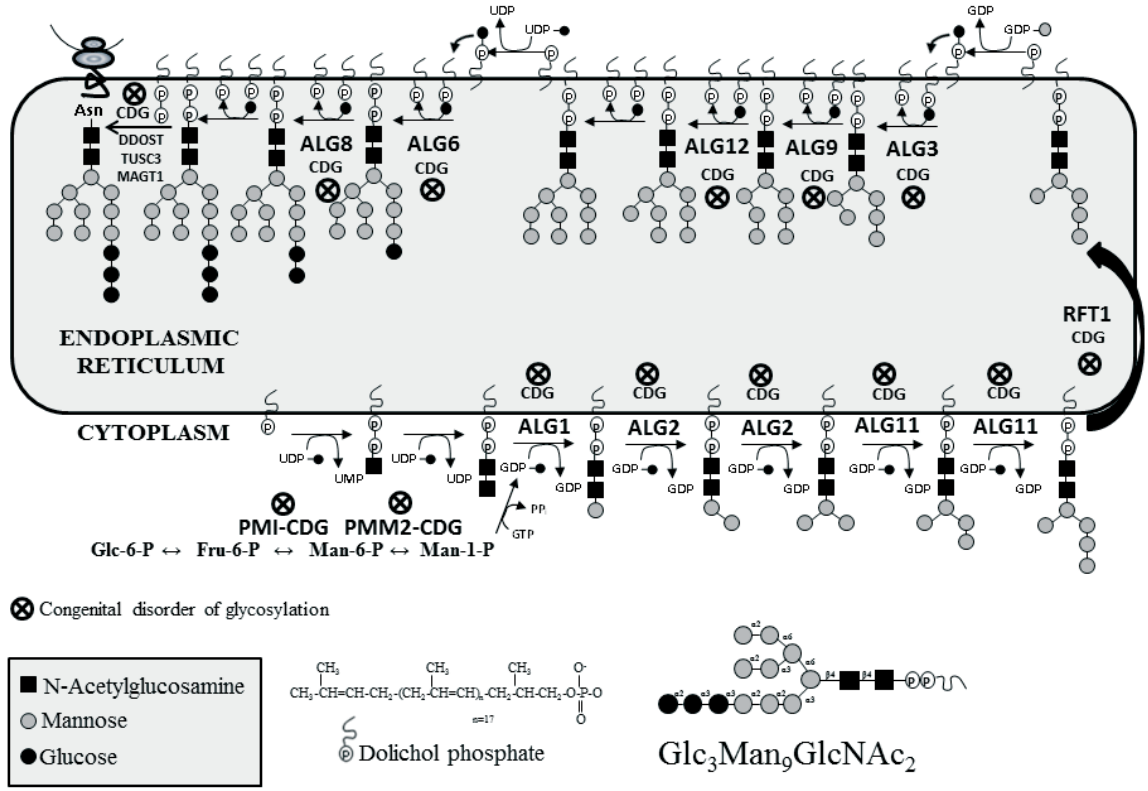

Figure 1a. Location of defects in the N-glycosylation pathway in CDG disorders.
CDG, congenital disorders of glycosylation; Fru, fructose; GDP, guanosine diphosphate; Glc, glucose; Man, mannose; GTP, guanosine triphosphate; PMI, phosphomannose-isomerase; PMM2, phosphomannomutase II; UDP, uridine diphosphate; UMP, uridine monophosphate

where it is present in nearly half of patients with this disorder (Jaeken & Matthijs, 2001). These mutations lead to the deficiency or instability of phosphomannomutase II (Matthijs *et al*., 1997; Matthijs *et al*., 2000). It has been shown that the activity of this enzyme is significantly diminished in fibroblasts, liver cells, and leukocytes, which in turn decreases the pool of GDP-Man and Dol-P-Man (van Schaftingen & Jaeken, 1995). For example, the GDP-Man pool in PMM2-CDG fibroblasts is reduced to 10% that of normal cells (Freeze & Westphal, 2001). Fibroblasts of patients with this defect predominantly synthesize incomplete oligosaccharides (truncated forms) containing four or five mannose residues, instead of nine. Impairment of protein N-glycosylation causes simultaneous occurrence in the serum the glycoproteins with normal structure as well as those in which the oligosaccharide part is deficient in sialic acid, galactose and N-acetylglucosamine. It also concerns membranous glycoproteins and lysosomal enzymes (Jaeken & Stibler, 1989). Often, patients are diagnosed as having the disorder, in their neonatal or early infantile period, on the basis of typical clinical presentation (Jaeken, 2010). A classical phenotype of this disease includes the following symptoms: psychomotor retardation, ataxia, strabismus, some dysmorphic features (inverted nipples and subcutaneous fat pads), and coagulopathy. Most children suffer from epilepsy and some from stroke-like episodes. Some patients have a very mild phenotype. It has been shown that the highest mortality occurs in the first years of life and is caused by malfunctions of many organs and systems (digestive tract, circulatory system, kidneys, liver) (Jaeken, 2010). Approximately 20% of newborns do not live past their first year of life. Mannose therapy is ineffective (Freeze & Westphal, 2001).

PMM2-CDG was first described in 1980 by Jaeken et al. (Jaeken *et al*., 1980) in twin sisters (as the Jaeken syndrome). It was the first-discovered genetic multisystem disorder characterized by defective glycosylation glycoconjugates, but during the next 15 years the underlying defect remained unknown. Only in 1995, it has been shown that this defect was caused by a deficiency of the phosphomannomutase enzyme. PMM2-CDG was the first form of CDG characterized at the molecular level (van Schaftingen & Jaeken, 1995; Carchon *et al*., 1999; Freeze & Westphal, 2001; Grunewald *et al*., 2002; Marquardt & Denecke, 2003). This defect is the most frequent type of CDG (70% of all CDG syndromes), with more than 700 cases identified worldwide (Carchon *et al*., 1999; Freeze & Westphal, 2001). This disorder occurs in people of many parts of the world (Northern and Western Europe, USA, Latin America, Iran, Japan), but almost half of patients are inhabitants of Scandinavia (Kjaergaard, 2004).

