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Issue: *The Year in Human and Medical Genetics***Congenital disorders of glycosylation**

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Congenital (genetic) disorders of glycosylation (CDG) are a rapidly growing disease family, with some 45 members reported since its first clinical description in 1980. Most of these are protein hypoglycosylation diseases, but recently three defects in lipid glycosylation have been identified. Most protein hypoglycosylation diseases are due to defects in the *N*-glycosylation pathway (16 diseases). The remaining ones affect the *O*-glycosylation pathway (8 diseases), both the *N*- and the *O*-glycosylation pathways, or other pathways (17 diseases). CDG can affect nearly all organs and systems, but there is often an important neurological component. The first-line screening for the *N*-glycosylation diseases is serum transferrin isoelectrofocusing (IEF), and for the *O*-glycosylation disorders apo CIII IEF. It has to be stressed that a normal test result does by no means exclude a CDG. In case of an abnormal result and as long as the basic defect has not been elucidated, the disease is labeled CDG-x (CDG-Ix when the transferrin IEF shows a type 1 pattern, and CDG-IIx when it shows a type 2 pattern).

Keywords: apo-CIII isoelectrofocusing; lipid glycosylation; *N*-glycosylation; *O*-glycosylation; transferrin isoelectrofocusing

Introduction

Congenital (genetic) disorders of glycosylation (CDG) are a rapidly growing family of genetic diseases first reported in 1980.¹ They are due to defects in the synthesis of the glycan moiety of glycoproteins or glycolipids, and in the attachment of these glycans to proteins and lipids.^{2,3} There are two main types of protein glycosylation: *N*-glycosylation and *O*-glycosylation. *N*-glycosylation (*N*-glycans attached to an aminogroup of asparagines of proteins) comprises an assembly part and a processing part and extends over three cellular compartments: the cytosol, the endoplasmic reticulum (ER), and the Golgi. The assembly part of the *N*-glycosylation starts on the cytosolic part of the ER with the transfer of *N*-acetylglucosamine (GlcNAc) phosphate from UDP-GlcNAc to membrane-bound dolichyl monophosphate (Dol-P), forming GlcNAc-pyrophosphatedolichol (GlcNAc-PP-Dol). One GlcNAc and five mannose (Man) residues are subsequently attached to this dolichol-linked monosaccharide in a stepwise manner. The donor

of these mannoses is a nucleotide-activated sugar, guanosine diphosphate (GDP)-Man, which is synthesized from fructose 6-phosphate, an intermediate of the glycolytic pathway. The lipid-linked heptasaccharide $\text{Man}_5\text{GlcNAc}_2$ is translocated by a flippase across the ER membrane and is elongated at the luminal side by the attachment of four further mannose residues and, subsequently, three glucose residues. The four mannosyltransferases and three glucosyltransferases involved require dolichylphosphate-bound monosaccharides (Dol-P-Man and Dol-P-Glc). The completed $\text{Glc}_3\text{Man}_9\text{GlcNAc}_2$ oligosaccharide is then transferred to selected asparagine residues of nascent proteins by the oligosaccharyltransferase complex. The processing part of the *N*-glycosylation starts in the ER by trimming the glucoses (catalyzed by glucosidases I and II) and one mannose (catalyzed by alpha-mannosidase I). The residual glycoprotein intermediate is directed to the cis-Golgi where the processing pathway branches. A minor branch targets glycoproteins to the lysosomes (after the action of a

GlcNAc-phosphotransferase and removal of the GlcNAc residues, leaving high-mannose glycoproteins capped with Man 6-P). The main branch leads to further trimming of mannoses (leaving a trimannosyl core) and addition of, respectively, GlcNAc, galactose, and eventually, sialic acid in the medial- and trans-Golgi. Another modification of many *N*-glycoproteins in the Golgi is the attachment of fucose.

