

20.1 Introduction

Congenital defects of glycosylation (CDG), formerly called carbohydrate deficient glycoprotein syndromes, are genetic diseases due to deficient glycosylation of glycoconjugates, particularly glycoproteins. The glycans on proteins are either N-linked or O-linked. The large majority of known CDG in man are N-glycosylation defects. This chapter is entirely devoted to primary N-glycosylation defects excluding disorders of phosphate and sulphate modifications of N-glycans. The N-glycosylation pathway embraces three cellular compartments: the cytosol, the endoplasmatic reticulum (ER) and the Golgi. It starts in the cytosol with the formation of the mannose donor GDP-mannose from fructose 6-phosphate, an intermediate of the glycolytic pathway. In the ER, the dolichol-linked oligosaccharide $\text{GlcNAc}_2\text{-Man}_9\text{Glc}_3$ is assembled and transferred to selected asparagines of the nascent proteins. Still in the ER this glycan starts to be processed by trimming off the three glucoses. This processing is continued in the Golgi by trimming off six mannoses and replacing them by two residues each of N-acetylglucosamine, galactose and finally sialic acid.

Most CDG are multisystem diseases comprising more or less severe brain involvement [1]. The first patients with a disease subsequently shown to be a CDG were reported in 1980 [2]. Seven disorders in N-glycan synthesis are known: five in the N-glycan assembly (phosphomannomutase [PMM2] deficiency (CDG-Ia), phosphomannose isomerase [PMI] deficiency (CDG-Ib), glucosyltransferase I [GT I] deficiency (CDG-Ic), mannosyltransferase VI [MT VI] deficiency (CDG-Id), and dolichol-phosphate-mannose [DPM] synthase-1 deficiency (CDG-Ie), and two in the glycan processing (N-acetylglucosaminyltransferase II [GnT II] deficiency (CDG-IIa), and glucosidase I [G I] deficiency (CDG-IIb).

CDG-Ia patients (some 300 known worldwide) show mild to severe neurological disease and variable involvement of many organs. Dysmorphism ranges from mild and aspecific to a characteristic abnormal subcutaneous adipose tissue distribution with fat pads and nipple retraction. Mortality is about 20% in the first years of life [3–5].

CDG-Ib has been reported in at least 16 patients. It is a hepatic-intestinal disease with liver fibrosis and protein-losing enteropathy and can be associated with coagulation disturbances, hyperinsulinemic hypoglycemia and/or prolonged episodic vomiting [6–8].

CDG-Ic is mainly a neurological disease but milder than CDG-Ia. Some 30 patients are known to the authors [9, 10].

CDG-Id has only been reported in 1 patient with an extremely severe encephalopathy and structural eye and brain abnormalities [11].

CDG-Ie was identified in 4 children with pronounced encephalopathy, failure to thrive and mild dysmorphism [12, 13].

CDG-IIa has been reported in 3 patients with severe mental retardation and a characteristic, mainly facial dysmorphism [14, 15].

CDG-IIb was identified in a neonate with dysmorphism, hypotonia, seizures and fatal outcome at 2.5 months [16].

The most widely used diagnostic test for CDG is isoelectrofocusing of serum sialotransferrins. Patients with CDG-I show a type 1 pattern (cathodal shift with increase of di- and asialotransferrin) while patients with CDG-IIa show a type 2 pattern (increase of tri- and monosialotransferrin). The patient with CDG-IIb showed a normal pattern. This test is also normal in those CDG not accompanied by a deficiency of sialic acid. An efficient treatment, oral mannose, is available only for CDG-Ib [6].

20.2 Nomenclature

No.	Disorder	Tissue distribution	Chromosomal localisation	MIM
20.1	PMM2 deficiency (CDG-Ia)	WBC, FB	16p13	212065 601785
20.2	PMI deficiency (CDG-Ib)	WBC, FB	15q22	154550 602579
20.3	GT I deficiency (CDG-Ic)	FB	1p22.3	603147 604566
20.4	MT VI deficiency (CDG-Id)	FB	–	–
20.5	DPM synthase-1 deficiency (CDG-Ie)	FB	20q13	603503
20.6	GnT II deficiency (CDG-IIa)	LYM, FB	14q21	212066 602616
20.7	G I deficiency (CDG-IIb)	FB	2p2–13	601336

For abbreviations see legend to Fig. 20.1.

