

# Congenital Disorders of Glycosylation: A Review

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**Abstract:** Congenital disorders of glycosylation (CDG) are a rapidly growing group of inborn errors of metabolism with abnormal glycosylation of proteins and lipids. Nearly 70 inborn errors of metabolism have been described due to congenital defects of glycosylation, present as clinical syndromes, affecting multiple systems, impacting nearly every organ. No specific tests are available yet for screening all types of CDG, analysis of serum Tf by isoelectric focusing (IEF) or high-performance liquid chromatography (HPLC) / (matrix-assisted laser desorption/ionization MALDI) or serum N-glycans (by MS), enzyme activity assays and DNA sequence analysis are the most frequently used methods for CDG screening and diagnosis. We here review the clinical phenotypes in CDG defects.

**Keywords:** Congenital Disorders of Glycosylation, Cdg, Transferrin, O-Glycosylation

## 1. Introduction

Protein post-translational modification increases the functional diversity of the proteome by the covalent addition of functional groups or proteins, proteolytic cleavage of regulatory subunits or degradation of entire proteins. These modifications include phosphorylation, glycosylation, ubiquitination, nitrosylation, methylation, acetylation, lipidation and proteolysis and influence almost all aspects of normal cell biology and pathogenesis.

Glycosylation is one of the most frequent and important post-translation modifications, 1–2% of the genome encodes enzymes involved in glycan formation, approximately half of all proteins typically expressed in a cell undergo glycosylation, 13 different monosaccharides and 8 amino acids are involved in glycoprotein linkages leading to a total of at least 41 bonds. These bonds represent the products of N- and O-glycosylation, C-mannosylation, phosphoglycation, and glypiation.

Deficiency of glycosylation enzymes or transporters results in impaired glycosylation, and consequently pathological modulation of many physiological processes.

There are numerous different glycoproteins, exist abundant in living organisms, appearing in nearly every biological process. Their functions span the entire spectrum of protein activities, including those of enzymes, transport proteins, receptors, hormones and structural proteins. Carbohydrates serve as cell surface receptors, signals for protein targeting, mediators of cell-to-cell interaction, and

protectors of polypeptides from proteases (Varki A 1998).

Protein glycosylation includes four important steps: synthesis of the carrier lipid dolichyl diphosphate, assembly of oligosaccharide-lipid intermediate, transfer of the oligosaccharide precursor from the dolichol to an asparagine residue on the nascent polypeptide, and finally, oligosaccharide modification in rER and GA.

N-glycosylation, in this process carbohydrates are attached covalently to asparagine (N-glycans), runs through cytosol, rough endoplasmic reticulum (rER) and Golgi apparatus (GA), or serine/threonine (O-glycans) residues of proteins.

### **Congenital disorders of glycosylation (CDG)**

Congenital disorders of glycosylation (CDG) comprise a group of inborn errors of metabolism with abnormal glycosylation of proteins and lipids. Defects, first described as “Carbohydrate Deficient Glycoprotein syndrome (CDGS)” (Jaeken J 1980) were later renamed CDG. A CDG might occur due to a defect in any of the following: activation or transport of sugar residues in the cytoplasm, dolichol synthesis and dolichol-linked glycan synthesis, ER-related glycan synthesis or compartment shifting (flipping), glucose signaling, transfer to the protein, trafficking or processing of the glycoprotein through the Golgi apparatus or transport, or secretion at the end of the multistep pathway (Jaeken J 2010).

CDGs were first classified as type I (CDG-I) related to

the disrupted synthesis of the lipid-linked oligosaccharide precursor and type II (CDG-II) involving malfunctioning processing/assembly of the protein-bound oligosaccharide chain. However, since 2009, most of the researchers use a novel nomenclature based on the name of the affected gene (*e.g.* *CDG-Ia* = PMM2-CDG, *CDG-Ib* = MPI-CDG). According to the novel classification, CDGs are divided into 4 categories as defects of: protein N-glycosylation, protein O-glycosylation, lipid glycosylation and glycosylphosphatidylinositol anchor glycosylation, defects in multiple glycosylation pathways and in other pathways (Jaeken J 2009).

In addition, several CDGs of so far unknown etiology (CDG-x) have been recognized. CDG symptoms highly vary, but some are common for several CDG types, such as

psychomotor retardation, failure to thrive, coagulopathies, dysmorphic features, seizures and stroke-like episodes. Clinical manifestation of CDGs ranges from very mild to extremely severe.

CDGs still remain under- or misdiagnosed. In addition, the population studies on the frequency of the mutations causing CDGs are still scarce. Based on the determined frequency of heterozygotes, the estimated incidence of homozygotes for certain mutations are as high as 1:20,000, suggesting the existence of much higher number of cases than documented.

The following chapter offers an overview of the CDG types (Table 1, 2 and 3), symptomatology, diagnostics, and possibilities of therapy.

**Table 1.** *N-glycosylation defects - CDG type I.*

Type	Gene	Locus	Prevailing symptoms
Ia (PMM2-CDG)	PMM2	16p13.3-p13.2	Dysmorphism, hypotonia, cerebellar hypoplasia
Ib (MPI-CDG)	MPI	15q22-qter	Hepatic fibrosis, enteropathy, coagulopathy
Ic (ALG6-CDG)	ALG6	1p22.3	Moderate form of CDG-Ia
Id (ALG3-CDG)	ALG3	3q27	Profound form of CDG-Ia
Ie (DPM1-CDG)	DPM1	20q13.13	Similar to CDG-Ia, cortical blindness, microcephaly
If (MPDU1-CDG)	MPDU1	17p13.1-p12	Typical CDG-Ia symptoms, ichthyosis
Ig (ALG12-CDG)	ALG12	22q13.33	Common CDG-Ia symptoms, low IgG
Ih (ALG8-CDG)	ALG8	11pter-p15.5	Similar to that of CDG-Ib
Ii (ALG2-CDG)	ALG2	9q22	Typical symptoms of CDG-Ia
Ij (DPAGT1-CDG)	DPAG1	11q23.3	Similar to that of CDG-Ia
Ik (ALG1-CDG)	ALG1	16p13.3	Common CDG-Ia symptoms, ↓ B-cells, IgG
Il (ALG9-CDG)	ALG9	11q23	Microcephaly, hypotonia, seizures, hepatomegaly
Im (DOLK-CDG)	DOLK	9q34.11	Ichthyosis, Dilated cardiomyopathy, Seizures, hypersarhythmia, PMR
In (RFT1-CDG)	RFT1	3p21.1	Seizures, PMR, Hypotonia, Hepatomegaly, Coagulopathy, Sensorineural hearing loss
Io (DPM3-CDG)	DPM3	1q22	Low-normal IQ, mild proximal muscle weakness, Dilated cardiomyopathy
Ip (ALG11-CDG)	ALG11	13q14	Hypotonia, failure to thrive, seizures. gastric bleeding; scoliosis, dry scaly skin
Iq (SRD5A3-CDG)	SRD5A3	4q12	Coloboma, hypoplasia optic disc, anemia
Ir (DDOST-CDG)	DDOST	1p36.12	MR, facial dysmorphism, coagulopathy, cerebellar atrophy, vermis malformations, ichthyosiform erythroderma
Is (ALG13-CDG)	ALG13	Xq23	Hypotonia, strabismus, liver dysfunction, PMR, never developed speech
It (PGM1-CDG)	PGM1	1p31	PMR, epilepsy, recurrent infections, optic nerve atrophy, dysmorphic features, bleeding tendency
Iu (DPM2-CDG)	DPM2	9q34.13	Rhabdomyolysis, elevation liver enzymes + CK, cerebral thrombosis, dilated cardiomyopathy
Iw (STT3A-CDG)	STT3A	11q23	Hypotonia, strabismus, scoliosis, cong. contractures, cerebellar hypoplasia
Iy (CDG-SSR4)	SSR4	Xq28	PMR, microcephaly, seizures, hypotonia, cerebellar atrophy
TUSC3-CDG	TUSC3	8p22	Microcephaly, delayed development, hypotonia, seizure, dysmorphic features
MAGT1-CDG	IAP	X21.1	Nonsyndromic moderate to severe cognitive impairment, normal brain MRI
DHDDS-CDG	DHDDS	1p36.11	Nonsyndromic X-linked MR
GMPPA-CDG	GMPPA	2q35	Recessive retinitis pigmentosa
			Cognitive impairment, a triple-A-like Syn. (achalasia-addisonianism-alacrima)

**Table 2.** *N/N+O-glycosylation defects - CDG type II.*

Type	Gene	Locus	Prevailing symptoms
Ila (MGAT2-CDG)	MGAT2	14q21	Developmental delay, dysmorphism, seizures
Iib (GCS1-CDG)	GCS1	2p13-p12	Dysmorphism, hypotonia, seizures, hepatic fibrosis
Iic (SLC335C1-CDG)	SLC351	11p11.2	Recurrent infections, PMR, hypotonia
Iid (B4GALT1-CDG)	B4GALT1	9p13	Myopathy, coagulopathy Dandy-Walker malfor.
Iie (COG7-CDG)	COG7	16p.12.2	Dysmorphism, hypotonia, recurrent infections
IIf (SLC35A1-CDG)	SLC351	6q15	Thrombocytopenia, no neurological symptoms
Ilg (COG1-CDG)	COG1	17q25.1	Failure to thrive, hypotonia, short stature, cerebral and cerebellar atrophy, cardiac abnormalities, hepatosplenomegaly, costocerebromandibular syndrome, Pierre-Robin sequence
Iih (COG8-CDG)	COG8	16q22.1	Normal to severe PMR, hypotonia, multiple organ involvement, protein-losing enteropathy seizures, esotropia, ataxia
Iii (COG5-CDG)	COG5	7q31	PMR , diffuse atrophy of the cerebellum
Iij (COG4-CDG)	COG4	16q22.1	Seizure, hypotonia, microcephaly, ataxia, absent speech, motor delays, recurrent respiratory
IIL (COG6-CDG)	COG6	13q14.11	PMR, dysmorphism, microcephaly, seizures, intracranial bleeding, vomiting, multiorgan involvement, chronic inflammatory bowel disease, T- and B-cell dysfunction
IIk (TMEM165)	TMEM165	4q12	PMR, facial dysmorphism, wrinkled skin, amelogenesis imperfecta, skeletal dysplasia, short stature , pituitary hypoplasia
IIm (SLC35A2)	SLC35A2	Xp11.23	PMR, seizures, feeding problems
ATP6V0A2-CDG	ATP6V02	12q24.31	Generalized cutis laxa, ophthalmological abnormalities, delayed motor development
MAN1B1-CDG	MAN1B1	9q34.3	Facial dysmorphism, PMR, truncal obesity
ST3GAL3-CDG	ST3GAL3	1p34.1	Mental retardation, autosomal recessive
PGM3-CDG	PGM3	6q14.1-q14.2	Severe atopic dermatitis, renal failure, immune dysfunction, connective /motor impairment

**Table 3.** *O - glycosylation disorders.*

Gene	Chromosome	Disease
Defects in O-xylosylglycan synthesis		
EXT1/EXT2	8q23-q24 +11p11-p12	Multiple cartilaginous exostoses
B4GALT7	5q35.1-q35.3.	Progeroid variant of Ehlers-Danlos syndrome
Defects in O-N-acetylgalactosaminylglycan synthesis		
GALNT3	2q24-q31	Familial tumoral calcinosis
Defects in O-xylosyl/N-acetylgalactosaminylglycan synthesis		
SLC35D1	1p31.3	Schneckenbecken dysplasia (Platyspondyly, extrem short long bones
Defects in O-mannosylglycan synthesis		
POMT1/POMT2	9q34.1	Walker-Warburg syndrome (Brain + eye involvement associated congenital muscular dystrophy
POMGNT1	1p34.1	Muscle-eye-brain disease
Fukutin	9q31.2	Fukuyama congenital muscular dystrophy
FKRP	19q13.3	limb girdle muscular dystrophy
LARGE	22q12.3	Muscular dystrophy, mental retardation, brain and eye anomalies.
ISPD	7p21.2	limb-girdle muscular dystrophy, brain + eye abnormalities
Defects in O-fucosylglycan synthesis		
POFUT1	20q11	Dowling-Degos disease (Hyperpigmentation hyperkeratotic dark brown papules (flexures and great skin folds)
EOGT	3p14	Adams-Oliver syndrome 4 (aplasia cutis congenita and terminal transverse limb defects)
SCDO3-CDG	7p22.2	Spondylocostal dysostosis type 3 (vertebral malsegmentation disorders)
B3GALTL-CDG	13q12.3	Peters'-plus syndrome (anterior eye-chamber defects, short stature, PMR

## 2. Defects of Protein N-Glycosylation

### *Types of CDG I*

#### *CDG-Ia (PMM-CDG)*

CDG-Ia was first observed in homozygous twin sisters (Jaeken J 1980). It is the most frequent CDG type (over 85%) with more than 700 patients described worldwide; it is caused by a deficiency of phosphomannomutase (PMM), which converts Man-6-P to Man-1-P. The PMM2 gene is located on chromosome 16p13 and is composed of 10 exons that encode a 246 amino acid protein. Over 100 different mutations have been found at the corresponding gene of CDG Ia (Haeuptle MA 2009).