O-glycosylation (*O*-glycans attached to the hydroxyl group of threonine or serine of proteins) has no processing and thus only consists of assembly. Contrary to *N*-glycosylation, this assembly mainly occurs in the Golgi. *O*-glycans show a greater diversity than *N*-glycans. Examples of important *O*-glycans are *O*-*N*-acetylglucosaminylglycans (mucin-type glycans), *O*-xylosylglycans (glycosaminylglycans), *O*-mannosylglycans, and *O*-fucosylglycans. There is a growing group of CDG with combined *N*- and *O*-glycosylation defects; a number of these are defects in proteins with a broader range of functions besides glycosylation. Recently, defects have been identified in glycosphingolipid glycosylation (ST3GAL5-CDG or Amish infantile epilepsy) and in glycosylphosphatidylinositol (GPI)-anchor glycosylation (PIGM-CDG and PIGV-CDG). This text uses the novel nomenclature, that is, the gene symbol followed by “-CDG.”^{4,5} The old designation is given between brackets.

Defects of protein *N*-glycosylation

Sixteen diseases are known in protein *N*-glycosylation: 14 assembly defects (CDG-I group) and 2 processing defects (CDG-II group). Only those CDG will be described here with more than two reported patients.

PMM2-CDG (*CDG-Ia*)

Phosphomannomutase (PMM)2 deficiency is a (cytosolic) defect in the second step of the mannose pathway (transforming mannose 6-phosphate into mannose 1-phosphate), which normally leads to the synthesis of GDP-mannose. This nucleotide sugar is the donor of the mannoses used in the ER to assemble the dolichol-pyrophosphate oligosaccharide precursor. Deficiency of GDP-mannose causes hypoglycosylation of numerous glycoproteins, including serum proteins, lysosomal enzymes, and membranous glycoproteins.

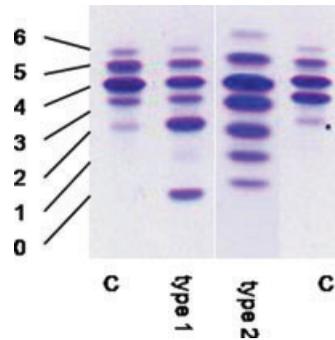


Figure 1. Serum transferrin isoelectrofocusing. Numbers 0 to 6 on the left indicate different sialotransferrins. C: control; type 1: type 1 pattern; type 2: type 2 pattern.

The diagnosis of *PMM2*-CDG (and of congenital disorders of *N*-glycosylation in general) is usually made by isoelectrofocusing (IEF) and immunofixation of serum transferrin⁶ (Fig. 1) or by capillary zone electrophoresis of total serum.⁷ Normal serum transferrin is mainly composed of tetrasialotransferrin and small amounts of mono-, di-, tri-, penta-, and hexasialotransferrins. The partial deficiency of sialic acid (a negatively charged and end-standing sugar) in CDG causes a cathodal shift. Two main types of cathodal shift can be recognized: type 1 is characterized by an increase of both disialo- and asialotransferrin, and a decrease of tetrasialotransferrin; in type 2 there is also an increase of the tri- and/or monosialotransferrin bands. In *PMM2*-CDG a type 1 pattern is found. In addition to the above-mentioned serum glycoprotein abnormalities, laboratory findings include increase of serum transaminases, hypoalbuminemia, hypcholesterolemia, and tubular proteinuria. The diagnosis is confirmed by finding a decreased activity of *PMM2* in leukocytes or fibroblasts. Prenatal diagnosis is possible by enzymatic analysis of amniocytes or chorionic villus cells. This should be combined with mutation analysis of the *PMM2* gene.