For the diagnosis of PMM2-CDG (and of congenital disorders of N-glycosylation in general), the assessment of the serum transferrin isoform profile by the isoelectrofocusing method (IEF) is widely used. In this defect, the cathodal shift was shown, because of partial deficiency of sialic acid. Therefore, the transferrin isoform pattern presents an increase of both, asialo- and disialo-, and a decrease of tetrasialotransferrin bands (Jaeken, 2010). This is a typical profile for type I CDG and that pattern indicates the defects in the earliest synthetic steps of the N-linked oligosaccharide pathway. Also, the

Table 1. Genetic defects in the N-glycosylation pathway- related CDG disorders (Freeze & Schachter, 2009; OMIM, 2013).

CDG Type	Number of reported cases	Gene symbol	Protein name	Phenotype OMIM* number
PMM2-CDG (CDG-Ia)	700	*PMM2*	Phosphomannomutase II	212065
PM1-CDG (CDG-Ib)	20	*PMI*	Phosphomannose isomerase	602579
ALG6-CDG (CDG-Ic)	30	*ALG6*	α-1,3-glucosyltransferase I	603147
ALG3-CDG (CDG-Id)	6	*ALG3*	α-1,3-mannosyltransferase VI	601110
ALG12-CDG (CDG-Ig)	6	*ALG12*	α-1,6-mannosyltransferase VIII	607143
ALG8-CDG (CDG-Ih)	5	*ALG8*	α-1,3-glucosyltransferase II	608104
ALG2-CDG (CDG-Ii)	1	*ALG2*	α-1,3-mannosyltransferase II	607906
DPAGT1-CDG (CDG-Ij)	2	*DPAGT1*	N-acetylglucosaminyltransferase I	608093
ALG1-CDG (CDG-Ik)	8	*ALG1*	β-1,4-mannosyltransferase I	608540
ALG9-CDG (CDG-Il)	2	*ALG9*	α-1,2-mannosyltransferases VII/IX	608776
RFT1-CDG (CDG-In)	6	*RFT1*	Flippase	612015
ALG11-CDG (CDG-Ip)	5	*ALG11*	α-1,2-mannosyltransferases IV/V	613661
DDOST-CDG (CDG-Ir)	1	*DDOST*	oligosaccharyltransferase complex	614507
TUSC3-CDG	5	*TUSC3*	Subunit TUSC3 of oligosaccharyltransferase complex	611093
MAGT1-CDG	4	*MAGT1*	Subunit MAGT1 of oligosaccharyltransferase complex	300716
MGAT2-CDG (CDG-IIa)	4	*MGAT2*	β-1,2-N-acetylglucosaminylo-transferase II	212066
GCS1-CDG (CDG-IIb)	1	*GCS1*	α-1,2-glucosidase I	606056

*OMIM, Online Mendelian Inheritance in Man (updated 1 March 2013).

IEF of serum transferrin can be normal in patients with PMM2-CDG (Fletcher *et al.*, 2000). Additionally, other laboratory tests showed an increase in serum transaminases, hypoalbuminemia, hypocholesterolemia, and tubular proteinuria. Finally, the diagnosis is confirmed by finding a diminished activity of enzyme phosphomannomutase in leukocytes or fibroblasts (van Schaftingen & Jaeken, 1995).

DPAGT1-CDG (CDG-Ij): deficiency of N-acetylglucosaminyltransferase I (EC 2.7.8.15)

The biosynthesis of fourteen-sugar lipid linked oligosaccharide (LLO) precursor begins on the cytoplasmic side of the ER membrane with the transfer of N-acetylglucosamine residue from an active donor - UDP-GlcNAc — to membrane-bound lipid-like precursor dolichol phosphate (Dol-P) to create dolichol pyrophosphate N-acetylglucosamine (GlcNAc-PP-Dol) (Freeze & Schachter, 2009). This process is catalyzed by the N-acetylglucosaminyltransferase I enzyme (Fig. 1a). CDG-Ij is caused by a deficiency in N-acetylglucosaminyltransferase I activity encoded by the *DPAGT1* gene (Table 1) (Wu *et al.*, 2003). A few mutations were found in this gene (OMIM, 2013).

The genetic defect DPAGT1-CDG was first described in 2003 in a female patient (Wu *et al.*, 2003), and in 2012 in a male patient (Timal *et al.*, 2012). The first patient showed severe psychomotor retardation, hypotonia, seizures, dysmorphy, exotropia, and microcephaly (Wu *et al.*, 2003), while the second patient had multisystem problems and died at the age of 2.5 (Timal *et al.*, 2012). An abnormal transferrin isoforms profile was consistent with CDG type I and a significant decrease in activity of N-acetylglucosaminyltransferase I was observed (Wu *et al.*, 2003).

ALG1-CDG (CDG-Ik): deficiency of β-1,4-mannosyltransferase I (EC 2.4.1.142)

Following the attachment of two N-acetylglucosamine residues to membrane-bound dolichol phosphate (Dol-P), the dolichol-linked oligosaccharide chain is further extended in a step-by-step mannosylation process (attachment of nine mannose residues) (Freeze & Schachter, 2009). The elongation of this chain begins on the cytoplasmic side of ER membrane by the addition of five mannose and ends on the luminal side of this membrane by the attachment of next four mannose residues. The first elongation step is catalyzed by a β-1,4-mannosyltransferase I that transfers the first-mannose (of nine which are in the full-sized LLO glycan) from GDP-Man onto the two-sugar LLO precursor — $GlcNAc_2$-PP-Dol (Fig.1a). CDG-Ik is caused by a deficiency in β-1,4-mannosyltransferase I activity encoded by the *ALG1* gene (tab.1). A few mutations were identified in this gene (Dupre *et al.*, 2010).

Genetic defect ALG1-CDG was first described in 2004 (Grubenmann *et al.*, 2004). Until now, only 8 patients have been diagnosed (OMIM, 2013). These patients presented severe psychomotor retardation with muscular hypotonia, seizures, microcephaly, and nephrotic syndrome leading to early death (Dupre *et al.*, 2010). Laboratory studies showed a typical pattern of

transferrin isoforms for CDG type I, an accumulation of $GlcNAc_2$-PP-Dol and a significant decrease in the activity of β-1,4-mannosyltransferase I in skin fibroblasts (Dupre *et al.*, 2010).