Patients can be often diagnosed in the neonatal or early infantile period on the basis of typical clinical features, such as inverted nipples and fat pads, in addition to strabismus, muscular hypotonia, failure to thrive, and elevated transaminases. A very common sign is cerebellar hypoplasia, which can usually be documented at, or shortly after birth. There is a substantial childhood mortality of approximately 20%, owing to severe infections or organ failure. At a later age, the impairment of the nervous system becomes more evident, presenting by a variable degree of mental retardation, cerebellar dysfunction, pigmentary retinopathy, and peripheral neuropathy, skeletal abnormalities.

Due to defective synthesis of coagulation factors by the liver (primarily factor XI, antithrombin III, protein C and protein S), patients have severe coagulation defects. Adding to the situation is hepatomegaly with consequent liver dysfunction. Some children experience seizures or exhibit stroke-like episodes with complete recovery, which can occur mainly during feverish infections. Adult female patients can present with hypergonadotrophic hypogonadism.

The number of patients with a less typical presentation is increasing, many children present with nearly normal psychomotor development (Marquardt T 2003, Pancho C 2005).

#### *CDG-Ib (MPI-CDG)*

CDG-Ib is caused by a deficiency of phosphate isomerase (PMI), which affects the endogenous productions of Man-6-P. The MPI gene is located on chromosome 15q24.1 and is composed of 8 exons. In contrast to CDG-Ia, mental and motor development is normal. The predominant symptom of CDG-Ib is chronic diarrhoea, commonly starting during the first year of life. Cyclic vomiting can be the leading symptom. Failure to thrive and protein-losing enteropathy can occur. Partial villus atrophy can be present in duodenal biopsies and might lead to suspicion of celiac disease (Jaeken J 1998, Niehues R 1998). Hypoglycaemia occurs frequently; some patients present with congenital hepatic fibrosis (Babovic-Vuksanovic D 1999). Hypoalbuminemia, elevated aminotransferases and low antithrombin III (AT III) activity are common findings in CDG-Ib patients. Thrombotic episodes and severe bleeding may complicate the course.

One explanation for the lack of demonstrable neurologic

deficit in CDG-Ib compared to CDG-Ia is that brain hexokinase can phosphorylate mannose to Man-6-P thereby, bypassing the need for PMI. However, liver glucokinase does not phosphorylate mannose thus, there are the associated hepatic anomalies with CDG-Ib.

#### *CDG-Ic (ALG6-CDG)*

Defect of the 1,3-glucosyltransferase causes CDG type Ic. The enzyme catalyses attachment of the first glucose to the LLO intermediate Man<sub>9</sub>-N-acetyl-glucosamine(GlcNAc<sub>2</sub>)-PP-dolichol in the rER (gene symbol: ALG3). The ALG6 gene is located on chromosome 1p31.3 and is composed of 15 exons spanning 55kbp encoding a 507 amino acid transmembrane protein. CDG-Ic It is the second most frequent N-glycosylation disorder after PMM2-CDG; some 37 patients have been reported with 21 different ALG6 gene mutations.

Symptoms of CDG-Ic are similar to those of CDG-Ia but much less severe. Patients have frequent seizures, psychomotor retardation that is milder than in CDG-Ia, pronounced axial hypotonia, and strabismus. Intestinal symptoms of CDG-Ic are markedly exacerbated by intestinal viral infections (Jaeken J 2010).

#### *CDG-Id (ALG3-CDG)*

CDG-Id results from deficiencies in mannosyltransferase VI (Dol-P-Man: Man<sub>5</sub>GlcNAc<sub>2</sub>-P-P-Dol α-1,3-mannosyltransferase; gene symbol: ALG3). This enzyme transfers Man from Dol-P-Man to Dol-PP-Man<sub>5</sub>GlcNAc<sub>2</sub> of the growing en bloc oligosaccharide. CDG-Id individuals suffer severe neurological impairment including profound psychomotor retardation and intractable seizures, dysmorphic features, eye abnormalities, optic atrophy, postnatal microcephaly, and hypsarrhythmia (Stibler H 1995, Denecke J 2004, Sun L 2005).

#### *CDG-Ie (DPM1-CDG)*

CDG-Ie is caused by a defect in the dolichol-P-Man synthase 1 (DPM1), which is required to generate the dolichol-P-Man, a donor of mannose for the growing LLO on the luminal side of the rER. The DPM1 gene is located on chromosome 20q13.13 and is composed of 10 exons that encode a protein of 260 amino acids. Mutations causing a complete loss of enzymatic activity might be lethal. Clinical manifestations include severe psychomotor retardation, hypotonia, cerebral atrophy, epilepsy, cortical blindness, hepatosplenomegaly, coagulopathy, and dysmorphic features (gothic palate, hypertelorism, dysplastic nails and knee contractures). Liver transaminases are raised. Body weight, length and head circumference might be normal at birth, but later on microcephaly is typical of CDG-Ie (Imbach T 2000, Kim S 2000).

#### *CDG-If (MPDU1-CDG)*

CDG If results from defects in the protein responsible for utilization of Dol-P-Man, independent of DPM1 which is defective in CDG-Ie. The gene encoding this activity is identified as Man-P-Dol utilization defect 1 (gene symbol: MPDU1) and it is required for the utilization of Dol-P-Man

and Dol-P-Glc. The MPDU1 gene is located on chromosome 17p13.1–p12 and is composed of 7 exons encoding a 247 amino acid transmembrane protein. CDG-If have clinical symptoms including psychomotor retardation, muscular hypotonia, seizures, and absence of speech development, short stature, failure to thrive, feeding problems, impaired vision and pigmentary retinopathy. Two of them have shown ichthyosis (Kranz C 2001, Schenk B 2001).

#### *CDG-Ig (ALG12-CDG)*

The defect in the CDG type Ig is located in the ALG12 mannosyltransferase. This enzyme adds the eighth mannose to the growing LLO in the rER (Chantret I 2002).

The ALG12 gene is located on chromosome 22q13.33 and is composed of 13 exons that encode a protein of 488 amino acids. The common clinical features associated with CDG-Ig are psychomotor retardation, facial dysmorphism, and hypotonia. In some patients there are feeding problems, microcephaly, convulsions, and frequent respiratory tract infections.

#### *CDG-Ih (ALG8-CDG)*

CDG type Ih is due to the deficiency of the glycosyltransferase II (gene symbol: ALG8) adding the second glucose onto the growing LLO in the rER. The ALG8 gene is located on chromosome 11q14.1. To date five children have been identified with CDG-Ih, clinical presentation is similar to that of CDG-Ib: hypoalbuminaemia, protein-losing enteropathy, hepatomegaly and coagulopathy, but without central nervous system (CNS) involvement, lung hypoplasia, anemia, and thrombocytopenia (Chantret I 2003, Schollen E 2004).

#### *CDG-Ii (ALG2-CDG)*

CDG-Ii is caused by the deficiency of  $\alpha$ -1,3-mannosyltransferase, which catalyses the transfer of mannosyl residues from GDP-Man to Man (1)GlcNAc(2)-PP-dolichol; gene symbol: ALG2. The ALG2 gene is located on chromosome 9q22.33 and is composed of 3 exons that encode a protein of 416 amino acids.

Only one patient with this type was reported; he had mental and motor retardation, colobomas, and cataract, nystagmus, seizures, hepatomegaly, and coagulation abnormalities. Cranial MRI showed a severely retarded myelinization (Thiel C 2003).

#### *CDG-Ij (DPAGT1-CDG)*

The CDG-Ij results from deficiency in UDP-GlcNAc: dolichol phosphate

N-acetyl-glucosamine-1-phosphate transferase (gene symbol: DPAGT1). The DPAGT1 gene is located on chromosome 11q23.3 and is composed of 9 exons that encode a protein of 408 amino acids. The patient presents with severe hypotonia, medically intractable seizures, mental retardation, microcephaly, arched palate, micrognathia, strabismus, fifth finger clinodactyly, single flexion creases, and skin dimples on the upper thighs (Jaeken J 2010).

#### *CDG-Ik (ALG1-CDG)*

The defect in the CDG-Ik patients affects the mannosyltransferase I, an enzyme necessary for the elongation of dolichol-linked chitobiose during N-glycan biosynthesis (gene symbol: ALG1), the ALG1 gene is located

on chromosome 16p13.3 and is composed of 14 exons that encode a protein of 464 amino acids.

Reduced enzyme activity in two patients led to severe disease and death in early infancy. Grubenmann reported a patient without dysmorphic features and normal MRI scan of the brain; he suffered from multiple intractable seizures, generalized muscular hypotonia, blindness, liver dysfunction and coagulation problems related to low AT III (Grubenmann CE 2004).

Kranz described a CDG patient with seizures, severe muscular hypotonia, cerebral atrophy, nephrotic syndrome and a severe decrease of circulating B-cells with a complete absence of IgG, the boy died from respiratory failure at 11 weeks of age (Kranz C 2004).

De Koning also described two patients; in the first, ultrasound analysis at the 30th week of pregnancy revealed foetal hydrops and hepatosplenomegaly. The boy showed multiple dysmorphic features with a large fontanelle, hypertelorism, micrognathia, hypogonadism, contractures, areflexia, cardiomyopathy, and multifocal epileptic activity. The patient died at 2 weeks of age. The clinical features of the second patient included facial dysmorphism with hypertelorism, micrognathia, low-set ears, coloboma iridis, multiple contractures, and genital abnormalities. The boy died on the second day of life because of severe septicaemia (De Koning TJ 1998).

#### *CDG-IL (ALG9-CDG)*

CDG-IL results from deficiencies in mannosyltransferase VII-IX (Dol-P-Man: Man<sub>6</sub>- and Man<sub>8</sub>-GlcNAc<sub>2</sub>-P-P-Dol  $\alpha$ -1,2-mannosyltransferase; gene symbol: ALG9). The ALG9 gene is located on chromosome 11q23 and is composed of 22 exons that generate several alternatively spliced mRNAs.

