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Congenital disorders of glycosylation: new defects and still counting

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Abstract

Almost 50 inborn errors of metabolism have been described due to congenital defects in N-linked glycosylation. These phenotypically diverse disorders typically present as clinical syndromes, affecting multiple systems including the central nervous system, muscle function, transport, regulation, immunity, endocrine system, and coagulation. An increasing number of disorders have been discovered using novel techniques that combine glycobiology with next-generation sequencing or use tandem mass spectrometry in combination with molecular gene-hunting techniques. The number of “classic” congenital disorders of glycosylation (CDGs) due to N-linked glycosylation defects is still rising. Eight novel CDGs affecting N-linked glycans were discovered in 2013 alone. Newly discovered genes teach us about the significance of glycosylation in cell–cell interaction, signaling, organ development, cell survival, and mosaicism, in addition to the consequences of abnormal glycosylation for muscle function. We have learned how important glycosylation is in posttranslational modification and how glycosylation defects can imitate recognizable, previously described phenotypes. In many CDG subtypes, patients unexpectedly presented with long-term survival, whereas some others presented with nonsyndromic intellectual

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disability. In this review, recently discovered N-linked CDGs are described, with a focus on clinical presentations and therapeutic ideas. A diagnostic approach in unsolved N-linked CDG cases with abnormal transferrin screening results is also suggested.

Introduction

Biochemical classification of CDGs

Congenital disorders of glycosylation (CDGs) are inborn errors of glycan metabolism and can be divided into different biochemical groups (Jaeken et al. 2009a). The most well-known, common group results from several different defects in N-linked protein glycosylation. O-linked protein glycosylation is commonly tissue specific and clinical presentation is very different from the classic N-linked CDG group (Mohamed et al. 2011a). An increasing number of defects have been recognized in the last few years due to lipid-linked and glycosphosphatidylinositol (GPI) anchor glycosylation (Krawitz et al. 2013). GPI anchors are lipid-based glycans, assembled stepwise on phosphatidyl inositol in the endoplasmic reticulum (ER) membrane, and are further remodeled in the Golgi apparatus (Supplementary Fig. 1). Whereas the lipid-linked glycosylation group is very similar in clinical presentation to the N-linked CDG phenotype (Morava et al. 2010), GPI anchor-related disorders frequently underlie well-known clinical syndromes such as Mabry disease (MIM 239300) or paroxysmal nocturnal hemoglobinuria (MIM 300818), and their clinical presentation is commonly tissue or organ specific (Murakami et al. 2012). Clinically, the most interesting group is those with multiple affected glycosylation pathways, which teaches us how defects in different interconnecting pathways manifest as complex disorders (Lefeber et al. 2009).

Involvement of different cell compartments

CDGs are very diverse in their biochemical disease mechanism. 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 2010, Freeze 2013, Theodore and Morava 2011, Guillard et al. 2011). Transferrin isoform analysis offers characteristic, recognizable patterns depending on whether the defect is localized to the cytoplasm, the ER, or the Golgi apparatus. Defects in the first two are designated a type 1 pattern (CDG-I), and the latter is a type 2 pattern (CDG-II). This discrimination is important when deciding on a diagnostic plan and evaluating enzymes or genes with functions related to these different cell compartments. Transferrin analysis, as transferrin isoelectric focusing (TIEF), gives an initial idea of defect severity and classification, because CDG-I mostly shows elevated disialotransferrin isoform whereas CDG-II shows elevated asialo-, monosialo-, and trisialotransferrin isoforms of varying severity depending on the type of defect (Lefeber et al. 2011). Mass spectrometry (MS) and tandem mass spectrometry (MS/MS) might offer more details on the exact biochemical abnormality (Guillard et al. 2011).