PMM2-CDG is by far the most prevalent protein *N*-glycosylation disorder (more than 700 patients known). The clinical spectrum is very large. The nervous system is affected in all patients, and most other organs are involved in a variable way. The neurological picture comprises alternating internal strabism and other abnormal eye movements, axial hypotonia, psychomotor retardation, ataxia, and hypoflexia. After infancy, symptoms include retinitis pigmentosa, often stroke-like episodes, and sometimes

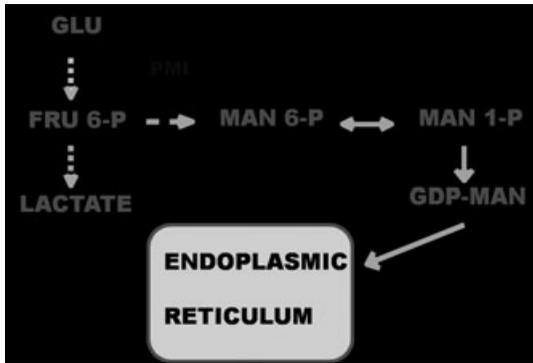


Figure 2. Scheme of the (cytosolic) mannose pathway with indication of the defect (vertical bar) in MPI-CDG. GLU, glucose; FRU, fructose; MAN, mannose; P, phosphate; GDP, guanosine diphosphate.

epilepsy. During the first year(s) of life, there are variable feeding problems such as anorexia, vomiting, and diarrhea. These can lead to severe failure to thrive. Other features are a variable dysmorphism (large hypoplastic/dysplastic ears, abnormal subcutaneous adipose tissue distribution), hepatomegaly, skeletal abnormalities, and hypogonadism. Some infants develop pericardial effusion and/or cardiomyopathy. At the other end of the spectrum are patients with a very mild phenotype (no dysmorphism, mild psychomotor retardation). Patients often have an extroverted and happy appearance. There is an increased mortality in the first years of life due to vital organ involvement or severe infection. Some 90 mutations have been identified in the PMM2 gene. The mutation leading to the R141H substitution is present in about 75% of the alleles of Central European patients.⁸

MPI-CDG (CDG-Ib)

The defect in phosphomannose-isomerase (MPI) deficiency is in the first step of the biosynthesis of the nucleotide sugar GDP-mannose (Fig. 2). The biochemical abnormalities are indistinguishable from those found in PMM2-CDG. Some 20 patients have been reported with this mainly hepatic-intestinal disorder. It is the only known *N*-glycosylation disorder without or with only minor neurological involvement. Symptoms start between 1 and 11 months of age and consist of various combinations of recurrent vomiting, abdominal pain, protein-losing enteropathy, recurrent thromboses, gastrointestinal bleeding, liver disease, and symptoms of hypoglycemia. Several patients have died

from this disorder. This is the only known CDG that is efficiently treatable (see Treatment).⁹

ALG6-CDG (CDG-Ic)

ALG6 encodes glucosyltransferase I. Its deficiency causes a defect in the attachment of the first glucose (of three) to the dolichol-linked $\text{Man}_9\text{GlcNAc}_2$ ER intermediate. This is the second most common protein *N*-glycosylation disease with at least 30 patients identified. As in PMM2-CDG, patients show hypotonia, strabism, and seizures but psychomotor development is less retarded, there is less dysmorphism and usually no retinitis pigmentosa or cerebellar hypoplasia. Remarkably, some glycoproteins have unusually low blood levels (particularly of factor XI, and coagulation inhibitors antithrombin and protein C). Analysis of the dolichol-linked oligosaccharides in fibroblasts shows an accumulation of the glycan intermediate $\text{Man}_9\text{GlcNAc}_2$.¹⁰

ALG3-CDG (CDG-I d)

Six patients have been reported with this mannosyltransferase VI deficiency. Symptomatology included severe psychomotor retardation, hypsarhythmia, postnatal microcephaly, optic atrophy, iris coloboma, hyperinsulinemic hypoglycemia with islet cell hyperplasia, and brain and corpus callosum atrophy. The hallmark biochemical feature of this CDG is an accumulation in fibroblasts of dolichyl pyrophosphate- $\text{Man}_5\text{GlcNAc}_2$.¹¹

ALG12-CDG (CDG-Ig)