Two patients with CDG-IL have been identified. Both exhibited psychomotor retardation, hypotonia, hepatomegaly, microcephaly, and seizures. (Frank CG 2004).

## 3. New Types of CDG 2006 – 2015

### 3.1. CDG I

#### *CDG-Im (DOLK gene on chromosome 9q34.11)*

Four affected infants had hypotonia and ichthyosis, and died between ages four and nine months. Additional features included seizures and progressive microcephaly in one and dilated cardiomyopathy in two sibs (Kranz C 2007). All patients showed a remarkable loss of oligosaccharide structures on serum transferrin (Tf), as shown by IEF and immunoprecipitation of the protein, implicating a disorder affecting N-glycosylation.

In all 4 patients with dolichol kinase deficiency examined by them, Kranz C et al. (2007) found homozygosity for 1 of 2 mutations in the DOLK gene. The DOLK gene encodes dolichol kinase, the enzyme responsible for the final step in the de novo synthesis of dolichol phosphate, which is involved in several glycosylation reactions, such as N-glycosylation, glycosylphosphatidylinositol (GPI)-anchor biosynthesis, and C- and O-mannosylation.

Lefeber et al. (2011) studied 11 children from 4 unrelated consanguineous families with CDG, who had predominantly nonsyndromic presentations of dilated cardiomyopathy (CMD) between 5 and 13 years of age.

Helander et al. (2013) reported 2 sibs, born of consanguineous Syrian Turkish parents, with CDG type Im. The patients presented at age 4 months with severe intractable seizures and hypsarrhythmia, consistent with a clinical diagnosis of West syndrome. Both had normal early development before the onset of seizures, but thereafter showed delayed psychomotor development with lack of speech. The seizures eventually remitted later in childhood in both patients after intense therapy. Neither patient had cardiac involvement. Serum Tf analysis showed a CDG type I pattern, and lipid-linked oligosaccharides were normal, suggesting an early defect in glycan assembly.

*CDG-In (RFT1 gene on chromosome 3p21.1)*

Stibler et al. (1998) identified a patient with an untyped disorder of N-linked glycosylation on the basis of detection of abnormal IEF of serum Tf. The patient, designated KS by Imtiaz et al. (2000), showed symptoms often encountered in CDG, namely, marked developmental delay, hypotonia, seizures, hepatomegaly, and coagulopathy. Six patients with type In were described, the common features in all six patients include severe developmental delay, hypotonia, visual disturbances, seizures, feeding difficulties, and sensorineural hearing loss, as well as features similar to other types of CDG including inverted nipples and microcephaly (Vleugels W 2009, Jaeken J 2009).

One key step in the biosynthesis of the Glc(3)Man(9)GlcNAc(2)-PP-dolichol precursor, essential for N-glycosylation, is the translocation of Man(5)GlcNAc(2)-PP-dolichol across the endoplasmic reticulum membrane. This step is facilitated by the RFT1 protein.

*CDG-Io (DPM3 gene on chromosome 1q22)*

A single described individual diagnosed with CDG Io at age 27 years had a low normal IQ and mild muscle weakness. She presented initially at age 11 years with mild muscle weakness and waddling gait. She was found to have dilated cardiomyopathy

without signs of cardiac muscle hypertrophy at age 20 years followed by a stroke-like episode at age 21 years (Lefeber DJ 2009). Metabolic investigations were normal, but results of Tf IEF showed an abnormal profile suggesting a CDG type I pattern. At age 27 years, she showed low-normal IQ and mild proximal muscle weakness. Further biochemical studies showed defective N-glycosylation of Tf in the endoplasmic reticulum and decreased dolichol-phosphate-mannose (Dol-P-Man) synthase activity.

In a Greek female patient with congenital disorder of glycosylation type Io, Lefeber DJ (2009) identified a homozygous mutation in the DPM3 gene. The authors noted that 4 biosynthetic pathways depend on DPM activity, including O-mannosylation of alpha-dystroglycan, and postulated that the isolated phenotype of muscular dystrophy in this patient most likely resulted from deficient

O-mannosylation of alpha-dystroglycan (DAG1). These findings linked the congenital disorders of glycosylation to the dystroglycanopathies.

*CDG-Ip (ALG11 gene on chromosome 13q14)*

ALG11 is a mannosyltransferase that uses GDP-mannose to sequentially add the fourth and fifth mannose residues to growing dolichol-linked oligosaccharide side chains at the outer leaflet of the endoplasmic reticulum. Upon completion, the lipid-linked polyoligosaccharides are translocated to the ER lumen for subsequent transfer to substrate asparagine residues of newly synthesized glycoproteins.

The first affected infant presented with microcephaly, high forehead, and low posterior hairline, hypotonia, and failure to thrive. She had severe neurologic impairment with frequent and difficult-to-treat seizures, and developed an unusual fat pattern around age six months and persistent vomiting and gastric bleeding; she died at age two years (Rind N 2010). The second affected child showed a similar disease course with hypotonia, generalized epilepsy, and opisthotonus, dysmorphic features were not noted. IEF of serum Tf from patient fibroblasts showed an increased amount of di- and asialo-transferrin with a decrease of tetrasialo-transferrin, consistent with CDG type I.

Subsequently, three additional individuals were identified with developmental delay, strabismus, and seizures in the first year of life, the most severely affected child had dysmorphic features, including long philtrum, retrognathia, and high forehead, scoliosis, fat pads, inverted nipples, oscillations of body temperature, dry scaly skin, and lack of visual tracking or light response. (Thiel C 2012). Biochemical analysis showed a CDG type I pattern. However, the pathologic glycosylation phenotype was only apparent after glucose starvation in patient fibroblasts; then, analysis of dolichol-linked oligosaccharides led to the emergence of pathologic shortened intermediate dolichol-linked oligosaccharides, indicating a defect in biosynthesis.

*CDG-Iq (SRD5A3 gene on chromosome 4q12)*

SRD5A3-CDG is caused by a mutation in the SRD5A3 gene. This gene codes for the enzyme 5 $\alpha$ -reductase type 3. The enzyme is responsible for the formation of polyprenol from dolichol, a reaction in lipid metabolism, required for the binding and carrying of glycans in the early steps of the glycosylation pathway.

Using laboratory studies of Tf, Cantagrel et al. (2010) demonstrated a type I glycosylation defect in affected individuals of the family reported by Al-Gazali et al. (2008). Biochemical analysis of this and other affected families showed that the metabolic block occurred early in the N-glycosylation pathway, altering synthesis or transfer of the glycan part of lipid-linked oligosaccharide (LLO) to recipient proteins.

SRD5A3-CDG is often called a cerebelloocular syndrome, individuals from seven families were identified, the most striking features were congenital eye malformations, such as ocular coloboma or hypoplasia of the optic disc, variable visual loss, nystagmus, hypotonia, motor delay, mental retardation, and facial dysmorphism. Brain abnormalities

included cerebellar atrophy or vermismalformations. Some patients had ichthyosiform erythroderma or congenital heart defects. Nine patients who were evaluated had microcytic anemia, increased liver enzymes, coagulation abnormalities, and decreased antithrombin III (Cantagrel et al 2010). Additional mutations in *SRD5A3* have been identified in people with Kahrizi syndrome, which consists of coloboma, cataract, kyphosis, and intellectual disability (Kahrizi K 2011).

*CDG-Ir (DDOST gene on chromosome 1p36.12)*

The oligosaccharyltransferase complex (OST) en bloc transfers the membrane-anchored dolichol-linked fourteen-sugar Glc3Man9GlcNAc2 glycan to a growing polypeptide chain of nascent protein by cleavage of the GlcNAc-P bond and release of dolichol diphosphate (Dol-PP) (Freeze HH 2009). This disease results from mutations in the *DDOST* gene, leading to the deficiency of this enzyme. Genetic defect *DDOST*-CDG was described in 2012, in a 6-month-old boy of European descent (Jones MA 2012). He showed hypotonia, external strabismus, mild to moderate liver dysfunction, delayed psychomotor development with walking, and never developed speech. The Tf isoform profile showed a typical for CDG type I pattern, in which both, mono- and aglycosylated Tf were markedly increased. Laboratory studies revealed a deficiency of coagulation factor XI, antithrombin III, protein C, and protein S (Jones MA 2012).

*CDG-Is (ALG13 gene on chromosome Xq23)*

Alg13 and Alg14 comprise a novel bipartite UDP-GlcNAc glycosyltransferase that catalyzes the second sugar addition in the synthesis of the dolichol-linked oligosaccharide precursor in N-linked glycosylation. Alg14 is a membrane protein that recruits the soluble Alg13 catalytic subunit from the cytosol to the face of the ER membrane where the reaction occurs. In a Caucasian boy with CDG1S, Timal S et al. (2012) identified a mutation in the *ALG13* gene. The mutation was identified by exome sequencing and confirmed by Sanger sequencing. The boy died at 1 year of age, he had refractory epilepsy with polymorphic seizures, hepatomegaly, swelling of hand, foot, and eyelid, recurrent infections, increased bleeding tendency, microcephaly, horizontal nystagmus, bilateral optic nerve atrophy, and extrapyramidal and pyramidal signs. Laboratory studies showed prolonged APPT. Tf IEF showed abnormal N-glycosylation and was consistent with CDG type I.

De Ligt et al. (2012) reported a 10-year-old girl who was born at 34 weeks' gestation and showed neonatal feeding problems, hypotonia, seizures, and severely delayed psychomotor development. She had a large head circumference, and brain MRI showed hydrocephalus, myelination delay, and wide sulci. Other features included self-mutilation, sleep disturbance, and dysmorphic features, such as hypertelorism, broad coarse face, low-set ears, mild retromicrognathia, small hands and feet, joint contractures, and scoliosis. IEF of Tf was not reported.

*CDG-It (PGM1 gene on chromosome 1p31)*

The protein encoded by *PGM1* gene is an isozyme of

phosphoglucomutase (PGM) and belongs to the phosphohexose mutase family. it catalyzes the interconversion of glucose 1-phosphate and glucose 6-phosphate. The influence of *PGM1* deficiency on protein glycosylation patterns is also widespread, affecting both biosynthesis and processing of glycans and their precursors. There are several *PGM* isozymes, which are encoded by different genes and catalyze the transfer of phosphate between the 1 and 6 positions of glucose. Affected patients show multiple disease phenotypes, reflecting the central role of the enzyme in glucose homeostasis. *PGM1* deficiency is classified as both a muscle glycogenosis (type XIV) and a congenital disorder of glycosylation of types I and II.

Stojkovic et al. (2009) reported a 35-year-old man with recurrent muscle cramps provoked by exercise. He had 2 episodes of dark-brown urine after strenuous exercise, suggesting rhabdomyolysis. Neurologic examination showed mild weakness of the pelvic-girdle muscles; serum creatine kinase and ammonia were increased after strenuous exercise. Muscle biopsy showed abnormal subsarcolemmal and sarcoplasmic accumulations of normally structured, free glycogen. In a follow-up report.

Tegtmeyer et al. (2014) found that the patient reported by Stojkovic et al. (2009) had abnormal liver enzymes and an abnormal pattern of Tf glycosylation, consistent with a congenital disorder of glycosylation.