ALG2-CDG (CDG-Ii): deficiency of α-1,3-mannosyltransferase II (EC 2.4.1.132)

On the cytoplasmic side of the ER, three separate mannosyltransferases catalyze the sequential transfer of mannoses from GDP-Man onto the LLO intermediate precursors (Fig. 1a) (Freeze & Schachter, 2009). Thus, α-1,3-mannosyltransferase attaches the second mannose (of nine) residue to the one-mannose (three-sugar) LLO precursor — $ManGlcNAc_2$-PP-Dol (Thiel *et al.*, 2003). It should be noted that all previously known defects in the biosynthesis of LLOs concerned the impaired activity of glycosyltransferases acting at the luminal side of the ER, but this defect was observed at the cytosolic side of the ER. That defect is associated with mutations in the *ALG2* gene, which leads to the deficiency of α-1,3-mannosyltransferase (Table 1) (Thiel *et al.*, 2003). As a result, a lack of the two-mannose LLO intermediate precursor — $Man_2GlcNAc_2$-PP-Dol is identified.

The genetic defect ALG2-CDG was first described in 2003 in a female patient (Thiel *et al.*, 2003) and up until now no new cases were diagnosed. This patient showed a severe psychomotor retardation with hypomyelination, seizures, bilateral colobomas of the iris, and hepatomegaly. The laboratory findings show a typical pattern of transferrin isoforms for CDG type I, an accumulation of $ManGlcNAc_2$-PP-Dol, a significant decrease in the activity of α-1,3-mannosyltransferase II, and diminished level of coagulation factors (Thiel *et al.*, 2003).

ALG11-CDG (CDG-Ip): deficiency of α-1,2-mannosyltransferases IV/V (EC 2.4.1.131/EC 2.4.1.131)

On the cytoplasmic side of the ER membrane, α-1,2-mannosyltransferases IV/V add fourth and fifth-mannose (of nine) residue from GDP-Man onto the three-mannose and four-mannose LLO precursors ($Man_3GlcNAc_2$-PP-Dol and $Man_4GlcNAc_2$-PP-Dol, respectively), creating four- and five-mannose intermediate precursors ($Man_4GlcNAc_2$-PP-Dol and $Man_5GlcNAc_2$-PP-Dol, respectively) (Fig.1a) (Freeze & Schachter, 2009). This disorder results from mutations in the *ALG11* gene, leading to the deficiency of α-1,2-mannosyltransferases IV/V (Table 1) (Rind *et al.*, 2010). The deficit of the enzyme leads to the accumulation of incomplete glucosylated LLO precursors (with three or four mannose residues, instead of four or of five, respectively).

The genetic defect ALG11-CDG was first described in 2010 in two siblings, born of consanguineous Turkish parents (Rind *et al.*, 2010). They showed feeding problems, hypotonia, epilepsy, and bilateral deafness. The girl presented severe psychomotor retardation, facial dysmorphism, and died at the age of 2. In 2012, another 3 unrelated patients have been diagnosed (Thiel *et al.*, 2012). Biochemical analysis of serum transferrin isoforms showed a CDG type I pattern (Rind *et al.*, 2010). The typical feature of this disease is the accumulation of shortened LLO intermediate precursors — $Man_3GlcNAc_2$-PP-Dol and $Man_4GlcNAc_2$-PP-Dol in fibroblasts (Jaeken, 2011).

RFT1-CDG (CDG-In): deficiency of flippase

Flippase translocates the seven-sugar LLO precursor ($Man_5GlcNAc_2$-PP-Dol) across the ER membrane towards the luminal side (Fig. 1a) (Freeze & Schachter, 2009). This disease results from mutations in the *RFT1* gene leading to the deficiency of this enzyme and an accumulation of the above mentioned precursor (Table .1) (Haeuptle *et al.*, 2008).

The genetic defect RFT1-CDG was first identified in 2008 (Haeuptle *et al.*, 2008). Six patients from six families have been diagnosed so far (Jaeken, 2010). All these patients showed a severe neurological syndrome including deafness as a consistent feature of this defect. Besides, they had marked developmental retardation, hypotonia, epilepsy, poor to absent visual contact, feeding problems, microcephaly, and dysmorphic features (Jaeken, 2011). The accumulation of LLO intermediate precursor ($Man_5GlcNAc_2$-PP-Dol) and a strong reduction of complete LLO were observed in fibroblasts (Haeuptle *et al.*, 2008). RFT1-CDG can be detected by capillary zone electrophoresis that showed a pattern of serum transferrin isoforms characteristic for CDG type I (Jaeken *et al.*, 2009).

ALG3-CDG (CDG-Id): deficiency of α-1,3-mannosylotransferase VI (EC 2.4.1.258)

On the ER lumen, specific mannosyltransferases (from VI to IX) catalyze the sequential attachment of the four-mannose residues to the LLO precursor ($Man_5GlcNAc_2$-PP-Dol) (Freeze & Schachter, 2009). Finally, the nine-mannose (eleven-sugar) structure is formed ($Man_9GlcNAc_2$-PP-Dol). α-1,3-mannosyltransferase VI catalyzes the attachment of the sixth-mannose (of nine) from Dol-P-Man onto the five-mannose LLO precursor ($Man_5GlcNAc_2$-PP-Dol), generating six-mannose intermediate precursor (Fig.1a) (Freeze & Schachter, 2009). This defect arises due to mutations in the *ALG3* gene, which leads to the deficiency of α-1,3-mannosyltransferase VI (Table 1) (Korner *et al.*, 1999). The deficiency of this enzyme leads to the truncation of the oligosaccharide chain containing five mannose residues, instead of nine. The five-mannose intermediate precursor is a poor substrate for the oligosaccharyltransferase (OST) complex (because this enzyme prefers full-sized LLO glycans), making it difficult to move to the growing chain of protein. This incomplete and immature precursor begins to accumulate in the cell. It is known that many deficiencies in LLO synthesis produce incomplete intermediates. Therefore, the hypoglycosylation of multiple proteins occurs in this defect, in which glycoproteins are partially or completely devoid of N-glycans (Korner *et al.*, 1999).