20.3 Metabolic Pathway

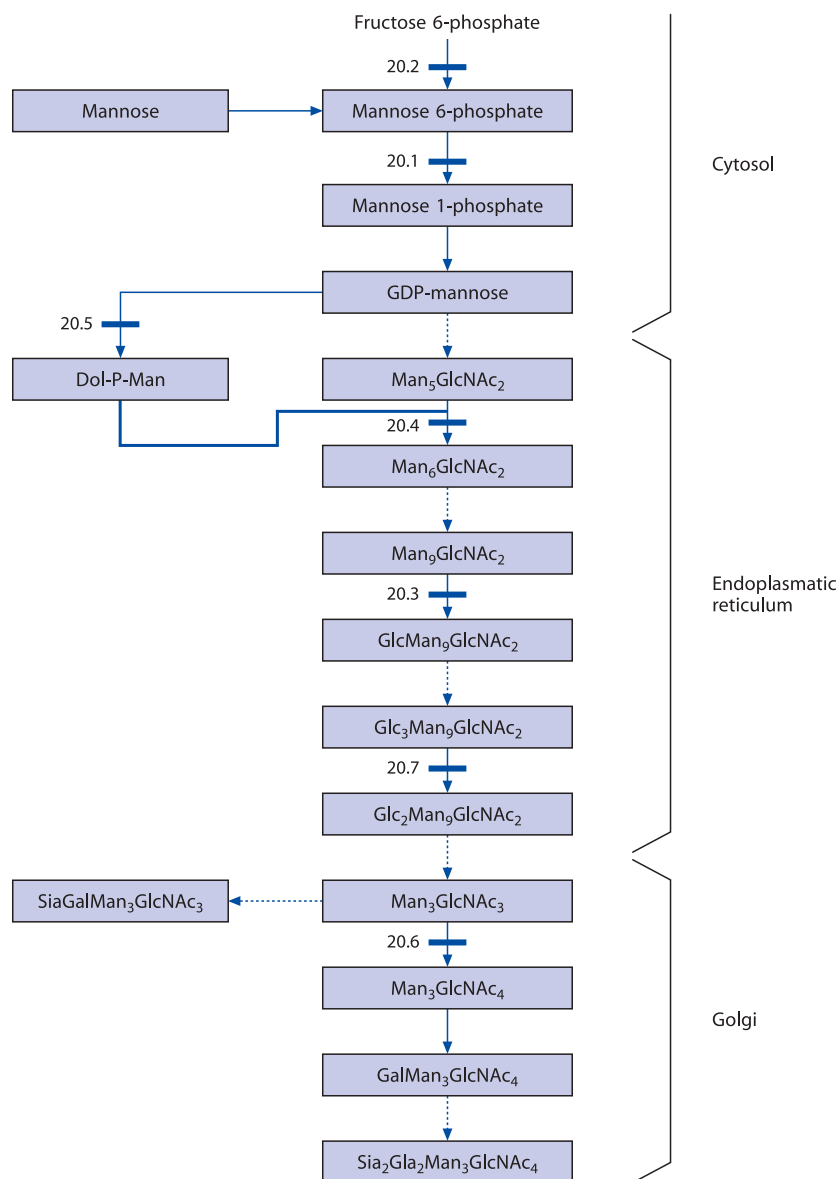


Fig. 20.1. Abbreviated scheme of the synthesis of a diantennary N-glycan. Fucose modification has been omitted. 20.1, Phosphomannomutase 2 (PMM2); 20.2, phosphomannose isomerase (PMI); 20.3, glucosyltransferase I (GT I); 20.4, mannosyltransferase VI (MT VI); 20.5, dolichol phosphate-mannose synthase-1 (DPM synthase-1); 20.6, N-acetylglucosaminyltransferase II (GnT II); 20.7, glucosidase I (G I); *GDP*, guanosine diphosphate; *Man*, mannose; *GlcNAc*, N-acetylglucosamine; *Dol*, dolichol; *P*, phosphate; *Glc*, glucose; *Gal*, galactose; *Sia*, sialic acid. Dotted arrow indicates multiple steps