In the six reported patients with this mannosyltransferase VIII deficiency, the phenotypes showed various combinations of facial dysmorphism, psychomotor retardation, hypotonia, inverted nipples, subcutaneous fat pads, skeletal dysplasia, and decreased serum IgG levels. The typical biochemical feature of this disorder is an accumulation of dolichyl pyrophosphate- $\text{Man}_7\text{GlcNAc}_2$.¹²

ALG8-CDG (CDG-Ih)

This CDG is due to glucosyltransferase II deficiency. Five patients have been reported from four families. Three showed a severe disease with dysmorphism and multiorgan failure resulting in early death. The fourth patient had a milder phenotype with hepatomegaly and protein-losing enteropathy. In the fifth patient there was prominent central nervous system involvement besides enterohepatic and renal disease. Dolichyl pyrophosphate- $\text{Glc}_1\text{Man}_9\text{GlcNAc}_2$ accumulated in fibroblasts.¹³

ALG1-CDG (CDG-Ik)

The four reported patients with mannosyltransferase I deficiency showed epilepsy, severe psychomotor retardation, and variable features such as dysmorphism, liver dysfunction, cardiomyopathy, nephrotic syndrome, hypogonadism, and depletion of beta-cells. Lipid-linked oligosaccharide analysis showed an accumulation of GlcNAc₂-PP-dolichol in fibroblasts.¹⁴

RFT1-CDG (CDG-In)

This is a defect in the flippase that transfers Man₅GlcNAc₂-PP-Dol from the cytoplasmic to the luminal side of the ER. The six reported patients showed mainly severe psychomotor retardation, hypotonia, drug-resistant epilepsy, and sensorineural deafness. This is the first CDG with sensorineural deafness as a consistent feature. Lipid-linked oligosaccharide analysis showed an accumulation of the above-mentioned intermediate in fibroblasts.¹⁵

TUSC3-CDG

This is a defect in one of the subunits of the oligosaccharyltransferase complex. It has been reported in two families with autosomal recessive, nonsyndromic mental retardation and suggests a crucial role of this complex in cognitive functioning. Remarkably, IEF of serum transferrin showed a normal pattern.¹⁶

MAGT1-CDG

Like TUSC3-CDG, this is a disorder of one of the subunits of the oligosaccharyltransferase complex, and has been reported in a family with X-linked, nonsyndromic, and nonprogressive mental retardation. This is the only known CDG with X-linked inheritance.¹⁷

MGAT2-CDG (CDG-IIa)

Four patients have been reported with *N*-acetylglucosaminyltransferase II deficiency, the first identified *N*-glycan processing defect. Besides neurological involvement (psychomotor retardation, epilepsy, behavioral disturbances), these patients present with craniofacial dysmorphism, skeletal abnormalities, gastrointestinal disturbances, and growth retardation. As in other CDG-II group patients, serum glutamic oxaloacetic transaminase is increased but glutamic pyruvic transaminase is normal. IEF of serum transferrin shows a type 2 pattern and structural analysis of the transferrin oligosaccharides indicates an abnormal glycan structure (a



Figure 3. Radiological picture of lower limb exostoses in EXT1/EXT2-CDG. The exostoses are indicated by white arrows.

monoantennary *N*-acetylglucosamine type glycan) as seen in some other CDG-II diseases.¹⁸

Defects of protein O-glycosylation

Defects have been identified in the synthesis of *O*-xylosylglycans (EXT1/EXT2-CDG and B4GALT7-CDG), of *O*-*N*-acetylgalactosaminylglycans (GALNT3-CDG), of *O*-xylosyl/*N*-acetylgalactosaminylglycans (SLC35D1-CDG), of *O*-mannosylglycans (POMT1/POMT2-CDG and POMGNT1-CDG), and of *O*-fucosylglycans (SCDO3-CDG and B3GALT-CDG).