Clinical phenotype and recognizable phenotypes in CDGs involving N-linked glycosylation

Here the focus is on the clinical aspects of N-linked glycosylation, lipid-linked glycosylation, and combined N- and O-glycosylation defects. In 2013 we counted 40 glycosylation defects with N-glycan involvement that led to abnormal transferrin screening results and hypoglycosylation in most patients. This number changes monthly with each new discovery. CDGs involving the N-glycans frequently lead to recognizable syndromes (Fig. 1). The best example is phosphomannomutase 2 (PMM2)-CDG, previously labeled CDG-Ia. Most patients with PMM2-CDG have abnormal fat pads, inverted nipples, arachnodactyly, mild muscle hypotonia, and strabismus at birth (Fig. 1a). However, abnormal fat distribution might disappear with older age, and milder cases frequently present without any of these dysmorphic features (Funke et al. 2013). Endocrine disturbance and thrombotic complications are common at any age (Linssen et al. 2013). Patients might have severe communication problems but a cheerful habitus (van de Loo et al. 2013). Many patients survive to adulthood, losing the syndromal aspects of PMM2-CDG.

Patients with phosphomannose isomerase (MPI)-CDG have a syndromal presentation with a hepatointestinal phenotype of chronic diarrhea (protein-losing enteropathy), coagulation defects, and liver disease, without the presence of dysmorphic features and with normal development (de Lonlay and Seta 2009). Liver fibrosis might appear in childhood or in young adulthood (Janssen et al. 2014) (Fig. 1b). The dolichol-synthesis-pathway-related disorders are frequently clinically recognizable, as well; SRD5A3-CDG is an oculocerebellar syndrome with occasional presence of ichthyosis (Morava et al. 2010) (Fig. 1d), DOLK-CDG is associated with dilated cardiomyopathy (Kapusta et al. 2013), and dolicholphosphomannose synthase (DPM)-CDGs are syndromal dystroglycanopathies (Mercuri and Muntoni 2012, Lefeber et al. 2009). DPGAT1-CDG is a form of CDG presenting either with severe early symptoms, including eye malformations, muscle weakness, and developmental delay, or with mild congenital myasthenia leading to muscle weakness, sometimes even as an isolated symptom in older patients (Wu et al. 2003, Belaya et al. 2012) (Fig. 1f). ALG11-CDG and RTF1-CDG can be recognized due to severe hearing loss (Jaeken et al. 2009b), and about one third of ALG6-CDG patients have skeletal abnormalities and distal limb malformations (Drijvers et al. 2010) (Fig. 1c). Thrombosis is a common feature in PMM2-CDG, MPI-CDG, and ALG1-CDG (Fig. 1g). Several CDG forms involve immune deficiency. Skeletal dysplasia has been described in only a few disorders of glycosylation in the ER, including ALG6-CDG and ALG8-CDG (Fig. 1e). Abnormal skeletal development is one of the major clinical features in cerebrocostomandibular syndrome due to COG1 deficiency (Zeevaert et al. 2009), in the newly discovered TMEM165-CDG (described later) (Foulquier et al. 2012), in ATP6V0A2-CDG with short stature and cutis laxa (also known as ARCL2B) (MIM 219200) (Mohamed et al. 2011c) (Fig. 2a), and in COG7-CDG in association with microcephaly, ventricular septal defect, failure to thrive, and hyperthermia with elevated levels of creatine kinase (CK) (Morava et al. 2007), and with the diagnostic feature of adducted thumbs (Fig. 2b). MGAT2-CDG presents with neurologic disease and radioulnar synostosis (Lefeber et al. 2011). Other recently defined recognizable CDGs are MAN1B1-CDG, characterized by intellectual

disability (ID) with speech delay, dysmorphic features, and truncal obesity (Scherpenzeel et al. 2014) (Fig. 2c and d); and SLC35A1-CDG, presenting with conotruncal malformation, ID, bleeding diathesis, and renal disease (Mohamed et al. 2013).