20.4 Signs and Symptoms

Table 20.1. Phosphomannomutase 2 deficiency (CDG-Ia)

System	Symptoms/markers	Neonatal	Infancy	Childhood	Adolescence	Adulthood
Characteristic clinical findings	Abnormal subcutaneous fat distribution	++	++	++	+	+
Routine laboratory	Thyroxin-binding globulin (S)	↓	↓	↓	↓	↓
	Antithrombin (B)	↓	↓	↓	↓	↓
	Other glycoproteins (S, B)	↓, n or ↑	↓, n or ↑	↓, n or ↑	↓, n or ↑	↓, n or ↑
Special laboratory	Sialotransferrins (S) cathodal shift (type 1 pattern)	++	++	++	+	-/+
CNS	Cerebellar atrophy	+	+	+	+	+
	Psychomotor retardation	++	++	++	++	++
	Hypotonia	++	++	++	+	+
	Squint	+	+	+	+	+
	Strokes	±	±	+	+	±
	Convulsions	±	±	±	±	±
Peripheral nervous system	Hypomyelination	+	+	+	+	+
	Hyporeflexia	±	+	+	+	+
Eyes	Retinitis pigmentosa	±	+	+	+	+
GI	Hepatomegaly	+	+	-	-	-
	Anorexia	±	±	-	-	-
	Vomiting	±	±	-	-	-
	Diarrhea	±	±	-	-	-
Skeletal system	Osteopenia	±	±	+	++	++
	Thorax and vertebral column abnormalities	±	±	+	+/++	+/++
Genitourinary	Enlarged kidneys	±	±	±	±	±
	Proteinuria	+	+	+	+	+
Gonads	Hypogonadism in females				+	+
Cardiovascular system	Cardiomyopathy	±	±	-	-	-
	Pericardial effusion	±	±	±	-/+	-

Table 20.2. Phosphomannose isomerase deficiency (CDG-Ib)

System	Symptoms/markers	Neonatal	Infancy	Childhood
Characteristic clinical findings	Protein-losing enteropathy	–	+ /+++	+ /+++
	Hepatic fibrosis	–	+	+
Routine laboratory	Thyroxin-binding globulin (S)	↓	↓	↓
	Antithrombin (B)	↓	↓	↓
Special laboratory	Other glycoproteins (S, B)	↓, n or ↑	↓, n or ↑	↓, n or ↑
	Sialotransferrins (S) cathodal shift (type 1 pattern)	+	+	+
CNS	Psychomotor retardation	±	±	±
	Hypotonia	±	±	±
	Thromboses	–	+	+
GI	See under “Characteristic clinical findings”			

Table 20.3. Glucosyltransferase I deficiency (CDG-Ic)

System	Symptoms/markers	Neonatal	Infancy	Childhood
Characteristic clinical findings	Axial hypotonia	++	++	+
	Epilepsy	+	++	+
Routine laboratory	Transaminases (S) (during infection)	+	+	+
	Cholesterol (S)	↓/↓↓	↓/↓↓	↓/↓↓
Special laboratory	Factor XI (B)	↓↓	↓↓	↓↓
	Other glycoproteins (S, B)	↓, n or ↑	↓, n or ↑	↓, n or ↑
Special laboratory	Sialotransferrins (S) cathodal shift (type 1 pattern)	+	+	+
	Lipid-linked Man ₉ GlcNAc ₂ (FB)	↑	↑	↑
CNS	Psychomotor retardation	+ /+++	+ /+++	+ /+++
	Strabismus	++	++	++
	Ataxia	–/+	–/+	–/+

Table 20.4. Mannosyltransferase VI deficiency (CDG-Id)

System	Symptoms/markers	Neonatal	Infancy
Characteristic clinical findings	Iris coloboma	+	+
	Optic atrophy	+	+
	Corpus callosum atrophy	+	+
Routine laboratory	Antithrombin (B)	↓	↓
	Apolipoprotein B (S)	↓	↓
Special laboratory	Lipid-linked Man ₅ GlcNAc ₂ (FB)	↑	↑
	Sialotransferrins (S) cathodal shift (type 1 pattern but no increased asialotransferrin)	+	+
CNS	Psychomotor retardation	+++	+++
	Hypsarrhythmia	+++	+++
	Postnatal microcephaly	+	+