EXT1/EXT2-CDG

This is the defect of multiple cartilaginous exostoses and the only known CDG with autosomal dominant inheritance. It is characterized by osteochondromas of the ends of long bones (Fig. 3) and is thus a monosystemic CDG. These tumors are often present at birth, and their growth slows during adolescence and stops in adulthood. A small percentage of these lesions shows malignant degeneration. Complications may arise from compression of peripheral nerves and blood vessels. The EXT1/EXT2 complex is localized in the Golgi and has both glucuronyltransferase and *N*-acetyl-*D*-hexosaminyltransferase

activities involved in the polymerization of heparan sulfate. It has been hypothesized that mutations in these glycosyltransferases impair the synthesis of a glycosaminoglycan that exerts a tumor-suppressor function. This would explain the higher risk of patients to develop chondrosarcomas and osteosarcomas. Mutations in *EXT1* and in *EXT2* are responsible for over 70% of the cases of hereditary multiple exostoses.¹⁹

B4GALT7-CDG

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-galactose-galactose), specifically in the attachment of the first galactose to xylose.²⁰

GALNT3-CDG

Deficiency of isoform 3 of *N*-acetylgalactosaminyltransferase causes recurrent, painful calcified subcutaneous masses known as familial, hyperphosphatemic tumoral calcinosis. This can be complicated by secondary infections and incapacitating mutilations. The hyperphosphatemia is due to increased renal phosphate retention.²¹

SLC35D1-CDG

The solute carrier 35D1 encodes an ER UDP-glucuronic 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.²²

POMT1/POMT2-CDG

POMT1 mutations can cause Walker-Warburg syndrome, a rare neuronal migration disorder 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. Psychomotor development is absent. In this disorder there is an aberrant glycosylation of alpha-dystroglycan, an external membrane protein expressed in muscle, brain, and other tissues. The

enzyme catalyses the first step in the synthesis of the *O*-mannose-linked core Galβ1-4GlcNAcβ1-2Man-*O*-Ser/Thr. Some patients with Walker-Warburg syndrome have mutations in the protein *O*-mannosyltransferase 2 gene (*POMT2*), in the fukutin gene, or in the fukutin-related protein gene.²³

POMGNT1-CDG

This disease is also known as muscle-eye-brain disease. It is a neuronal migration/congenital muscular dystrophy syndrome similar to but less severe than Walker-Warburg syndrome. These patients have mutations in the gene encoding protein *O*-mannosyl-β1,2-*N*-acetylglucosaminyltransferase 1, catalyzing the second step in the synthesis of the *O*-mannosylglycan core.²³

SCDO3-CDG

Spondylocostal dysostosis type 3 is a notch pathway defect in lunatic fringe, an *O*-fucose-specific β1,3-*N*-acetylglucosaminyltransferase. Patients show a severe vertebral phenotype with malsegmentation due to disruption of somitogenesis. Somites are precursors of the axial skeleton and associated musculature. It should be noted that spondylocostal dysostosis type 1 and type 2 are also due to defects in the notch pathway.²⁴

B3GALTL-CDG

This so-called Peters’-plus syndrome is characterized by peculiar eye malformations including corneal opacities and iridocorneal adhesions besides growth retardation and variable abnormalities in other organs. Mutations are in a beta1,3-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.²⁵

Defects of glycosphingolipid and GPI-anchor glycosylation

Only three disorders have been reported in this CDG group: *SIAT9-CDG* or Amish infantile epilepsy, and GPI deficiency due to *PIGM* deficiency and to *PIGV* deficiency.

SIAT9-CDG

This defect causes Amish infantile epilepsy, an infantile-onset epilepsy syndrome associated with developmental retardation and blindness. The

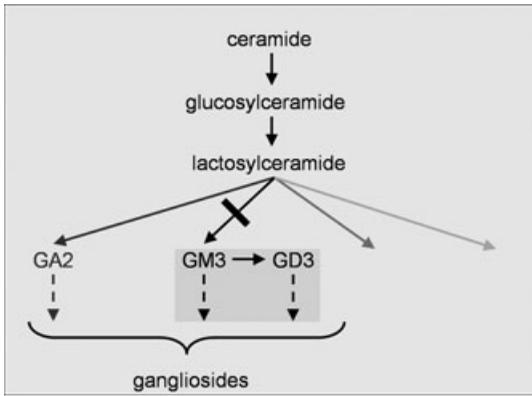


Figure 4. Scheme of the ganglioside synthesis pathway with indication of the defect (horizontal bar) in SIAT9-CDG.