Some clinical symptoms with suspicion of a new subtype of CDG were first described in 1995, in a German boy (Stibler *et al.*, 1995), but biochemical and molecular diagnosis and the confirmation of this defect were given later, in 1999 (Korner *et al.*, 1999). Up till now, only six patients have been diagnosed (Rimella-Le-Huu *et al.*, 2008). Patients show severe psychomotor retardation, microcephaly, coloboma of the iris, atrophy of the optic nerve, as well as brain and corpus callosum (Freeze & Schachter, 2009). The hallmark biochemical feature of this defect is the accumulation of the truncated, incomplete LLO precursor - $Man_5GlcNAc_2$-PP-Dol — in fibroblasts (Kranz *et al.*, 2007). Plasma glycoproteins are markedly hypoglycosylated and the IEF pattern of serum transferrin reveals an additional band at the position of disialotransferrin (Denecke *et al.*, 2005). The asialotransferrin is not detectable. SDS/PAGE method indicates the lack of at least one of the two complete oligosaccharides normally attached to transferrin (Denecke *et al.*, 2005).

ALG9-CDG (CDG-Il): deficiency of α-1,2-mannosyltransferases VII/IX (EC 2.4.1.259/EC 2.4.1.261)

α-1,2-Mannosyltransferases VII/IX, on the ER lumen, catalyze the attachment of the seventh and ninth-mannose (of nine) from Dol-P-Man onto the six-mannose and eight-mannose LLO precursors ($Man_6GlcNAc_2$-PP-Dol and $Man_8GlcNAc_2$-PP-Dol, respectively), forming seven and nine-mannose intermediate precursors ($Man_7GlcNAc_2$-PP-Dol and $Man_9GlcNAc_2$-PP-Dol, respectively) (Fig. 1a) (Freeze & Schachter, 2009). Mutations in *ALG9* gene that leads to the deficit of α-1,2-mannosyltransferases VII/IX (Table 1) (Frank *et al.*, 2004) are known to causes this disease. Deficiencies of these enzymes lead to the accumulation of incomplete glucosylated and immature LLO precursors (with six or eight mannose residues, instead of seven or of eight, respectively).

Genetic defect ALG12-CDG was first described in 2004 (Frank *et al.*, 2004). Until now, only 2 patients are known. Patients have mild psychomotor retardation, seizures, muscular hypotonia, diffuse brain atrophy with delayed myelination, severe macrocephaly, and hepatomegaly (Frank *et al.*, 2004). In laboratory studies, an abnormal transferrin isoform profile has been found, typical for CDG type I pattern, with elevated disialo- and asialotransferrin, and dual accumulation of $Man_6GlcNAc_2$-PP-Dol and $Man_8GlcNAc_2$-PP-Dol (Frank *et al.*, 2004; Weinstein *et al.*, 2005).

ALG12-CDG (CDG-Ig): deficiency of α-1,6-mannosyltransferase VIII (EC 2.4.1.260)

On the luminal side of the ER membrane, α-1,6-mannosyltransferase VIII catalyzes the attachment of the eighth-mannose (of nine) from Dol-P-Man onto the seven-mannose LLO precursor ($Man_7GlcNAc_2$-PP-Dol), creating eighth-mannose intermediate precursor ($Man_8GlcNAc_2$-PP-Dol) (Fig. 1a) (Freeze & Schachter, 2009). This defect results from mutations in *ALG12* gene and leads to the deficiency of α-1,6-mannosyltransferase VIII (Table 1) (Grubenmann *et al.*, 2002). In turn, enzyme deficit leads to the accumulation of incomplete glucosylated LLO precursor (with seven mannose residues instead of 8).

Genetic defect ALG12-CDG was first reported in 2002, in a girl born of nonconsanguineous parents (Chantret *et al.*, 2002; Grubenmann *et al.*, 2002). Only 6 patients have been recognized so far (Jaeken, 2010). Patients present moderate to severe psychomotor retardation, muscular hypotonia, facial dysmorphism, progressive microcephaly, seizures, and frequent upper respiratory tract infections (Chantret *et al.*, 2002; Grubenmann *et al.*, 2002). Biochemical studies showed decreased serum immunoglobulin levels (IgG) and diminished coagulation factors (Chantret *et al.*, 2002; Grubenmann *et al.*, 2002). Serum transferrin isoform pattern was consistent with CDG type I (Grubenmann *et al.*, 2002). Structural analysis of LLO presents an accumulation of intermediate precursor in fibroblasts — $Man_7GlcNAc_2$-PP-Dol — (typical biochemical feature of this defect). The activity of α-1,6-mannosyltransferase VIII was significantly reduced in skin fibroblasts (Grubenmann *et al.*, 2002).

ALG6-CDG (CDG-Ic): deficiency of α-1,3-glucosyltransferase I (EC 2.4.1.267)

Following the mannosylation process the dolichol-linked oligosaccharide chain is further extended in a step-by-step glucosylation process to generate a mature, full-sized LLO glycan (fourteen sugars) (Freeze & Schachter, 2009). α-1,3-glucosyltransferase I catalyzes the transfer of the first glucose residue (out of three) from dolichol-P-glucose (Dol-P-Glc) onto the eleven-sugar LLO precursor ($Man_9GlcNAc_2$-PP-Dol) forming the twelve-sugar LLO precursor ($GlcMan_9GlcNAc_2$-PP-Dol) (Fig. 1a) (Freeze & Schachter, 2009). ALG6-CDG is caused by mutations in the *ALG6* gene which leads to the deficiency of α-1,3-glucosyltransferase I (tab.1) (Burda *et al.*, 1998; Imbach *et al.*, 1999). In turn, it causes the accumulation of the nonglucosylated LLO intermediate precursor ($Man_9GlcNAc_2$-PP-Dol) in fibroblasts, which is weakly transmitted to the polypeptide protein chain (Al-Owain *et al.*, 2010).