Table 20.5. Dolichol phosphate-mannose synthase-1 deficiency (CDG-Ie)

System	Symptoms/markers	Neonatal	Infancy	Childhood
Characteristic clinical findings	Epilepsy	–	++	++
	Hypotonia	–	++	++
Routine laboratory	Transaminases (S)	+ / ++	+ / ++	+ / ++
	Creatine kinase (S)	+ / ++	+ / ++	+ / ++
Special laboratory	Factor XI (B)	↓↓	↓↓	↓↓
	Other glycoproteins (S, B)	↓, n or ↑	↓, n or ↑	↓, n or ↑
	Sialotransferrins (S) cathodal shift (type 1 pattern)	+	+	+
	Lipid-linked Man ₉ GlcNAc ₂ (FB)	↑	↑	↑
CNS	Microcephaly	– / +	– / +	– / +
	Psychomotor retardation	+++	+++	+++
Skeletal system	Dysmorphism	+ / ++	+ / ++	+ / ++

Table 20.6. N-Acetylglucosaminyltransferase II deficiency (CDG-IIa)

System	Symptoms/markers	Neonatal	Infancy	Childhood	Adolescence
Characteristic clinical findings	Facial dysmorphism	++	++	++	++
Routine laboratory	AST (S)	↑	↑	↑	↑
	Factor XI (B)	↓↓	↓↓	↓↓	↓↓
Special laboratory	Other glycoproteins (S, B)	↓, n or ↑	↓, n or ↑	↓, n or ↑	↓, n or ↑
	Sialotransferrins (S) cathodal shift (type 2 pattern)	++	++	++	++
CNS	Psychomotor retardation	++	++	++	++
	Epilepsy	– / ++	– / ++	– / ++	– / ++
	Abnormal behaviour	– / +	– / +	– / +	– / +
GI	Volvulus of the stomach	– / +	– / +	– / +	– / +
	Chronic diarrhoea	– / +	– / +	– / +	– / +
Cardiovascular system	Ventricular septal defect	– / +	– / +	– / +	– / +
Skeletal system	Short neck	– / +	– / +	– / +	– / +
	Kyphoscoliosis	– / +	– / +	– / +	– / +

Table 20.7. Glucosidase I deficiency (CDG-IIb)

System	Symptoms/markers	Neonatal	Infancy
Characteristic clinical findings	Craniofacial dysmorphism	++	++
Routine laboratory	AST (S)	↑	↑
	Thin-layer chromatography oligosaccharides (U)	++	++
Special laboratory	Hexosaminidase	-/+	-/+
	Isoelectrofocusing		
	Cathodal shift (S)		
	Tetrasaccharide (U)	+	+
CNS	Psychomotor retardation	++	++
	Hypotonia	++	++
	Epilepsy	++	++
PNS	Demyelinating polyneuropathy	+	+
Skeletal system	Thoracic scoliosis	+	+
Respiratory system	Respiratory insufficiency	+	+
GU	Hypoplastic genitalia	+	+

20.5 Reference Values

■ Serum Sialotransferrins (isoelectrofocusing) (centile values: P3–P97, $n=96$; all ages)

Monosialotransferrins	0.0–3.7
Disialotransferrins	2.0–6.1
Trisialotransferrins	5.5–15.1
Tetrasialotransferrins	48.5–65.3
Pentasialotransferrins	14.9–28.7
Hexasialotransferrins	2.3–8.1

■ Enzyme Analyses

Phosphomannomutase (mU/mg protein)	
Leukocytes	1.8–3.2 (range); 2.2 (median)
Fibroblasts	3.8±0.9 (mean±1 SD)
Phosphomannose isomerase (mU/mg protein)	
Leukocytes	860–1800 (nmol/h/mg protein; range)
Fibroblasts	6.8±2.4 (mean±1 SD)
N-Acetylglucosaminyltransferase II (nmol/mg protein/h)	
Fibroblasts	2.2±1.0 (mean±1 SD)