SIAT9 gene encodes GM3 synthase, a sialyltransferase (lactosylceramide α -2,3 sialyltransferase). This enzyme catalyzes the initial step in the biosynthesis of most complex gangliosides from lactosylceramide (Fig. 4). The defect causes an accumulation of lactosylceramide associated with a decrease of the gangliosides of the GM3 and GD3 series.²⁶

PIGM-CDG

This is a new form of inherited (autosomal recessive) GPI deficiency presenting in infancy. It is characterized by splanchnic vein thrombosis and epilepsy. As compared with paroxysmal nocturnal hemoglobinuria, it does not result in clinically significant hemolysis and bone marrow failure. The defect results in histone hypoacetylation at the promoter of the PIGM gene. This gene codes for the first mannosyltransferase in the GPI-anchor biosynthesis pathway. The GPI anchor is synthesized in the ER through at least nine sequential reaction steps mediated by at least 18 proteins. Thus a number of other disorders have to be expected in this pathway. A partial treatment is available (see Treatment).²⁷

PIGV-CDG

PIGV codes for the second mannosyltransferase in the GPI anchor biosynthesis. Mutations in this gene have recently been identified in the hyperphosphatasia mental retardation syndrome, also known as Mabry syndrome. More particularly, mutations have been found in a subgroup of this syndrome with facial dysmorphism (hypertelorism, long palpebral fissures, a broad nasal bridge and tip, thin upper lip), and variable neurological features. The hyperphosphatasia can be explained by the fact

that alkaline phosphatase (as more than 100 other proteins) is a GPI-anchored protein.²⁸

Defects of multiple glycosylation and other pathways

This group comprises combined defects in *N*- and *O*-glycosylation (DPM1-CDG, MPDU1-CDG, B4GALT1-CDG, GNE-CDG, SLC35A1-CDG), a fucosylation defect (SLC35C1-CDG), two dolichol synthesis defects (DK1-CDG, SRD5A3-CDG), defects in subunits of the COG complex (COG7-CDG, COG1-CDG, COG8-CDG-COG4-CDG-COG5-CDG), a defect in a V-ATPase (ATP6V0A2-CDG), and a defect in SEC23B, a COPII component.

DPM1-CDG (CDG-Ie)

Six children been reported with this defect in the catalytic subunit of Dol-P-Man synthase. They showed a severe neurological involvement as in NOT56L-CDG and also an accumulation of Man₅GlcNAc₂-PP-dolichol in fibroblasts.²⁹

MPDU1-CDG (CDG-I_f)

The deficient protein is considered to be a chaperone involved in the use of both Dol-P-Man and Dol-P-Glc. The four reported patients showed a severe encephalopathy, and three of them had a scaly, erythematous skin disorder. One patient had dwarfism with growth hormone deficiency. Fibroblasts accumulated Man₅GlcNAc₂-PP-dolichol as well as Man₉GlcNAc₂-PP-dolichol.³⁰

GNE-CDG

Mutations in UDP-GlcNAc 2-epimerase/*N*-acetylmannosamine kinase cause sialuria, recessive hereditary inclusion body myopathy and Nonaka myopathy. The gene encodes a bifunctional enzyme that catalyses the first two (and rate-limiting) steps in sialic acid biosynthesis. This myopathy has an adult onset with progressive distal and proximal muscle weakness. A peculiar feature is that it spares the quadriceps muscles. Muscle histology shows rimmed vacuoles on Gomori's trichrome stain, small fibers in groups, and tubulofilaments without evidence of inflammation. IEF of serum transferrin is normal in this disorder.³¹

SLC35C1-CDG (CDG-IIc)