Genetic defect ALG6-CDG was first described in 1980 in four children from two families (Burda *et al.*, 1998; Korner *et al.*, 1998). This is the second most frequent protein N-glycosylation disorder, and until now 30 patients have been diagnosed (Jaeken, 2010). Patients reveal moderate psychomotor retardation (compared to patients with PMM2-CDG), muscular hypotonia, strabismus, and seizures (Chantret *et al.*, 2003). The biochemical phenotype was characterized by an accumulation of nonglucosylated LLOs precursors, a marked reduction of glucosylated LLOs, incomplete utilization of N-glycosylation sites in nascent glycoproteins, and hypoglucosylation of serum proteins (Burda *et al.*, 1998; Korner *et al.*, 1998). The analysis of serum transferrin isoforms pattern by IEF reveals the CDG type I, in which disialo- and tetrasialotransferrin are the most abundant glycoforms, but asialotransferrin occurs in low amounts (Burda *et al.*, 1998). Laboratory tests indicate low blood levels of factor XI, coagulation inhibitors, and protein C (Jaeken, 2010).

ALG8-CDG (CDG-Ih): deficiency of α-1,3-glucosyltransferase II (EC 2.4.1.265)

α-1,3-Glucosyltransferase II transfers the second (out of three) glucose residue from dolichol-P-glucose (Dol-P-Glc) onto the twelve-sugar LLO precursor ($GlcMan_9GlcNAc_2$-PP-Dol), creating the thirteen-sugar LLO precursor ($Glc_2Man_9GlcNAc_2$-PP-Dol) (Fig. 1a) (Freeze & Schachter, 2009). Genetic defect ALG8-CDG (CDG-Ih) is linked with mutations in the *ALG8* gene, that leads to the deficiency of α-1,3-glucosyltransferase II (Table 1) (Chantret *et al.*, 2003). Deficit of this enzyme causes accumulation of incomplete glucosylated precursor ($GlcMan_9GlcNAc_2$-PP-Dol) and lack of the LLO intermediate precursor ($Glc_2Man_9GlcNAc_2$-PP-Dol).

Genetic defect ALG8-CDG was first described in 2003 (Chantret *et al.*, 2003). Until now, only 5 patients from four families have been recognized (Jaeken, 2010). One patient showed normal psychomotor development and had no dysmorphic manifestations, but had severe diarrhea and moderate hepatomegaly (OMIM, 2013). The following clinical symptoms were observed in other patients: dysmorphy, edema, massive ascites, and renal failure (Chantret *et al.*, 2003). The accumulation of incomplete LLO intermediate precursor — $GlcMan_9GlcNAc_2$-PP-Dol — in fibroblasts constitutes a typical feature of this disease (Schollen *et al.*, 2004). The combination of coagulation factor anomalies and protein-losing enteropathy suggests CDG. Laboratory findings diagnose anemia, severe thrombocytopenia, and primary hypothyroidism (Chantret *et al.*, 2003). Routine hematological tests showed that factor X, protein C, and antithrombin III were decreased (Chantret *et al.*, 2003). The transfer-

rin isoform profile is presented as a typical pattern for CDG type I.

DDOST-CDG (CDG-Ir): deficiency of oligosaccharyltransferase complex (OST) (EC 2.4.99.18)

The oligosaccharyltransferase complex (OST) *en bloc* transfers the membrane-anchored dolichol-liniked fourteen-sugar $Glc_3Man_9GlcNAc_2$ glycan to a growing polypeptide chain of nascent protein by cleavage of the GlcNAc-P bond and release of dolichol diphosphate (Dol-PP) (Fig. 1a) (Freeze & Schachter, 2009). This disease results from mutations in the *DDOST* gene, leading to the deficiency of this enzyme (Table 1) (Jones *et al*., 2012).

Genetic defect DDOST-CDG was described in 2012, in a 6-month-old boy of European descent (Jones *et al*., 2012). He showed hypotonia, external strabismus, mild to moderate liver dysfunction, delayed psychomotor development with walking, and never developed speech. The transferrin isoform profile showed a typical for CDG type I pattern, in which both, mono- and aglycosylated transferrins were markedly increased. Laboratory studies revealed a deficiency of coagulation factor XI, antithrombin III, protein C, and protein S (Jones *et al*., 2012).

TUSC3-CDG: deficiency of subunit TUSC3 of oligosaccharyltransferase complex (EC 2.4.99.18)

The human oligosaccharyltransferase complex contains 7 subunits (Mohorko *et al*., 2011). One of them is TUSC3 or IAP (MAGT1). These two are paralogous and mutually exclusive subunits of this enzyme (Fig. 1a). These subunits are proposed to display oxidoreductase activity. This disorder results from mutations in the *TUSC3* gene (Table 1) (Garshasbi *et al*., 2011).

Genetic defect TUSC3-CDG was first described in 2008, in two families (Iranian and French) (Molinari *et al*., 2008; Garshasbi *et al*., 2011). Five patients have been recognized so far, in which nonsyndromic moderate mental retardation was observed. The transferrin isoform profile showed a normal pattern (Garshasbi *et al*., 2008).

MAGT1-CDG: deficiency of subunit MAGT1 of oligosaccharyltransferase complex (EC 2.4.99.18)

The deficiency of subunit MAGT1 of the oligosaccharyltransferase complex of second paralog, is caused by mutations in the *IAP* gene (Fig. 1a) (Table 1) (Molinari *et al*., 2008).

Genetic defect MAGT1-CDG was first described in 2008, in an Australian family, and presented nonsyndromic X-linked mental retardation (Molinari *et al*., 2008). Two girls had mild mental retardation, and two boys severe mental retardation. Glycosylation analyses of patients' fibroblasts showed normal N-glycan synthesis and transfer, suggesting that normal N-glycosylation observed in patients fibroblasts may be observed due to functional compensation (Molinari *et al*., 2008). The transferrin isoform profile by IEF method was not performed (Molinari *et al*., 2008).