■ Lipid-linked Oligosaccharides in Fibroblasts (HPLC)

Man ₅ GlcNAc ₂	Not detectable
Man ₉ GlcNAc ₂	Not detectable

20.6 Pathological Values/Differential Diagnosis

Disease	Sialotransferrins (S)	Lipid-linked oligosaccharides (FB)
20.1 CDG-Ia	<div>Asialotransferrin ↑/↑↑</div> <div>Monosialotransferrin n</div> <div>Disialotransferrin ↑↑</div> <div>Trisialotransferrin n</div> <div>Tetrasialotransferrin ↓</div> <div>Pentasialotransferrin ↓</div> <div>Hexasialotransferrin ↓</div>	Normal pattern
20.2 CDG-Ib	Same as CDG-Ia	Normal pattern
20.3 CDG-Ic	Same as CDG-Ia but asialotransferrin usually less increased	Man ₉ GlcNAc ₂ ↑
20.4 CDG-Id	Same as CDG-Ia	Man ₅ GlcNAc ₂ ↑
20.5 CDG-Ie	Same as CDG-Ia except that asialotransferrin is not increased	Man ₅ GlcNAc ₂ ↑
20.6 CDG-IIa	<div>Asialotransferrin n</div> <div>Monosialotransferrin ↑</div> <div>Disialotransferrin ↑↑</div> <div>Trisialotransferrin ↑↑</div> <div>Tetrasialotransferrin ↓↓</div> <div>Pentasialotransferrin ↓↓</div> <div>Hexasialotransferrin ↓↓</div>	Normal pattern
20.7 CDG-IIb	Normal pattern	?

20.7 Loading Tests

No acute loading tests are performed in these disorders.