A few patients have been reported with this so-called leukocyte adhesion deficiency type II syndrome (LAD II). This syndrome comprises craniofacial dysmorphism, severe growth and psychomotor

retardation, as well as recurrent bacterial infections with unusually high leukocytosis. Due to hypofucosylation, neutrophils of these patients lack sialyl-Lewis X, a fucose-containing carbohydrate ligand of the selectin family of cell adhesion molecules. This ligand is required for the recruitment of neutrophils to infection sites. A partial treatment is available for some patients, depending on the mutation (see Treatment).³²

DK1-CDG (CDG-Im)

This is a defect in dolichol kinase catalyzing the final step of the *de novo* biosynthesis of dolichol phosphate. This compound is involved in several glycosylation reactions: *N*-glycosylation, GPI-anchor biosynthesis, and *C*- and *O*-mannosylation. Two families with two affected sibs each have been reported. All four had ichthyosis of the skin and died before the age of nine months. Inconstant features included dilated cardiomyopathy, hair problems, failure to thrive, postnatal microcephaly, epilepsy, hypotonia, bilateral nystagmus, and hypoketotic hypoglycaemia.³³

SRD5A3-CDG

This is the second reported defect in the synthesis of dolichol phosphate, more specifically in the conversion of polyprenol to dolichol. Clinical data are from 11 patients belonging to seven families. Besides psychomotor retardation the most prominent symptoms are cerebellar (atrophy, vermis malformations), ophthalmological (hypoplasia or coloboma of the iris, retina, choroid, optic disc; nystagmus, optic atrophy, microphthalmia, glaucoma, cataract), and cutaneous (ichthyosis, erythroderma, dry skin, atopic dermatitis).³⁴

COG-CDG

The conserved oligomeric Golgi (COG) complex is an eight subunit (COG1-8) peripheral Golgi membrane hetero-oligomeric protein complex. It is organized into lobes A (COG2-4) and B (COG5-7) with COG1 and COG8 bridging these lobes. This complex is thought to play a critical role in vesicle tethering processes involving retrograde Golgi transport of resident proteins responsible for glycan biosynthesis. Defects have been reported in COG1 (COG1-CDG; *CDG-IIg*), COG7 (COG7-CDG; *CDG-IIe*), COG8 (COG8-CDG; *CDG-IIh*), COG4 (COG4-CDG; *CDG-IIj*), COG5 (COG5-CDG), and COG6 (COG6-CDG). Common fea-

tures were feeding problems, growth retardation, microcephaly, dysmorphism, hypotonia, and cerebral atrophy. Two of the 3 COG1-deficient patients showed a cerebrocostomandibular-like syndrome. The seven reported COG7-deficient patients were all of North African origin. They had a lethal disorder (six of them died in their first year of life) and showed in addition hyperthermia, ventricular/atrial septum defect, and cholestatic liver disease. They showed a type 2 pattern on serum transferrin IEF and an abnormal pattern of serum apolipoprotein C-III IEF. Studies of fibroblast glycoproteins showed a partial *N*- and *O*-glycosylation defect caused by a decreased transport of CMP-sialic acid and UDP-galactose into the Golgi, and a reduced activity of two glycosyltransferases involved in the galactosylation and sialylation of *O*-glycans. The six patients with early lethality were homozygous for the same intronic mutation in *COG7*.³⁵ Since the COG complex is most probably not only involved in glycosylation but also in other cellular functions, we propose to call the COG defects "CDG-plus."

ATP6V0A2-CDG

In a subgroup of patients with autosomal recessive cutis laxa type 2 and of patients with wrinkly skin syndrome defects have been demonstrated in the $\alpha 2$ subunit of the vesicular ATPase H⁺-pump. These patients have generalized cutis laxa at birth that becomes less obvious with age. They also present with increased joint laxity, ophthalmological abnormalities (mainly strabismus, myopia or amblyopia, and sometimes corneal dystrophy), microcephaly, and delayed motor development that improves with age. Tropoelastin aggregates are found in their Golgi apparatus.³⁶ This seems to be another "CDG-plus."