GCS1-CDG (CDG-IIb): deficiency α-1,2-glucosidase I (EC 3.2.1.106)

The fourteen-sugar $Glc_3Man_9GlcNAc_2$ glycan, after being attached to the protein, undergoes further modi-

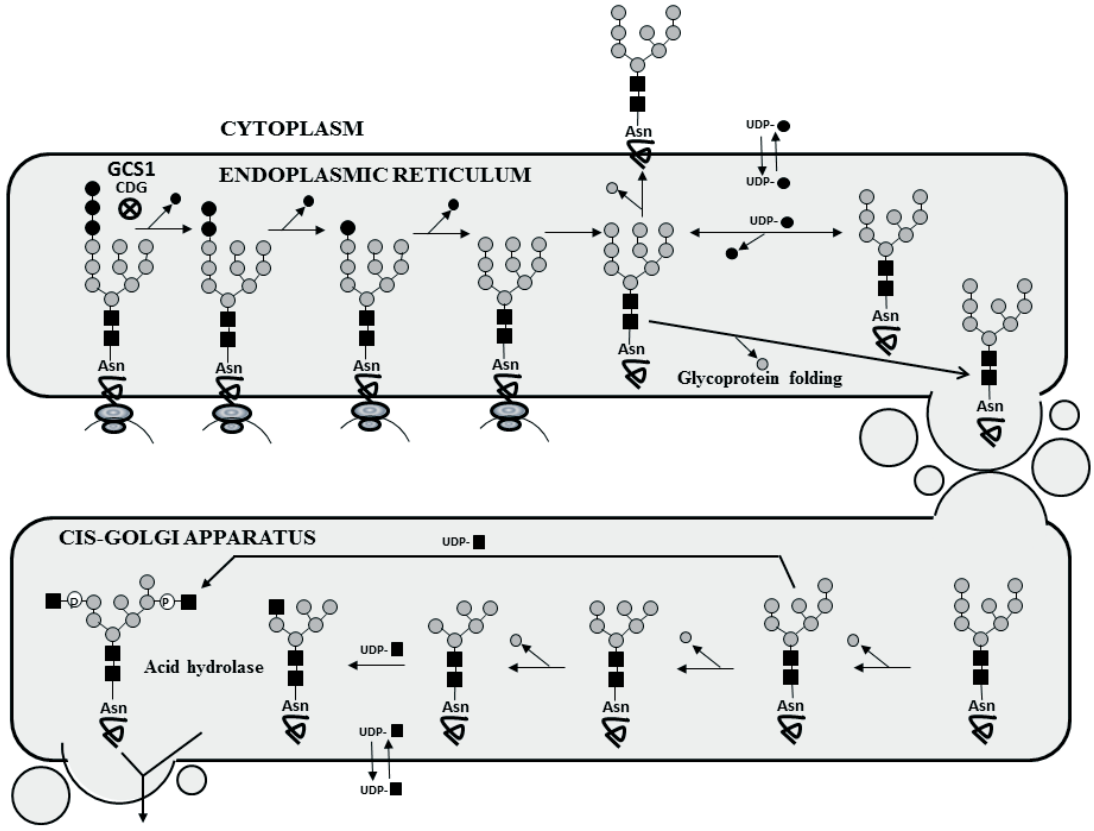

Figure 1b. Processing and maturation of an N-glycan in the endoplasmic reticulum and *cis*-Golgi apparatus.
Asn, asparagine; UDP, uridine diphosphate

fication by a series of trimming/processing reactions to the forming multiantennary complex-type glycans (Freeze & Schachter, 2009). Trimming of the oligosaccharide chain begins with the sequential removal of three glucose and one mannose residues creating ten-sugar chain ($Man_8GlcNAc_2$), which leaves the ER and passes to the Golgi apparatus. On the ER lumen, α-1,2-glucosidase I removes the first terminal α1,2-linked glucose (Fig. 1b). This disturbance results from mutations in the *GCS1* (Table 1) (de Praeter *et al*., 2000). α-1,2-glucosidase I deficiency causes an increase in the activity of the pathway involving endo α-1,2-mannosidase that cuts off the four-sugar structure (Glc_3Man) from the oligosaccharide chain (Volker *et al*., 2002). The remaining part of the chain can be further extended.

Genetic defect GCS1-CDG was first identified in 2000, in a female neonate (de Praeter *et al*., 2000). Only one case has been described so far. The patient displayed generalized hypotonia, dysmorphic features, hepatomegaly, hypoventilation, feeding problems, seizures, and fatal outcome at the age of 74 days (de Praeter *et al*., 2000). In laboratory studies, a normal profile of serum transferrin isoforms, accumulation of abnormal tetrasaccharide (Glc_3Man) in urine, and severe reduced of α-1,2-glucosidase I activity in the liver tissue and cultured skin fibroblasts have been shown (de Praeter *et al*., 2000).

MGAT2-CDG (CDG-IIa): deficiency of β-1,2-N-acetylglucosaminyltransferase II (EC 2.4.1.143)

For most glycoproteins (mature membrane and secreted glycoproteins), three mannose residues are removed and the first (out of two) N-acetylglucosamine residue is added to the chain in the *cis* compartment of the Golgi, forming eight-sugar chain ($GlcNAcMan_5GlcNAc_2$). Then, this glycan passes to the *medial* compartment (Fig. 1b). In this compartment, two outer mannose residues are removed, and the second N-acetylglucosamine as well as fucose residues are attached to the core of $Man_3GlcNAc_2$ (Fig. 1c). This glycan passes to the *trans* Golgi compartment, where two galactose and two sialic acid residues are added, generating an N-glycan complex with two branches. All N-glycans have a common five-sugar core (two N-acetylglucosamine and three mannose residues) and are classified into the following three types: oligomannose, complex, and hybrid. β-1,2-N-acetylglucosaminyltransferase II adds the second N-acetylglucosamine to the β-linked mannose of biantennary complex-type chains (Freeze & Schachter, 2009). This disorder results from mutations in the *MGAT2* gene causing the deficiency of β-1,2-N-acetylglucosaminyltransferase II (Table 1) (Fig. 1c) (Tan *et al*., 1996).