20.8 Diagnostic Flow Chart

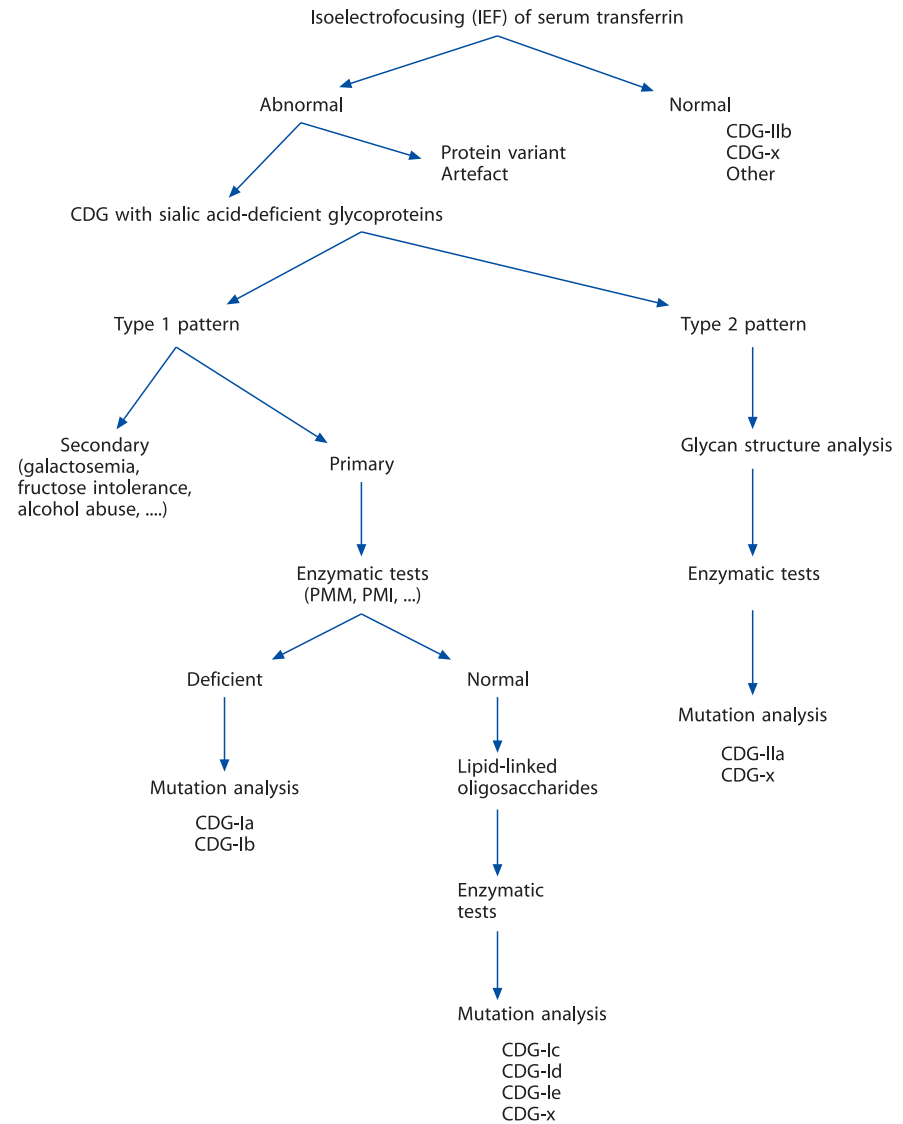


Fig. 20.2

20.9 Specimen Collection

Test	Precondition	Material	Handling	Pitfalls
Sialotransferrins		S	Frozen (−20 °C)	No EDTA plasma
Lipid-linked oligo-saccharides		FB	Frozen (−20 °C)	
PMM		WBC, FB	Frozen (−20 °C)	
PMI		WBC, FB	Frozen (−20 °C)	
GT I		FB	Frozen (−20 °C)	
MT VI		FB	Frozen (−20 °C)	
DPM synthase-1		FB	Frozen (−20 °C)	
Gn T II		Lym, FB	Frozen (−20 °C)	
G I		FB	Frozen (−20 °C)	

20.10 Prenatal Diagnosis

Disorder		Material	Timing, trimester
20.1	CDG-Ia	CV sampling cultured AFC	I, II
20.2	CDG-Ib	CV sampling cultured AFC	I, II
20.3	CDG-Ic	CV sampling cultured AFC	I, II
20.4	CDG-Id	CV sampling cultured AFC	I, II
20.5	CDG-Ie	CV sampling cultured AFC	I, II
20.6	CDG-IIa	CV sampling cultured AFC	I, II
20.7	CDG-IIb	CV sampling cultured AFC	I, II

20.11 DNA Analysis

Disorder	Material	Methodology
20.1 CDG-Ia	F, WBC	direct sequencing of genomic DNA; SSCP; D-HPLC
20.2 CDG-Ib	F, WBC	direct sequencing of genomic DNA
20.3 CDG-Ic	F, WBC	direct sequencing of genomic DNA
20.4 CDG-Id	F, WBC	direct sequencing of genomic DNA
20.5 CDG-Ie	F, WBC	direct sequencing of genomic DNA
20.6 CDG-IIa	F, WBC	direct sequencing of genomic DNA
20.7 CDG-IIb	F, WBC	direct sequencing of genomic DNA

SSCP, single-stranded conformational polymorphism analysis; D-HPLC, denaturing HPLC analysis.

20.12 Initial Treatment

The only CDG responsive to treatment is CDG-Ib. Mannose circumvents the defective step (phosphomannose isomerase) because it can be directly converted to mannose 6-phosphate by hexokinases.

It can be given orally or intravenously in a dose of 150 mg/kg/d (in 4 to 6 divided doses). Response to treatment takes weeks to months.

20.13 Summary/Comments

Congenital disorders of glycosylation (CDG) are a rapidly growing group of genetic diseases due to defects in the synthesis of glycans. Seven disorders have been well characterized in N-glycan assembly and processing: two in the cytosol, four in the ER and one in the Golgi. Except for CDG-Ib all affect the brain besides many other organs. A CDG should be considered in any unexplained clinical disorder. A valuable screening test is isoelectrofocusing of serum transferrins but a normal result does not exclude a CDG. Only for one CDG (CDG-Ib) an efficient treatment is available namely D-mannose. The number of patients with an unexplained CDG (CDG-x) is rapidly growing. Glycan structure analysis, yeast genetics and knock-out animals are important tools in the elucidation of these CDG [17].

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