SEC23B-CDG

Congenital dyserythropoietic anemia type II (CDAIL) has recently been shown to be due to mutations in the gene coding for SEC23B, a COPII component. One reason why this disorder is limited to the erythroblastic lineage might be that in the other tissues a SEC23A isoform compensates for the SEC23B deficiency.³⁷⁻³⁹

Diagnostic approaches in CDG

CDG should be looked for in any unexplained syndrome. Serum transferrin IEF remains the golden standard for the diagnosis of CDG due to an *N*-glycosylation defect. In case of an abnormal result,

an artifact, a transferrin protein variant, and a secondary CDG (mainly galactosemia and hereditary fructose intolerance), should be excluded. There are two types of patterns due to a CDG; the type 1 pattern points to an assembly defect (in the cytosol or ER; CDG-Ix) and the type 2 pattern to a processing defect (in the ER or Golgi; CDG-IIx).

The first step in CDG-Ix is measurement of the enzymatic activity of phosphomannomutase and phosphomannose isomerase in fibroblasts or leukocytes. When PMM2-CDG and MPI-CDG are excluded, the next step is dolichol-linked oligosaccharide (LLO) analysis in fibroblasts. An increase of one or more glycan intermediates, permits to formulate a diagnostic hypothesis that can be tested by enzymatic analysis and/or mutation analysis. If there is no specific abnormal pattern, a defect in the dolichol-phosphate pathway should be considered.

CDG-IIx should be further investigated by two different analyses: transferrin glycan analysis, and apolipoprotein C-III IEF to look for core 1 mucin-type glycosylation defects. In a small minority of cases the transferrin glycan analysis reveals a specific pattern with an accumulation of (a) Golgi glycan intermediate(s). This permits to formulate a hypothesis. In most cases however, an aspecific pattern will be obtained (usually some degree of hyposialylation and/or hypogalactosylation). The further approach should then be guided by the clinical presentation. In case of a cutis laxa syndrome, mutational analysis of the ATP6V0A2 gene is indicated. If not, we recommended to perform mutation analysis of the COG subunit genes.

Treatment of CDG

CDG is still a poorly treatable family of disorders. Only in three CDG is a treatment option available.

- (1) MPI-CDG: this is the only efficiently treatable CDG. The treatment consists of oral mannose (1 g/kg body weight per day, divided in 4–6 doses). The rationale for this treatment is that hexokinases phosphorylate mannose to mannose 6-phosphate, thus bypassing the defect.
- (2) SLC35C1-CDG: some patients with GDP-fucose transporter deficiency respond to fucose, depending on the nature of the mutation. The treatment is only effective with regard to the typical recurrent infections with hyperleukocytosis.

- (3) PIGM-CDG: the mutation causes histone hypoacetylation at the *PIGM* promoter, and this feature has been exploited as the basis for a treatment with butyrate, a histone deacetylase inhibitor. Butyrate markedly increases *PIGM* transcription, and is able to control the seizures.

Conclusions and perspectives

The field of CDG continues to grow at a surprisingly rapid rate. Forty-five CDG have been unraveled in the course of 30 years. Never before have so many disorders from the same family been identified in this time lapse. Major developments are to be expected in the following areas: defects in dolichol metabolism, defects in lipid glycosylation, defects in organ-specific glycosylation, and defects in other multifunctional proteins, that is, proteins that are not only involved in glycosylation but also in other functions. Advances in treatment are eagerly awaited since an efficient treatment is available for only one CDG (MPI-CDG). Efforts should be directed in particular toward a therapy for the important and often devastating disorder, PMM2-CDG. Since about 250 genes are considered to be involved in glycosylation, it is evident that we know only a small minority of existing CDG. The search for novel CDG will at the same time greatly boost our knowledge of the many unknown aspects of this most important protein and lipid modification.

Conflicts of interest

The author declares no conflicts of interest.

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