Timal et al. (2012) reported 2 unrelated children with congenital disorder of glycosylation type It. One boy was adopted and of Colombian origin. He had cerebral thrombosis and dilated cardiomyopathy, and died at age 8 years. Laboratory studies showed low levels of antithrombin III and elevated liver enzymes. The other child was a 16-year-old Caucasian girl who had Pierre Robin sequence, cleft palate, fatigue, dyspnea, tachycardia, dilated cardiomyopathy, and chronic hepatitis. Laboratory studies showed increased serum creatine kinase and liver enzymes. Tf- IEF in both patients showed abnormal N-glycosylation. In addition to the loss of complete N-glycans, there were minor bands of monosialo- and trisialotransferrin, suggesting the presence of incomplete glycans. Thus, the pattern could best be described as CDGI/II.

Tegtmeyer et al. (2014) reported 19 patients from 16 families with CDG It, including the 3 patients reported by Stojkovic et al. (2009) and Timal et al. (2012). Patients displayed a wide range of clinical features, but all had signs of hepatopathy with abnormal liver enzymes and sometimes with steatosis and fibrosis. The majority of patients had muscle symptoms, including exercise intolerance and muscle weakness; 5 had a history of rhabdomyolysis. Serum creatine kinase was often elevated, and hypoglycemia was common. Most patients were noted to have cleft palate and bifid uvula at birth, and many of these patients had short stature later in life. Six patients developed dilated cardiomyopathy, including 3 who were listed for heart transplantation.

Two patients developed malignant hyperthermia after the administration of general anesthesia. Two unrelated girls had hypogonadotropic hypogonadism with delayed puberty.

Patient cells showed considerable variability in the transferrin-glycoform profile, with forms lacking one or both glycans as well as forms with truncated glycans, consistent with a mixed type I/II pattern.

*CDG-Iu (DPM2 gene on chromosome 9q34.13)*

DPM2 gene regulates the biosynthesis of dolichol phosphate-mannose (DPM) synthase complex, DPM serves as a donor of mannosyl residues on the luminal side of the ER. Barone et al. (2012) reported 3 patients from 2 unrelated families with a severe multisystem and neurologic phenotype resulting in early death. Two brothers, born of consanguineous Sicilian parents, had originally been reported by Messina S et al. (2009). At birth, both boys showed severe hypotonia, myopathic facies, and dysmorphic features. One had micrognathia, malocclusion, and strabismus. Both had severe congenital contractures of the joints and scoliosis.

Onset of severe focal, generalized, or myoclonic seizures began between 3 and 5 months of age. Both had profoundly delayed psychomotor development without visual tracking, head control, or speech. Microcephaly was also present, and brain MRI of 1 showed cerebellar hypoplasia. The boys died of respiratory infections at ages 16 and 7 months, respectively. Skeletal muscle biopsy of 1 boy showed a dystrophic pattern, and immunohistochemical studies showed a reduction of the O-mannosyl glycans on DAG1, suggestive of a dystroglycanopathy. Fibroblasts showed an accumulation of Dol-PP-GlcNAc2Man5, consistent with a CDG.

The third child, also of Sicilian origin, showed respiratory distress and severe hypotonia at birth. She had facial dysmorphism, including trigonocephaly, hypotelorism, small nose, high-arched palate, and micrognathia. She developed seizures at age 1 week. Over the first 2 years of life, she had lack of psychomotor development, was unaware of her surroundings, and had poor visual fixation. Brain MRI showed loss of periventricular and subcortical white matter. Laboratory studies showed increased serum transaminases and creatine kinase, and decreased antithrombin activity. Serum Tf showed abnormal N-glycosylation, consistent with CDG type I. She died at age 36 months.

*CDG-Iw (STT3A gene on chromosome 11q23)*

The protein encoded by STT3A gene is Oligosaccharyl transferase subunit STT3A which catalyzes the transfer of a high mannose oligosaccharide from a lipid-linked oligosaccharide donor to an asparagine residue within an Asn-X-Ser/Thr consensus motif in nascent polypeptide chains. CDG- STT3A patient cells showed reduced amounts of the STT3A protein.

Shrimal et al. (2013) reported 2 sibs, born of consanguineous Pakistani parents, the patients had delayed psychomotor development with mental retardation, microcephaly, failure to thrive, seizures, hypotonia, and cerebellar atrophy. Serum Tf studies showed abnormal glycosylation consistent with a type I pattern. At age 13 years, both patients showed developmental delay, failure to thrive, seizures, and hypotonia. One patient was more severely affected, with an inability to sit, weak visual tracking, and intractable seizures.

*CDG-Iy (SSR4 gene on chromosome Xq28)*

Losfeld et al. (2014) reported a 16-year-old boy, born of unrelated parents, he presented at birth with microcephaly and respiratory distress. Later in infancy, he showed delayed development, hypotonia, and developed a mild seizure disorder that did not require treatment. Dysmorphic features included micrognathia, excess skin around the neck, increased fat pads, mild hypospadias, and clinodactyly of the fourth and fifth toes. Biochemical studies showed a mildly abnormal IEF Of Tf profile suggestive of a type I CDG, but all known CDG defects were excluded. The patient also had von Willebrand disease, which was thought to be unrelated to the CDG.

The mutation was found by whole-exome sequencing. In vitro functional expression studies indicated that the mutation caused a loss of function and defective N-glycosylation of proteins. Losfeld et al. (2014) hypothesized that the SSR4 defect would induce ER stress, lead to the accumulation of misfolded proteins, and further the hypoglycosylation of proteins. The findings suggested that the TRAP complex directly functions in N-glycosylation.

*TUSC3-CDG (TUSC3 gene on chromosome 8p22)*

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. These subunits are proposed to display oxidoreductase activity. This disorder results from mutations in the TUSC3 gene. Genetic defect TUSC3- described in 12 individuals (including two French sibs and three Iranian sibs) with nonsyndromic moderate to severe cognitive impairment and normal brain MRI. The Tf isoform profile showed a normal pattern (Garshasbi et al 2011).

*MAGT1-CDG (IAP gene on chromosome X21.1)*

The deficiency of subunit MAGT1 of the oligosaccharyltransferase complex of second paralog, is caused by mutations in the IAP gene. Genetic defect MAGT1-CDG was first described in 2008, in an Australian family, and presented nonsyndromic X-linked mental retardation. 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. The Tf isoform profile by IEF method was not performed (Molinari F 2008).

*DHDDS-CDG (DHDDS gene on chromosome 1p36.11)*

A single-nucleotide mutation in the gene that encodes Cis-prenyltransferase (DHDDS) has been identified by whole exome sequencing as the cause non-syndromic recessive retinitis pigmentosa (RP) in a family of Ashkenazi Jewish origin in which three of the four siblings have early onset retinal degeneration (Lam BL 2014).

In plasma and urine of patients, a characteristic shortening of dolichols was identified by mass spectrometry. Instead of the common dolichol-19 species, dolichol-18 was the dominant species in patients. Interestingly, no significant



abnormality in protein glycosylation has been observed of plasma Tf in deficient patients. Suppression of DHDDS expression in zebrafish leads to the loss of photoreceptor outer segments and visual function. These observations support the hypothesis that insufficient DHDDS function leads to retinal degeneration. Still the cellular mechanisms explaining whether and how the shortened dolichol profiles contribute to the retinal degeneration phenotype awaits clarification (Wen et al 2014).

#### *GMPPA-CDG (GMPPA gene on chromosome 2q35)*

Human GMPPA encodes GMPPA with known domains in InterPro. The predicted nucleotidyltransferase domain (amino acids 3) is shared by a wide range of enzymes that transfer nucleotides onto phosphosugars.

In guanosine diphosphate (GDP)-mannose pyrophosphorylase A (GMPPA), it was identified a homozygous nonsense mutation that segregated with achalasia and alacrima, delayed developmental milestones, and gait abnormalities in a consanguineous Pakistani pedigree. Mutations in GMPPA were subsequently found in ten additional individuals from eight independent families affected by the combination of achalasia, alacrima, and neurological deficits.

Identified in several individuals with cognitive impairment and autonomic dysfunction including achalasia and alacrima. Gait abnormalities were also seen, the affected individuals and control subjects showed similar N-glycosylation profiles, both for Tf glycosylation and for N-glycans derived from either total serum. Moreover, serum Apo-CIII glycosylation did not differ between controls and our individuals. (Koehler et al 2013).

### **3.2. CDG II**

#### *CDG-IIa (MGAT2-CDG)*

CDG-IIa is caused by a deficiency of the N-acetylglucosaminyl transferase II (GnT II), which is encoded by the MGAT2. The MGAT2 gene is located on chromosome 14q21 and is an intronless gene encoding a protein of 447 amino acids.

Symptoms of CDG-IIa include severe psychomotor retardation, dysmorphic features, cortical atrophy, delayed myelination, generalized hypotonia, stereotypical behaviour, epilepsy, raised liver transaminases, decreased activities of AT III, factors IX and XII were present (Jaeken J 1993).

In animal experiments over 60% mouse embryos lacking the gene encoding GnT II develop fully, but 99% of newborns die during the first week of postnatal development. It is suggested that the majority of humans with CDG-IIa die during gestation or shortly after birth (Freeze H 2001). From these results it is speculated that the true incidence of human MGAT2 defects may go undetected due to spontaneous fetal abortion or death shortly after birth.

#### *CDG-IIb (GCS1-CDG)*

CDG-IIb is caused by a deficiency of glucosidase I (GCS1), an enzyme removing the terminal glucose from the oligosaccharide, after its transfer to the polypeptide in the

rER. The GCS1 gene is located on chromosome 2p13.1

Three patients with CDG-IIb have been identified so far, one patient presented with severe developmental delay, muscular hypotonia, oedema, seizures, hypoventilation, apnoea, hepatomegaly and peculiar dysmorphism, including retrognathia, high arched palate, broad nose, and overlapping fingers. Motor nerve conduction velocity was reduced. Following a rapid decline and a stuporous state, the patient died at 2.5 months of age (De Praeter CM 2000),

Two patients presented with dysmorphic facial features, generalized hypotonia, seizures, global developmental delay, cerebral atrophy, a small corpus callosum, optic-nerve atrophy, sensorineural hearing loss, hypoplastic genitalia, chronic constipation, and recurrent bone fractures; severe hypogammaglobulinemia and increased resistance to particular viral infections (A. Sadat M 2014).

#### *CDG-IIc (SLC35C1-CDG)*

CDG-IIc was discovered by Etzioni in 1992, and named leucocyte adhesion deficiency type II (LAD II) (Etzioni A 1992); latter on it was enlisted to CDG. Fucosylated glycoconjugates are severely diminished in this disorder, due to a defect of GDP-fucose import into the GA (Lübke T 2001), encoded by SLC35C1 gene which is located on chromosome 11p11.2.

Dysmorphic features of reported patients include short limbs and stature,

a flat face with a broad and depressed nasal bridge, long eyelashes and broad palms (Etzioni A 1992, Marquardt T 1999). Moderate to severe psychomotor retardation, hypotonia and increased peripheral leucocyte counts are the predominant findings already present in newborns, recurrent infections and immune deficiency are due to the absence of fucosylated selectin ligands, decreasing the adhesion of leucocytes to endothelial cells, and migration of neutrophils to infection focuses (Etzioni A 1992, Marquardt T 1999).

#### *CDG-IId (B4GALT1-CDG)*

CDG-IId is caused by a deficiency of  $\beta$ -1,4-galactosyltransferase, an enzyme adding galactose to the oligosaccharide of the newly synthesized glycoprotein in the GA (gene symbol: B4GALT1). The B4GALT1 gene is located on chromosome 9p13.