Genetic defect MGAT2-CDG was first described in 1991 in an Iranian child (Ramaekers *et al*., 1991). This disorder has been identified only in four patients (OMIM, 2013). These patients had severe psychomotor retrdation, hypotonia, seizures, craniofacial dysmorphy, and gastrointestinal disturbances (Tan *et al*., 1996). The diagnosis was confirmed by isoelectric focusing of serum transferrin, which showed an abnormal profile, typical for CDG type II pattern (increase of disialo-, and asialo-, trisialo-and/or monosialotransferrin (Jaeken *et al*., 1994). The activity of β-1,2-N-acetylglucosaminyltransferase II, an enzyme localized in the Golgi apparatus, was reduced in fibroblasts (Jaeken *et al*., 1994). Biochemical differences from classic CDG-Ia are: the absence of proteinuria, no change in serum alanine transaminase activity, normal serum albumin level, deficiency of clotting factors IX

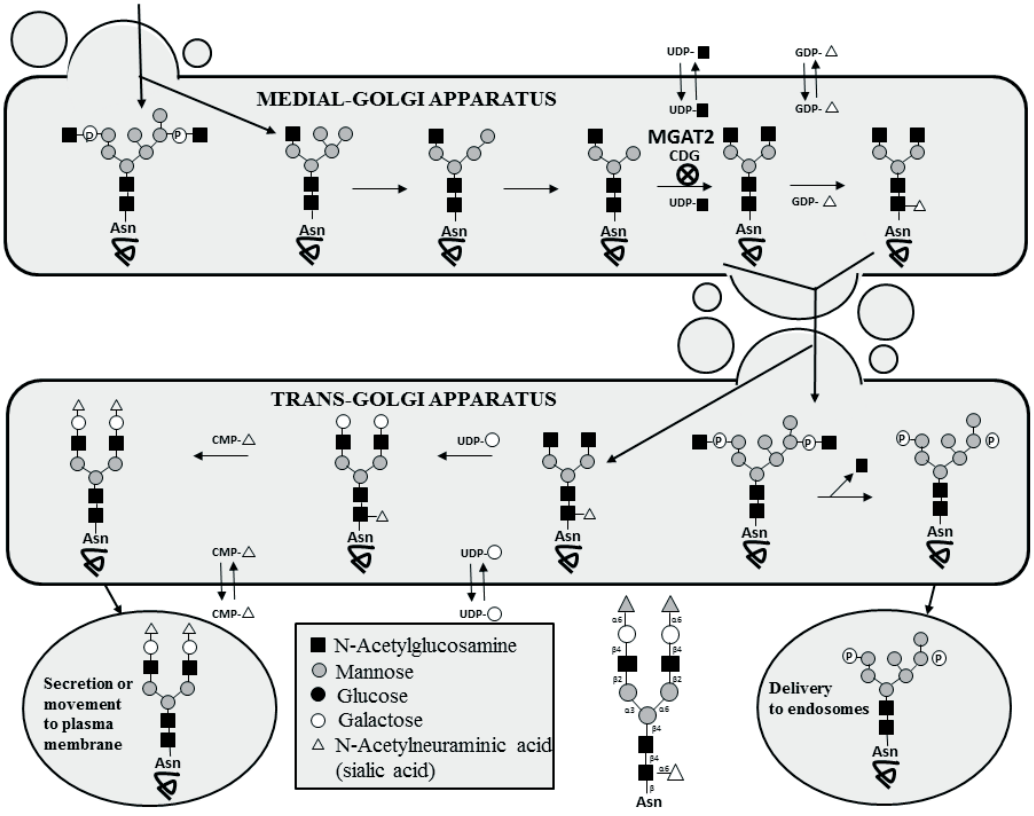

Figure 1c. Processing and maturation of an N-glycan in the *medial*- and *trans*-Golgi apparatus.
Asn, asparagine; CMP, cytidine monophosphate; UDP, uridine diphosphate; GDP, guanosine diphosphate