Two patients with this disorder are known to date; in the first patient, in addition to muscular hypotony, severe psychomotor and mental retardation, blood coagulation abnormalities, and myopathy with elevated creatine kinase levels, he presented with a Dandy-Walker malformation with macrocephalus at birth, and progressive hydrocephalus later on (Peters V 2002, Hansske B 2002). The second patient presented with recurrent episodes of diarrhea and mild hepatomegaly, transient axial hypotonia improved within the first year of life. At age 7 years, she had dysmorphic facial features involving hypertelorism, broad nasal bridge, full supra-orbital region, a long philtrum, thin upper lip, low-set ears, and severe myopia, laboratory investigations showed mild hepatopathy and coagulation anomalies (Guillard M 2011).

*CDG-IIe (COG7-CDG)*

In the type CDG-IIe, the alteration of glycosylation is secondary to the alteration of a GA protein, not primarily involved in glycosylation. CDG IIe is caused by a mutation that impairs the integrity of the conserved oligomeric Golgi complex (COG7) and alters Golgi trafficking, resulting in the disruption of multiple glycosylation pathways.

The protein encoded by the COG7 gene is one of eight subunits of COG. Because this gene defect disrupts proper Golgi trafficking its effects are evident in both the processes of N- and O-linked glycosylation pathways. The COG7 gene is located on chromosome 16p12.2.

Patients present with growth retardation, progressive severe microcephaly, hypotonia, adducted thumbs, feeding problems due to gastrointestinal pseudoobstruction, failure to thrive, cardiac anomalies, wrinkled skin, and episodes of extreme hyperthermia, haemolytic uraemia syndrome, thrombocytopenia, anaemia, hypoproteinemia, proteinuria, increased liver enzymes and creatine kinase.

COG7 deficiency is comparable to diseases such as Chediak-Higashi or Hermansky-Pudlak disease; this group of disorders affects different coat proteins later on in the secretory pathway (Wu X 2004).

*CDG-IIf (SLC35A1-CDG)*

CDG-IIf is caused by altered transport of cytidine monophosphate (CMP) -sialic acid into the GA (gene symbol: SLC35A1).. The SLC35A1 gene is located on chromosome 6q15. Only one patient with this type was reported so far; the clinical features included a spontaneous massive bleeding in the posterior chamber of right eye, and cutaneous haemorrhage, severe thrombocytopenia, respiratory distress syndrome and opportunistic infections. Pulmonary viral infection and massive pulmonary haemorrhage with refractory respiratory failure led to death at the age of 3 years (Martinez-Duncker I 2005).

About 20% of CDG patients remain untyped and are named CDG-x. Apart from the CDG typical clinical presentations, oligohydramnion, hydrops fetalis, absent psychomotor development, severe thrombocytopenia, ascites, demineralisation of distal bones, tubulopathia, and death in status epilepticus have been reported (Charlwood J 1997, Acarregui MJ 1998, Eyskens F 1994, Skladal D 1996).

*COG- CDG*

Multisubunit peripheral membrane protein complexes appear to play important roles in facilitating Golgi-associated membrane trafficking and glycoconjugate processing. One of these is the conserved oligomeric Golgi (COG) 2 complex comprising eight distinct subunits, previous biochemical, imaging, and genetic studies had suggested that the eight distinct COG subunits were organized into two subcomplexes, lobe A (Cog1–4) and lobe B (Cog5–8) (Oka T 2005).

Mutations in proteins of the COG complex that provides a scaffold important for Golgi membrane structure and tethering of retrograde vesicles, also cause alterations in glycosylation. Several COG subunits have now been shown to be mutated and to give rise to glycosylation defects in

patients with congenital diseases of glycosylation The mechanism by which COG defects alter multiple glycosylation pathways appears to be caused by partial relocation and degradation of Golgi glycosyltransferases and other glycosylation activities when COG is dysfunctional (Stanley P 2011).

*COG1-CDG (IIg) (COG 1 gene on chromosome 17q25.1)*

An affected infant presented in the first month of life with feeding difficulties, failure to thrive, and hypotonia. She had mild developmental delays, rhizomelic short stature, and progressive microcephaly with slight cerebral and cerebellar atrophy on brain MRI, as well as cardiac abnormalities (ventricular hypertrophy with diastolic abnormalities) and hepatosplenomegaly, IEF of the patient plasma Tf and ApoC-III showed an abnormal profile compared to the control (Foulquier F 2006).

*COG8-CDG (IIh) (COG 8 gene on chromosome 16q22.1)*

Phenotypes of this disorder are extremely variable. Manifestations range from severe developmental delay and hypotonia with multiple organ system involvement beginning in infancy, to hypoglycemia and protein-losing enteropathy with normal development. Two affected infants were reported who had severe developmental delay, hypotonia, seizures, esotropia, failure to thrive, and progressive microcephaly (Foulquier F 2007). More recently, a pair of sibs were described who had a milder presentation with pseudo-gynecomastia, hypotonia, intellectual disability, and ataxia. IEF of the plasma Tf and ApoC-III showed an abnormal profile (Stolting T 2009).

*COG5-CDG (IIi) (COG 5 gene on chromosome 7q31)*

A single individual with mild delay in motor and language development was described.

MRI analysis showed pronounced diffuse atrophy of the cerebellum and brain stem. The IEF of serum Tf in the patient showed increased levels of trisialo-transferrin that clearly differed from the pattern of a control subject or a patient with a N-glycosylation defect caused by a PMM2 deficiency. This accumulation of trisialo-transferrin is usually a sign of normal N-glycosylation site occupancy but incomplete N-glycan structures. This patient had abnormal IEF of ApoC-III (Paesold-Burda P 2009).

*COG4-CDG (IIj) (COG 4 gene on chromosome 16q22.1)*

A single child has been described who presented at age four months with a complex seizure disorder that was treated with phenobarbital. At age three years, additional findings included hypotonia, microcephaly, ataxia, brisk uncoordinated movements, absent speech, motor delays, and recurrent respiratory infections (Reynders E 2009). IEF of the patient plasma Tf showed an abnormal profile of CDG type II.

*COG6-CDG (IIl) (COG 6 gene on chromosome 13q14.11)*

First infant patient presented with severe neurologic disease including intractable seizures; vitamin K deficiency and intracranial bleeding; vomiting; and early death (Lubbehusen J 2010). Second patient presented at birth with dysmorphic features including microcephaly, post-axial polydactyly, broad palpebral fissures, retrognathia, and anal anteposition.

The clinical phenotype was further characterised by multiorgan involvement including mild psychomotor retardation, and microcephaly, chronic inflammatory bowel disease, micronodular liver cirrhosis, associated with life-threatening and recurrent infections due to combined T- and B-cell dysfunction and neutrophil dysfunction. The type 2 IEF pattern of serum Tf and the abnormal IEF of serum apolipoprotein C-III in was detected in this patient. (Huybrechts S 2012).

*TMEM165-CDG (Iik) (TMEM165 gene on chromosome 4q12)*

TMEM165 has a perinuclear Golgi-like distribution and is present mainly in the late Golgi region. It belongs to uncharacterized and highly conserved family of membrane proteins, the UPF0016 family. These proteins are involved in Ca<sup>2+</sup> and pH homeostasis, suggesting that they could be members of Golgi-localized Ca<sup>2+</sup>/H<sup>+</sup> antiporters. Deficiency or absence of TMEM165 was associated with an acidification of the lysosomal and Golgi apparatus and, gradually, of all of the downstream acidic compartments, causes of defects of glycosylation observed in TMEM165-deficient patients (Demaegda D 2013).

2-Sibs with a skeletal dysplasia presentation affecting the epiphyses, metaphyses, and diaphyse were described. Additional features included abnormal white matter and pituitary hypoplasia on brain MRI. One of the sibs also had recurrent, unexplained fevers and died at age 14 months. Evaluation of unsolved cases with a type II Tf -IEF pattern identified three additional patients, one of whom had no skeletal abnormalities (Foulquier F 2012).

Case 3 showed the same clinical, biochemical, and radiological features as cases 1 and 2. Case 4 with only psychomotor retardation, there was no dysmorphism except for mild rhizomelia, no hepatosplenomegaly, and no epilepsy. He has no clear skeletal anomalies. Case 5 presented with a short stature, facial dysmorphism, wrinkled skin, abnormal fat distribution, and dysplastic toenails. She had amelogenesis imperfecta and skeletal abnormalities, including osteoporosis, anterior beaking of lumbar vertebrae, dysplastic vertebrae and ribs, dysplastic fourth metacarpals and metatarsals, hypoplasia of femoral heads, and kyphoscoliosis. The type 2 IEF pattern of serum Tf and the abnormal IEF of serum apolipoprotein C-III in was detected (Zeevaert R 2013, Foulquier F 2012).

*SLC35A2-CDG (Iim) (SLC35A2 gene on chromosome Xp11.23)*

SLC35A2-CDG is an X-linked disorder caused by hemizygous or heterozygous mutation in the SLC35A2 gene on chromosome Xp11 leading to severe early-onset encephalopathy. UDP-galactose transporter (UGT) encoded by SLC35A2 leads to galactose-deficient glycoproteins. UDP galactose transporter is one of the nukleotide sugar transporters (NSTs) and imports UDPgalactose from the cytoplasm to the lumen of the golfu apparatus (Kodera et al 2013). All children with SLC35A2-CDG had developmental delay and neurological abnormalities. IEF of serum Tf showed an abnormal type II pattern. A possible treatment with galactose supplementation is demonstrated in one patient (Dörre K 2015), frequency of seizures has decreased,

pharmacological treatment is currently unnecessary, and there are no Frediny problems any more (Kodera H 2013).

*ATP6V0A2-CDG (ATP6V0A2 gene on chromosome 12q24.31)*

(Autosomal recessive cutis laxa (ARCL) type type IIA)

ATPase, H<sup>+</sup> transporting, lysosomal V0 subunit a2 (ATP6V0A2) encodes for the a2 subunit of the vacuolar H<sup>+</sup>-ATPase (V-ATPase), a proton pump involved in the maintenance of the pH gradient along the secretory pathway and the regulation of protein transport. Individuals with mutations in ATP6V0A2 have abnormal protein N- and O-linked glycosylations. Octly Abnormal protein glycosylation in patients with ATP6V0A2-CDG is due to vacuolar H<sup>+</sup>-ATPase deficiency leading to an increase in Golgi pH that affects glycosyltransferase activity and organele trafficking causing Golgi fragmentation and possible mislocalization of these enzymes (Bahena-Bahena D 2014).

laboratory findings of type 2 pattern on Tf-IEF, abnormal of apoC-III, and abnormal mass spectrometry of glycans of total serum proteins could be ascribed to the classical CDG type II caused by defects of enzymes involved in glycan processing,. All patients have generalized cutis laxa at birth, but ophtalmological abnormalities and delayed motor development that improves with age were also described (Goreta S 2012).

*MAN1B1-CDG (MAN1B1 gene on chromosome 9q34.3)*

MAN1B1 localizes to the Golgi complex in human cells and uncovered its participation in ERAD substrate retention, retrieval to the ER, and subsequent degradation from this organelle. MAN1B1 characterize as part of a Golgi-based quality control network (Iannotti M.J 2014). 12 cases with MAN1B1-CDG were found. All individuals presented slight facial dysmorphism, psychomotor retardation and truncal obesity. MAN1B1 is indeed localized to the Golgi complex, an altered Golgi morphology in all patients' cells, with marked dilatation and fragmentation was observed. Capillary zone electrophoresis (CZE) of serum Tf showed a type 2 Tf pattern in all affected cases (Rymen D 2013, Scherpenzeel V 2014).

*ST3GAL3-CDG (ST3GAL gene on chromosome 1p34.1)*

The protein encoded by ST3GAL3 gene is a type II membrane protein that catalyzes the transfer of sialic acid from CMP-sialic acid to galactose-containing substrates. The encoded protein is normally found in the Golgi apparatus but can be proteolytically processed to a soluble form. This protein is a member of glycosyltransferase family. Mutations in this gene have been associated with autosomal recessive nonsyndromic mental retardation-12 (MRT12) (Hu H 2011).

*PGM3-CDG (PGM3 gene on chromosome 6q14.1-q14.2)*

Phosphoglucomutase 3 (PGM3) deficiency is a recently characterized autosomal recessive disorder associated with decreased PGM3 enzyme activity and decreased O- and Nlinked protein glycosylation. PGM3 catalyzes the conversion of N-acetyl-glucosamine-6-phosphate (GlcNAc-6-P) to GlcNAc-1-P, a critical step in the biosynthesis of UDPGlcNAc. This precursor is then further

modified to make Nglycans, O-glycans, proteoglycans, and GPI-anchored proteins. The distinguishing clinical features of this syndrome include severe atopic dermatitis, immune dysfunction, autoimmunity, vasculitis, renal failure, ID, connective tissue involvement, and motor impairment (Zhang Y 2014). Eight patients in two unrelated families were initially referred for assessment because of atopic dermatitis, recurrent skin and pulmonary infections, and high serum immunoglobulin E (IgE) levels. Subsequent evaluation and whole-genome sequencing in both families identified PGM3 as a possible candidate gene, a finding confirmed by Sanger sequencing. Serum Tf glycosylation was normal, total N-linked glycans showed decreased galactosylation of N-linked oligosaccharides in three patients (Zhang Y 2014). Interestingly, UDP-GlcNAc and UDP N-acetylgalactosamine levels increased to control levels by GlcNAc-supplemented medium. Thus, it has been shown that PGM3 deficiency disrupts UDP-GlcNAc synthesis and N- and O-linked glycosylation though the exact contribution of impaired glycosylation to the significant atopic and immune-deficient phenotype of disorder is not yet well understood. Contrary to patients with PGM1-CDG, PGM3-CDG patients show no significant endocrine abnormalities, hypoglycemia, cardiomyopathy, or malformations but do present with significant CNS involvement. Clinical screening for disorders of glycosylation did not show abnormalities in IEF of Tf.

## 4. Defects of Protein O-Glycosylation

Biosynthesis of O-glycans (as well as N-glycans) can be divided into 3 stages: biosynthesis and activation of monosaccharides in the cytoplasm, transport of nucleotide sugar residues into the endoplasmic reticulum (ER) or the Golgi apparatus and attachment of sugar residues to a protein or to a glycan by specific transferases.

O-glycosylation has no processing and thus only consists of assembly that mainly occurs in the Golgi apparatus, contrary to N-glycosylation. O-glycans (O-linked saccharides) in O-glycoproteins are covalently linked to the hydroxyl group of serine or threonine (or hydroxylysine and hydroxyproline) of the protein.

In humans, seven different types of O-glycans are known that are classified on the basis of the first sugar residue attached to amino acid residues. The most common form of O-glycans are the mucin-type O-glycans. In this type, O-glycans are linked via N-acetylgalactosamine (GalNAc), to a hydroxyl group of serine or threonine residues of the protein core and can be further extended with sugar residues including galactose, N-acetylglucosamine, fucose or sialic acid into a variety of different structural core classes.

There are 7 mucin-type core structures distinguished according to the second sugar residue and its sugar residue linkage. Mucins are found in mucous secretions and as membrane glycoproteins of the cell surface.

Another common type of O-glycans are glycosaminoglycans (GAGs) that are a long, unbranched carbo-

hydrate part of proteoglycans. GAGs are attached to the serine of a protein via the linker tetrasaccharide (O-linked xylose-galactose-galactose-glucuronic acid), except for keratan sulfate, which is linked to proteins either through N- or core 1 O-glycans. There are 3 types of GAGs differentiated on the basis of the composition of the disaccharide repeat: dermatan sulfate/ chondroitin sulfate with GlcA and GalNAc, heparin sulfate/heparin with GlcA and GlcNAc and keratan sulfate with Gal and GlcNAc. Many forms of proteoglycans are present in virtually all extracellular matrices of connective tissues.

The less common types of glycoprotein linkages are nonmucin O-glycans, including  $\alpha$ -linked O-fucose,  $\beta$ -linked Oxylucose,  $\alpha$ -linked O-mannose,  $\beta$ -linked O-GlcNAc,  $\alpha$ - or  $\beta$ -linked O-galactose, and  $\alpha$ - or  $\beta$ -linked O-glucose glycans.

O-glycosylation is a very complex process involving a number of enzymes which are encoded by multiple genes. Mutations in these genes are the main cause of enzyme deficiency and lead to defects in the biosynthesis of different types of O-glycans. These defects are responsible for a number of diseases named congenital disorders of O-glycosylation. In contrast to N-glycosylation disorders, clinical manifestation of these disorders is usually limited to one organ or organ system without general symptoms.

These defects concern 8 disorders and are associated with the synthesis of O-xylosylglycans, O-N-acetylgalactosaminylglycans, Oxylsilyl/N-acetylglucosylglycans, and O-fucose glycans.

### 4.1. Defects in O-xylosylglycan synthesis

#### EXT1/EXT2 - CDG

EXT1/EXT2-CDG, also called hereditary multiple osteochondroma (Multiple cartilagenous exostoses) with autosomal dominant inheritance. It is a monosystemic CDG characterized by formation of benign osteochondromas at the ends of long bones in childhood, which, in some cases, can become malignant lesions resulting in osteosarcomas and chondrosarcomas in adult age. Hereditary multiple osteochondromas are usually caused by various mutations in EXT1 or EXT2 genes or in other homologous EXT1 genes. EXT1 and EXT2, are tumor suppressor genes that encode glycosyltransferases involved in heparan sulfate elongation. All members of this multigene family encode glycosyltransferases involved in the adhesion and/or polymerization of heparin sulfate chains at HS proteoglycans. EXT1 and EXT2 form heterooligomeric protein complex, which in Golgi apparatus (GA) catalyses addition of N-acetylglucosamine and glucuronic acid, thus elongating HS-chains.

The development of exostoses is proposed to be mediated by a lack of heparin sulphate proteoglycans, which play a crucial role in the negative feedback loop regulating chondrocyte proliferation and maturation (Duncan G 2001).

With more than 900 affected patients, EXT1/EXT2-CDG represents one of the most frequent CDGs.

#### B4GALT7-CDG

Progeroid variant of Ehlers-Danlos syndrome (EDS), this

defect in beta-1,4-galactosyltransferase 7 has been reported in three patients from two families with a premature aging phenotype, hyperelastic skin, microcephaly, and joint hyperlaxity. The defect disrupts the trisaccharide linker region of glycosaminoglycans (O-linked xylose-galactosegalactose), specifically in the attachment of the first galactose to xylose. The EDS progeroid form is caused by a protein O-glycosylation defect is the result of deficiency in the B4GALT7 gene encoding  $\beta$ -1,4-galactosyltransferase 7. This gene is also identified by the name xylosylprotein 4- $\beta$ -galactosyltransferase (XGALT1 or XGPT1). The proposed CDG nomenclature for the EDS progeroid variant is B4GALT7-CDG.

Laboratory tests for the assessment of thyroid, kidney, liver functions, serum creatine kinase, growth hormone levels are within the reference range. The diagnosis can be performed by the determination of  $\beta$ 4GalT7 activity in human fibroblast and confirmed by the B4GALT7 gene mutations (Cylwik B 2013).

Larsen of Reunion Island syndrome (LRS) include dislocations of large joints with ligamentous hyperlaxity, short stature and characteristic facial features, namely, round flat face, prominent forehead, prominent bulging eyes, under-eye shadows and microstomia). A homozygous p.R270C mutation in B4GALT7 gene caused LRS were reported in 22 patients (Cartaul F 2015).

#### 4.2. Defects in O-N-Acetylgalactosaminylglycan Synthesis

##### GALNT3-CDG

Deficiency of isoform 3 of N-acetylgalactosaminyltransferase causes recurrent, painful calci-fied subcutaneous masses known as familial, hyperphosphatemic tumoral calcinosis (FTC). The hyperphosphatemia is due to increased renal phosphate retention.

The calcium deposits are probably due to the fact that the enzyme GalNAc-T3

uses calcium and manganese as co- factors to catalyze the first reaction in mucin-type O- glycosylation. Laboratory tests show increased serum levels of phosphorus, calcium, active vitamin D, and parathyroid hormone. Radiographs presents osteopenia, patchy sclerosis in the hands, feet, long bones and calvaria, intracranial calcifications. The diagnosis of FTC can be carried out based on the immunostaining of skin biopsy samples with a monoclonal antibody against GalNT3. The recognition can be further confirmed by mutations of the GALNT3 gene (Topaz O 2004).

#### 4.3. Defects in O-Xylosyl/N-Acetylgalactosaminylglycan Synthesis

##### SLC35D1-CDG

This syndrome is caused by loss-of-function mutations of the SLC35D1 gene (1p32-p31), encodes an ER UDPglucuronic acid/UDP-N-acetylgalactosamine dual transporter needed for chondroitin sulfate biosynthesis. Loss-of-function mutations cause Schneckenbecken

dysplasia, a rare, severe skeletal dysplasia comprising mainly platyspondyly, extremely short long bones, and small ilia with snail-like appearance. Less than 20 cases have been reported in the literature so far (Sparrow D.B).

#### 4.4. Defects in O-Mannosylglycan Synthesis

One of the most predominant O-mannosyl glycan structures observed is the O-mannosyl tetrasaccharide (Sia $\alpha$ 3Gal $\beta$ 4GlcNAc $\beta$ 2Man $\alpha$ -Ser/Thr), which was first identified on  $\alpha$ -dystroglycan ( $\alpha$ -DG) purified from bovine peripheral nerve tissue.  $\alpha$ -DG is an integral glycoprotein of the dystrophin-glycoprotein complex. It connects the actin cytoskeleton with the extracellular matrix by interacting with ECM (extracellular matrix) proteins such as laminin in a glycosylation-dependent manner. Disruptions in the O-mannosylation pathway that lead to hypoglycosylation of  $\alpha$ -DG are causative for several forms of congenital muscular dystrophy.

DG consists of two sub-units ( $\alpha$ -DG and  $\beta$ -DG). The  $\beta$ -subunit is a transmembrane protein that interacts with dystrophin and utrophin serving to connect the extracellular protein to the actin cytoskeleton.  $\alpha$ -DG is an extensively O-glycosylated membrane protein that is predicted to have a molecular weight of ~72 kDa. However, due to extensive glycosylation,  $\alpha$ -DG is more commonly observed as a diffuse set of bands ranging from 150 to 200 kDa when separated by SDS-PAGE. DK expressed in muscle, brain, and other tissues.

Classical O-mannosyl glycan structures on  $\alpha$ -DG were thought to be necessary for  $\alpha$ -DG to bind to extracellular ligands such as laminin, agrin, and perlecan.

Duchenne's muscular dystrophy (MD) is linked to mutations within dystrophin and accounts for approximately 95 % of muscular dystrophy cases. Aberrant glycosylation of  $\alpha$ -DG has been associated with numerous forms of muscular dystrophy that have been dubbed the dystroglycanopathies. This large subset of congenital muscular dystrophy (CMD) ranges in phenotype from mild muscle wasting and basement membrane separation to severe muscle wasting and mental retardation.