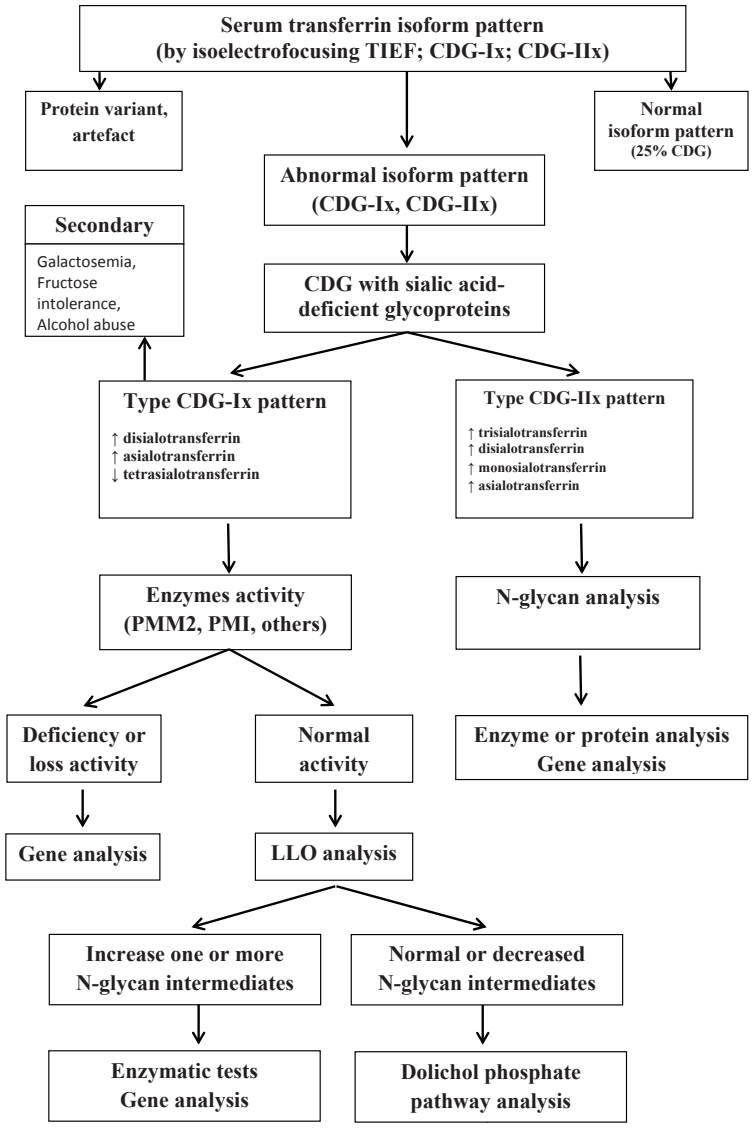

Figure 2. Outline of CDG laboratory diagnostics.
The first step in the laboratory diagnostics of congenital disorders of glycosylation (CDG) is the assessment of serum transferrin isoform pattern by TIEF transferrin isoelectrofocusing (TIEF) method. In case of an abnormal result, an artifact, a transferrin protein variant, and a secondary CDG, should be excluded. There are two types of profiles: the type I pattern points to an assembly defect (in the cytosol or endoplasmic reticulum; CDG-Ix, where x is the letter of alphabet corresponding to the type of disorder) and type II pattern to a processing defect (in the endoplasmic reticulum or Golgi apparatus; CDG-IIx). In the next step, a detailed diagnostics is performed separately for each type of CDG. In the CDG-Ix diagnosis, measurements of the enzymatic activity of phosphomannomutase II and phosphomannose isomerase in fibroblasts or leukocytes, and lipid-linked oligosaccharides analysis (LLO) in fibroblasts, and a detailed enzymatic analysis and/or mutation analysis are required. If there is no specific abnormal pattern, a defect in the dolichol-phosphate pathway should be considered. In the CDG-IIx diagnosis, the transferrin N-glycan structure analysis, and detailed enzymatic tests and/or mutation analysis are needed.

and XII, normal activity in serum of arylsulfatase A, and a decreased activity of β-glucuronidase. Some patients also had an increased serum carbohydrate-deficient transferrin (OMIM, 2013).

LABORATORY DIAGNOSTICS OF CDG

Laboratory findings for the diagnosis of congenital disorders of glycosylation should be performed as a first-line screening not only in patients with suspected genetic defects, but also in case of any unexplained syndromes. The assessment of the pattern of serum transferrin isoforms by the transferrin isoelectrofocusing method still remains the golden standard for the diagnosis of CDG (CDG-Ix i CDG-IIx, where x is the letter corresponding to the type of disease), due to N-glycosylation defect (TIEF) (Freeze & Schachter, 2009). In case of an abnormal result, an artifact, a transferrin protein variant or a secondary CDG (galactosemia, fructose intolerance, alcohol abuse, others) should be excluded. However, not all CDG types can be detected by TIEF. About 25% of the identified CDG may have a normal profile (Jaeken, 2010). For example, normal transferrin isoform pattern was described in patients with GCS1-CDG (CDG-IIb), SLC35C1-CDG (CDG-IIc) and SLC35A1-CDG (CDG-IIf). Patients with PMM2-CDG (CDG-Ia) may also have a normal TIEF profile (Fletcher *et al.*, 2000). It should be mentioned that some of the CDG patients younger than 3 or even 6 months of age may show normal transferrin glycosylation (Morava *et al.*, 2008; Morava *et al.*, 2010;

Lefeber *et al*., 2011). Modifications in the glycosylation pattern of serum transferrin occur in patients suffering from CDG where the normal transferrin isoform profile is shifted to the cathode in both type I and type II CDG with hypoglycosylation (Theodore & Morava, 2011). There is a difference between serum transferrin isoform patterns in type I and type II CDG. The type I profile is characterized by increased disialo- and asialo- and decreased tetrasialotransferrin, while in the type II CDG, by an increase of trisialo-, disialo-, monosialo- and asialotransferrin (Theodore & Morava, 2011).

The next step in the identification of congenital disorders of glycosylation type I (CDG-Ix) is the determination of two enzymes' activity: phosphomannomutase II (PMM2) and phosphomannose isomerase (PMI) in fibroblasts or leukocytes (Jaeken, 2010). If these results are negative, then the identification of lipid linked oligosaccharide precursor (LLO) accumulated in fibroblasts is performed. An increase of one or more N-glycan intermediates, permits to make a preliminary diagnosis that should be confirmed by further enzymatic tests and/or genetic analysis. In turn, if N-glycan intermediates values are normal or decreased, and there is no specific abnormal pattern, a defect in the dolichol-phosphate pathway should be taken into consideration (Jaeken, 2010).

If a type II TIEF pattern is found, the diagnosis of congenital disorders of glycosylation (CDG-IIx) should be further performed using two different analyses: N-glycan structures of serum transferrin, and O-glycan of apolipoprotein C-III by isoelectrofocusing (IEF), to find glycosylation defects of a mucin-type glycan (Jaeken, 2010). The pattern of the transferrin glycan has a specific profile with an accumulation of glycan intermediates in Golgi apparatus only in a small number of cases. In most cases, this pattern is nonspecific with some degree of hyposialylation and/or hypogalactosylation (Jaeken, 2010). Therefore, further diagnostics should include a clinical presentation. If there is still no diagnosis, mutation analysis (gene testing) should be performed. The outline of CDG laboratory diagnostics is presented in Fig. 2.

SUMMARY

Congenital disorders of glycosylation (CDG) constitute a rapidly growing disease family due to genetic defects in the glycosylation pathway of proteins and lipids. Since the first clinical description in year 1980, 45 CDG have been identified, and a novel nomenclature and classification were developed. This paper reviews CDG due to defects only in the N-glycosylation pathway of proteins, on the basis of current knowledge. In this group, 17 different genetic defects have been recognized. Most of these diseases have been discovered recently and frequently concern a small number of patients. CDG are an important clinical problem due to the fact that they cause severe multiorgan and multisystem disorders manifested as early as in the first months of life and about 20% of patients do not live past their fifth years of life. The variability of clinical presentation makes it difficult for physicians to diagnose these patients. Regarding that about 250 genes are considered to be involved in glycosylation, it should be expected that many diseases are yet to be identified in the near future. Rapidly developing modern laboratory diagnostics will help with that. Therefore, the cooperation between the physician and the laboratory is of great importance. The purpose of this paper is to draw attention to congenital disorders of glycosylation in cases when the disease is of unknown origin, especially when the patient has a broad spectrum of clinical symptoms and~~of~~ psychomotor retardation.

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