Mutations in known and putative glycosyltransferases that have been associated with defects in proper glycosylation of  $\alpha$ -DG include POMT1, POMT2, POMGnT1, LARGE, Fukutin, Fukutin-related protein, and ISPD.

##### 4.4.1. POMT1/POMT2

Protein O-mannosyltransferase 1 (POMT1) is the first protein involved in the mammalian O-mannosylation pathway. POMT1 and POMT2, a closely related protein, are type III transmembrane glycosyltransferases that co-localize in the endoplasmic reticulum. Together they catalyze the O-linked addition of a mannose from a dolichol-linked precursor onto a serine or threonine residue of a polypeptide. Of all diseases with molecular foundations in the mutation of POMT1, Walker-Warburg syndrome (WWS) is the most commonly observed. WWS is a recessive disorder that presents with a

severely affected physiological and anatomical phenotype, characterized by brain and eye involvement associated with congenital muscular dystrophy. The brain lesions consist of “cobblestone” lissencephaly, agenesis of the corpus callosum, cerebellar hypoplasia, hydrocephaly, and sometimes encephalocoele. This disease usually runs a fatal course before the age of 1 year. In this disorder there is an aberrant glycosylation of  $\alpha$ -DG. Infants diagnosed with WWS rarely live past 12 months of age. Some patients with WWS have mutations in the protein O-mannosyltransferase 2 gene (POMT2), in the fukutin gene, or in the fukutin-related protein gene (Buyse K 2013).

#### 4.4.2. *POMGnT1*

Human protein O-linked mannanose  $\beta$ -1,2 N-acetylglucosaminyltransferase, also known by its acronym POMGnT1, is a type II transmembrane glycosyltransferase that is found in the GA. POMGnT1 is expressed in a variety of mammalian tissue types, most prominently in skeletal muscle, brain tissue, and the eyes. After POMT1 adds the O-mannose structure, POMGnT1 catalyzes the extension of the reducing-end mannanose with the addition of a  $\beta$ -1,2 N-acetylglucosamine (GlcNAc). Additionally, this enzyme is essential for building the classical and the  $\beta$ -1,2/ $\beta$ -1,6 branched structures primarily only observed in neural tissue. The disease most often associated with mutation of the POMGnT1 gene is muscle-eye-brain disease (MEB). The clinical phenotype of MEB largely mirrors that of WWS; however, the phenotype of MEB is not as severe as WWS. The three major characterizing features of MEB are congenital muscular dystrophy, ocular abnormalities, and type II lissencephaly. Although these features are very similar to those of WWS, the life expectancy of a child born with MEB is 6–12 years, and in some cases even as high as 16 years; this is significantly longer than WWS patients (Buyse K 2013).

#### 4.4.3. *Fukutin*

Fukuyama congenital muscular dystrophy (FCMD) is largely caused by mutations in the fukutin gene, which codes for the putative glycosyltransferase fukutin result in a hypoglycosylated non-functional  $\alpha$ -DG. FCMD is very prevalent in Japanese populations, with a carrier frequency of 1 in 88. Mutations in the fukutin gene have also been detected in patients showing a wide range of variability in dystroglycanopathy disease phenotypes; also including WWS, MEB, and a variety of limb-girdle muscular dystrophies (Reed UC 2009).

#### 4.4.4. *FKRP*

Fukutin-related protein (FKRP) is expressed in a wide range of tissues with highest levels in the skeletal muscle, placenta and heart. Mutations in *FKRP* were originally identified in a form of CMD and in a clinically-defined group of limb girdle muscular dystrophy patients. The exact biochemical function of FKRP is not well characterized and may relate to the modification and possibly glycosylation of  $\alpha$ -DG (Reed UC 2009).

#### 4.4.5. *LARGE*

Studies indicate that like-acetylglucosaminyltransferase (LARGE) modifies O-linked mannosyl glycans, complex N-, and mucin O-glycans, and involved in extension of an unidentified phosphoryl glycosylation branch on O-linked mannanose (Zhang Y). LARGE encodes the glycosyltransferase that adds the final xylose and glucuronic acid, allowing  $\alpha$ -dystroglycan to bind ligands, including laminin 211 and neurexin. Only 11 patients with LARGE mutations have been reported (Meilleur KG 2014).

#### 4.4.6. *Isoprenoid Synthase Domain Containing*

Patients from family with Isoprenoid synthase domain containing (ISPD) mutations presented with hypotonia and delayed motor milestones at 4 months of age. ISPD probably acts as a nucleotidyltransferase involved in synthesis of a nucleotide sugar, required for dystroglycan O-mannosylation. Mutations in ISPD cause WWS and defective glycosylation of DG. Also recently, mutations in the ISPD gene have been reported as a common cause of CMD and LGMD. All affected individuals had a severe phenotype, with cobblestone lissencephaly, hydrocephalus, cerebellar hypoplasia, and hypoplasia of the corpus callosum, as well as eye abnormalities. Most died by age 2 years (Roscioli T 2012, Baranello G 2014).

#### 4.5. *O-Fucosylglycan Synthesis*

##### Notch-Related O-Fucose Glycosylation

The Notch signaling pathway is a highly conserved cell signaling system present in most multicellular organisms. Notch and most of its ligands are transmembrane proteins, so the cells expressing the ligands typically must be adjacent to the notch expressing cell for signaling to occur. The notch ligands are also single-pass transmembrane proteins and are members of the DSL (Delta/Serrate/LAG-2) family of proteins. In mammals there are multiple Delta-like and Jagged ligands, as well as possibly a variety of other ligands, such as F3/contactin. The notch extracellular domain is composed primarily of small cystine knot motifs called EGF-like repeats. Each EGF-like repeat can be modified by O-linked glycans at specific sites. These sugars are added by an as-yet-unidentified

O-glycosyltransferase, and GDP-fucose Protein O-fucosyltransferase 1 (POFUT1), respectively. The addition of O-fucose by POFUT1 is absolutely necessary for notch function, and, without the enzyme to add O-fucose, all notch proteins fail to function properly.

##### 4.5.1. *Dowling-Degos Disease*

Dowling-Degos disease-2 is caused by mutation in the POFUT1 gene on chromosome 20q11. It is a rare autosomal-dominant skin disorder, individuals with Dowling-Degos disease develop a postpubertal reticulate hyperpigmentation that is progressive and disfiguring, and small hyperkeratotic dark brown papules that affect mainly the flexures and great skin folds. Pitted perioral acneiform scars and genital and perianal reticulated pigmented lesions

have also been described. Patients usually show no abnormalities of the hair or nails. Histology shows filiform epithelial downgrowth of epidermal rete ridges, with a concentration of melanin at the tips (Basmanav FB).

#### 4.5.2. *Adams-Oliver Syndrome 4*

Adams-Oliver syndrome 4 (AOS4) caused by mutation in the EOGT gene on chromosome 3p14. EOGT functions as an O-GlcNAc transferase, Eogt utilized uridine diphosphate (UDP)-GlcNAc as a sugar donor to transfer GlcNAc to a conserved threonine residue within the EGF-like domain of Notch. Mutation in the EOGT gene cause aplasia cutis congenita and terminal transverse limb defects. Autozygosity mapping of five individuals from multiple consanguineous families revealed the presence of homozygous frameshift, deletion, or missense mutations in EOGT. Eogt loss causes a deficiency in cell-cell or cell-matrix interactions or in Notch-related signaling (Stittrich AB 2014).

#### 4.5.3. *SCDO3-CDG*

Spondylocostal dysostosis type 3 is a Notch pathway defect in lunatic fringe, an O-fucose-specific  $\beta$ 1,3- $\alpha$ -N-acetylglucosaminyltransferase, this leads to elongation of O-linked fucose residues on Notch, which alters Notch signaling. This gene is a member of the fringe gene family which also includes radical and manic fringe genes. They all encode evolutionarily conserved glycosyltransferases that act in the Notch signaling pathway to define boundaries during embryonic development. Mutations in this gene have been associated with autosomal recessive spondylocostal dysostosis 3.

Patients show a severe vertebral phenotype with malsegmentation due to disruption of somitogenesis (Sparrow D.B 2006).

#### 4.5.4. *B3GALTL-CDG*

This so-called Peters'plus syndrome is an autosomal recessive disorder characterized by a variety of anterior eye-chamber defects, of which the Peters anomaly occurs most frequently. Other major symptoms are a disproportionate short stature, developmental delay, characteristic craniofacial features, and cleft lip and/or palate. Mutations are in a  $\beta$ 1,3-glucosyltransferase that adds glucose to O-linked fucose. This disaccharide modification is specific to thrombospondin type 1 repeats, found in extracellular proteins that function in cell-cell and cell-matrix interactions (Faletra F 2011).

## 5. Diagnostics of CDG

Pathological changes of the common biochemical tests may be found as a consequence of defective pathways of protein glycosylation. Abnormal liver function tests, low plasma cholesterol and cholinesterase activity with proteinuria are common findings in patients with CDG type Ia. Frequently found hypoalbuminemia, hypoglycaemia with inadequately increased insulin production, and high activities of aminotransferases, are typical for CDG Ib. Conversely,

proteinuria is absent in CDG type II (Keir G 1999).

The levels of plasma glycoproteins, including transport proteins, e.g.  $\alpha_1$ -antitrypsin ( $\alpha_1$ -AT), thyroxin-binding globulin (TBG), Tf, glycoprotein hormones, coagulation and anticoagulation factors (particularly the factors V, XI, II, X, AT III), proteins C, S and heparin cofactor II are usually low, while the level of fibrinogen D-dimer is frequently raised.

#### *Screening Tests for CDG*

Most CDGs are associated with at least in some extent by changes of glycosylation. A large number of serum glycoproteins have been shown to have abnormal IEF pattern. The common diagnostic test for CDG is IEF of serum Tf and ApoC-III for N- and O-glycan synthesis defects, respectively; (Stibler H 1998, Wopereis S 2003, Albahri Z 2005).

High-performance liquid chromatography (HPLC) and capillary zone electrophoresis (CZE) have been applied for diagnostics of CDG.

N-glycosylation defects can be divided into two main groups, CDG-I and CDG-II. CDG-I are defects in the assembly of a precursor, consisting of 14 oligosaccharides, on the lipid carrier dolichol or in the transfer of this precursor from dolichol to the NH<sub>2</sub> group of an asparagine of a nascent protein. CDG-II comprises defects in the processing of this precursor into a complex type N-glycan. During this processing, monosaccharides are sequentially removed and added by specific enzymes. IEF of serum Tf shows a so-called type 1 pattern in CDG-I, and in CDG-II often a type 2 pattern.

Analysis of other serum glycoproteins, e.g.  $\alpha_1$ -AT may help in documentation of generalized glycosylation defect in the patient

Some CDG types cannot be identified by Tf IEF analysis because in some of them Tf sialylation is not altered e.g. (CDG-IIb, CDG-IIc, IIc). Even some CDG-Ia patients might be missed by the IEF Tf test (Marklova E 2007, Marquardt T 2003).

Thin-layer chromatography (TLC) of urine oligosaccharides is the method of choice in the screening of CDG-IIb. Sialyl Lewis X antigen is absent on the neutrophils in CDG IIc and IIc, which also shows the Bombay blood group phenotype (Lübke T 2001, Marquardt T 2003, Marklova E 2004).

For diagnostics of the other glycosylation defects, in addition to a careful personal, family history and physical examination, a number of tests (Creatine Phosphokinase, Aldolase, SGOT and SGPT) point to the evidence of muscle damage. An EMG shows abnormal muscle function. Muscle biopsy is very important to establish the diagnosis of MEB and WWS.

The mannosylation and fucosylation related disease are not detectable by IEF of Tf / Apoc3. Moreover, electrophoretic analysis of  $\alpha$ -DG in skeletal muscle may be helpful for detection of some O-glycosylation defects (Marquardt T 2003).

The  $\alpha$ -dystroglycanopathies can be investigated by measurement of monoclonal antibodies to the O-mannosylated glycan in muscle biopsy samples.

The diagnosis of HME is based on clinical and/or radiographic findings of multiple exostoses in one or more members of a family. Sequence analysis of the EXT1 gene and the EXT2 gene is available.

Additional analysis of the glycan structure by MALDI-TOF mass spectrometry of serum Tf and/or total serum distinguishes defects in branching, demannosylation, galactosylation, sialylation and fucosylation. Based on the glycan structure, a hypothesis can be made on the possible defect. O-glycosylation can be checked by IEF of apoC-III, an O-glycosylated protein. Additional improvement of CDG diagnostics was achieved by the employment of mass spectrometric (MS) analyses. Because of high specificity and sensitivity of MS, and possibility to be fully automated, different kinds of MS found the application in CDG diagnostics, e.g. ESI-MS (electrospray ionization MS) of Tf, MALDI-TOF MS (matrix-assisted laser desorption/ionization time-of-flight MS) of Tf and  $\alpha$ -1-antitrypsin as well as isolated serum N-linked and O-linked glycans.

Nuclear magnetic resonance spectroscopy can determine the glycan structures and molecular mass of the glycovariants (Coddeville B 1998). Such glycan structure analysis may be instrumental for the elucidation of CDG-x cases, by pinpointing candidate enzymes and genes responsible for the abnormal glycan synthesis.

Specific diagnosis of all these disorders is made after genetic defect identification.

Over 100 mutations are known on PMM2, enzymatic activity of PMM2 in fibroblasts or leukocytes should be the first choice when CDG is suspected, since PMM2-CDG is the most frequent CDG. Normal activities of the mentioned enzyme indicate further analysis of the lipid-linked oligosaccharides (LLO) in fibroblasts or other assays to identify known or unknown CDG defect. Whole-exome sequencing led to the identification of defects in many different CDG-I genes (Timal S 2012). Molecular basis of most of all known CDGs has been elucidated.

## 6. CDG Therapy

The therapy for only three (MPI-CDG (*CDG-Ib*), SLC35C1-CDG (*CDG-IIc*) and PIGM-CDG) of almost known CDG defects is available so far. Yet, lot of efforts is putting in mouse models which were shown to be very useful not only in the studies of molecular basis of these diseases, but also in the therapeutic studies.

Unfortunately, an efficient treatment is still not available for the CDG-Ia patients. Moreover, any postnatal therapy of CDG-Ia would be difficult: one reason is the prenatal onset of CDG-Ia, demonstrated by the presence of dysmorphic features and neurological dysfunction at birth. On the other hand, the normal foetal growth and the failure to detect hypoglycosylation of Tf in CDG-Ia prenatally suggest that maternal compensation and/or a developmentally regulated alternate pathway may bypass PMM deficiency “in-utero”. Presently, the treatment offered to patients with CDG-Ia remains only supportive.

It was reported that mannose supplementation results in an increased incorporation of mannose in patient's fibroblasts (Panneerselvam K 1997), but mannose administration to CDG-Ia patients did not improve the clinical or biochemical features (Mayatepek E 1997, 1998).

Providing PMM-deficient cells with Man-1-P may be a way to increase the GDP-mannose pool, but Man-1-P is not able to penetrate cell membranes due to its high polarity (Rutschow S 2002).

The biguanide drug metformin corrected experimentally induced deficiencies in the synthesis of Glc<sub>3</sub>Man<sub>9</sub>GlcNAc<sub>2</sub>-P-P-dolichol and N-linked glycosylation. Metformin stimulates AMP-activated protein kinase, a master regulator of cellular energy metabolism, and it activates a novel fibroblast mannose-selective transport system. This suggests that AMP-activated protein kinase may be a regulator of mannose metabolism, thus implying a therapy for CDG-Ia (Shang J 2004).

Enzyme replacement is unequal accessibility to cells, especially CNS; and would not cross blood brain barrier, requires cytoplasmic targeting. MPI inhibition increase the Man-6-P flux toward glycosylation by reducing MPI activity increasing PMM2, disadvantages of MPI inhibition may not be effective in all tissues; likely to benefit those with higher residual activity (Freeze H.H 2012).

PMM2 activation with small molecule activates or stabilizes mutant enzyme and increasing its activity, but may not be useful for all mutant genotypes; will depend on whether specific mutation affects enzyme stability, Km, substrate binding or transcription.

Results in a hypomorphic mouse model for PMM2-CDG might give hope for a future therapy for women at risk for a PMM2-CDG child. After feeding pregnant dams with mannose, the lethality of compound-heterozygous embryo was overcome and normal life was possible thereafter, indicating that mannose treatment in the patients might have been started too late (Thiel C 2012).

CDG-Ib was the first disorder of glycosylation where a specific therapy was available. Symptoms can be effectively reduced with the oral mannose administration (Niehues R 1998). Oral mannose supply bypasses the enzymatic block using alternative way catalysed by hexokinase and leads to the significant metabolic normalization and disappearance of symptoms. Mannose also normalizes hypoproteinemia, blood coagulation and effectively treats the symptoms of CDG-Ib like protein-losing enteropathy and hypoglycaemia, with such therapy patients usually can live normally. Significant improvement of the Tf IEF pattern during mannose therapy takes several months of treatment to occur (De Lonlay P 1999, Niehues R 1998, Thiel C 2012).

Despite the successful correction of immunodeficiency-related defects in CDG IIc (LAD II), correction of the delayed psychomotor development was expected to be more difficult to achieve. However, the patient showed significant psychomotor improvement while on fucose therapy. In some patients, increased level of fucose achieved by oral supplementation might overcome low



affinity of the fucose transporters and in that way result in clinical improvements. However, the observations that fucose treatment did not have the effect in some other cases, suggest that the effectiveness of fucose therapy depends on the nature of the mutation (Goreta S 2012).

#### PIGM-CDG

Constitutional mutation in the promoter of a housekeeping gene *PIGM* causes histone hypoacetylation and disruption of binding of SP1 transcription factor, resulting in deficiency of the first mannosyltransferase in the GPI-anchor biosynthesis pathway and consequently low glycosylphosphatidylinositol content. PIGM-CDG is characterized by splanchnic vein thrombosis and epilepsy. Treatment with a histone deacetylase inhibitor, butyrate, was proposed as an effective therapy for PIGM-CDG in vitro as well as in vivo, since it was shown to increase PIGM transcription and GPI expression, and is able to cause complete cessation of intractable seizures on one PIGM-CDG patient. It may be an effective therapeutic option for other diseases caused by Sp1-dependent hypoacetylation, so further investigations are needed.

Unfortunately, the successful therapy for PMM2-CDG, the most prevalent CDG is not yet available, although many attempts to design the effective therapeutic approach have been undertaken. One of the examples is the application of cell permeable mannose-1-phosphate derivatives that succeeded to restore glycosylation to normal levels, but the half-life of these derivatives was too short. In addition, zaragozic acid A, a squalene synthase inhibitor, was shown to be able to improve protein N-glycosylation, by redirecting the flow of the polyisoprene pathway toward dolichol by lowering cholesterol biosynthesis. As mentioned before, deficiency of phosphomannomutase 2 and mannosephospho isomerase (MPI-CDG) reduces the metabolic flux of mannose-6-phosphate (Man-6-P), which results in impaired N-glycosylation. Both enzymes compete for the same substrate, Man-6-P. Mannose supplementation reverses most of the symptoms of MPI-CDG patients, but has no effect on PMM2-CDG patients because Man-6-P is catabolized by MPI. It was recently proposed that inhibition of MPI activity might provide more Man-6-P for glycosylation and possibly help PMM2-CDG patients with residual PMM2 activity. Application of a potent MPI inhibitor from the benzoisothiazolone series successfully diverted Man-6-P towards glycosylation in various cell lines including fibroblasts from PMM2-CDG patients and improved N-glycosylation. Hopefully, this novel therapeutic approach will be also effective in clinical trials and beneficial for at least a subset of PMM2-CDG patients.

The sugar crosses the blood-brain barrier, resulting in elevated free fucose levels in the CSF during therapy. Whether or not fucosylation of glycoproteins produced in the CNS and found in the CSF is influenced by fucose therapy is a topic of further investigation. (Marquardt T 1999). In different mouse lines showed that by adenoviral-transmitted gene transfer of Large, expression of the protein in Large-deficient mice or an upregulation of Large expression

in fukutin- and PomGnT1-deficient mouse lines was achieved, respectively.

This led to enhancement of the glycosylation status of alpha-dystroglycan and thus to a decrease in muscle disorder (Thiel C 2012).

There are no causal therapeutic options for the other CDG types and various O- glycosylation defects; treatment varies widely depending on the exact diagnosis.

Studies with a ketogenic diet in CDG-Ia are ongoing. The rationale for this treatment is the observation, that glucose starvation improves N-glycosylation in fibroblasts from CDG-Ia patients (Körner C 1998).

As to symptomatic treatment, prevention of stroke-like events by using 0.5 mg acetylsalicylic acid / kg per day is recommended. Also, bisphosphonates should be considered in patients with recurrent fractures (Grünwald S 2000). Oestradiol therapy has induced growth of breast tissue and pubic hair in two Danish females (Kjaergaard S 2001).

Most types of CDG have failure to thrive as one of their major medical problems. These children can be nourished with any type of formula for maximal caloric intake although early in life they seem to do better on elemental formulas. This diagnosis is not associated with any dietary restrictions; they can tolerate carbohydrates, fats and protein.

A developmental delay is typically recognized in CDG patients around four months of age. At this point early intervention with occupational therapy, physical therapy and speech therapy should be instituted.

Many patients with CDG have low levels of factors in the coagulation cascade. The clinical importance of this rarely manifests in every day activities, but must be acknowledged if an individual with CDG undergoes surgery. Consultation with a haematologist to document the coagulation status and factor levels of the patient and to discuss with situation with the surgeon is important. Infusion of fresh frozen plasma corrects the factor deficiency and clinical bleeding when indicated.

Seizures-Children with CDG-Ia may have seizures in their 2nd or 3rd year of life which are easily controlled with medication.

Appropriate orthopaedic management for thorax shortening, scoliosis/kyphosis, wheel chairs, appropriate transfer devices for the home, and continued physical therapy to prevent contractures is important.

Occupational therapy, physical therapy, and speech therapy should be instituted. As the developmental gap widens between children with CDG and their unaffected peers, parents need continued counseling and support.

## 7. Conclusions

CDG constitute a rapidly growing disease family due to genetic defects in the glycosylation pathway of proteins and lipids, a novel nomenclature and classification of CDG were developed. 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. CDG should be

suspected and screened in any child with a multisystem disease, especially in combination with neurologic symptoms. Most individuals with a N-glycosylation disorders are diagnosed because of an abnormal Tf IEF test. However, not all these types are characterized by an abnormal IEF of Tf. Moreover, abnormal Tf results can resolve with age, particularly after infancy, such patients can only be diagnosed via the identification of pathogenic mutations in glycosylation-related genes.

Clinical features of O-glycosylation disorders are usually limited to one organ or organ system without general symptoms. The diagnostics include a syndromic presentation and organ-specific expression of the disease and laboratory findings. Most of these defects have been found by genetic approaches.

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