Newly discovered CDGs involving N-linked glycosylation

DPM2-CDG

The DPM synthase complex is a very important enzyme complex related to the dolichol synthesis pathway. Guanosine diphosphate mannose (GDP-mannose) is used by the complex to mannosylate dolichol for N-linked protein glycosylation and offers mannose residues for O-linked glycosylation, C-mannosylation, and the GPI anchor-related glycosylation (Lefeber et al. 2009). C-mannosylation is less relevant for humans, but abnormal O-linked mannosylation has severe consequences for central nervous system (CNS) development and function, along with skeletal muscle function. Alpha-dystroglycan is an O-mannosylated protein glycosylated through sequential synthetic steps. Enzyme defects in these steps lead to alpha dystroglycanopathies; however, the availability of activated mannose is also essential to make the proper O-linked mannosylated glycan. DPM-complex-related defects lead to different alpha dystroglycanopathies, which are syndromes of variable severity imitating muscle-eye-brain disease (Mercuri and Muntoni 2012, Lefeber et al. 2009, Barone et al. 2012). Upon the initial definition of DPM1-CDG as a severe neurologic disease with developmental delay and muscle weakness with CK elevations (MIM 608799), and DPM3-CDG as a mild limb-girdle-type muscle dystrophy with cardiomyopathy, Barone et al. defined the DPM2-related CDG that leads to alpha dystroglycanopathy, elevated CK levels, abnormal muscle histology, and associations with microcephaly and severe seizure disorder (Barone et al. 2012).

MAN1B1-CDG

Another disorder caused by alpha-1,2-mannosidase defect was initially described as a novel ID gene (Rafiq et al. 2011). Although the authors noted some dysmorphic features, such as down-slanting palpebral fissures, broad nasal root, and prominent lips, no systemic or other organ involvement was noted. Based on the suspected role of mannosidase in the processing of N-linked glycans and application of next-generation sequencing (NGS) in patients with unsolved CDG, Rymen et al. (2013) and Scherpenzeel et al. (2014) discovered several patients with mutations in *MAN1B1* and defined the disorder as the novel MAN1B1-CDG. Glycan analysis in these patients revealed increased mannose residues on the glycans and a CDG-II transferrin pattern with elevated trisialotransferrin (Rymen et al. 2013). Abnormal transferrin glycosylation was mild in several cases, imitating the pattern of transferrin protein polymorphism; MS, however, was diagnostic (Scherpenzeel et al. 2012). Previously unreported clinical features included abnormal fat distribution, strabismus, muscle weakness, developmental and speech delay, and central obesity (Rymen et al. 2013, Scherpenzeel et al. 2014). Interestingly, only a very small proportion of patients had liver-function and coagulation abnormalities (Scherpenzeel et al. 2014).

SLC35A1-CDG

This autosomal recessive sialic-acid-transporter disorder, previously labeled CDG-II_f, has been redefined as a CDG with multisystem disease and secretory glycosylation anomalies (Mohamed et al. 2013). The single previously described case showed no abnormality in transferrin pattern and isolated macrothrombocytopathy, and pancytopenia as the single presentation of the abnormal cytidine monophosphate sialic acid (CMP-sialic acid) transport (Martinez-Duncker et al. 2005). How is this phenotypic and biochemical difference possible? A recent report on another genetic sialic-acid-transport defect of unknown etiology revealed that one mutation found in the initial CDG-II_f patient is a common polymorphism (Mohamed et al. 2013). This indicates that the initial case with no secretory glycosylation anomalies either has a second, as yet undiscovered, mutation or that the phenotype might be due to a single mutation, possibly in the presence of the additional polymorphism (Jones et al. 2011, Mohamed et al. 2013). The current case is an autosomal recessive ID syndrome due to missense mutations in *SLC35A1*, with bleeding diathesis and multiorgan involvement. In association with the deficient CMP-sialic acid transport, the patient had developmental delay, seizures, ataxia, aortic insufficiency, proteinuria, and macrothrombocytopenia (Mohamed et al. 2013). The biochemical abnormality was a mixed N- and O-linked glycosylation defect, with elevated tri-, di-, and monosialotransferrin and decreased glycosylation by apolipoprotein C-III (ApoC-III) isofocusing (Mohamed et al. 2011a, 2013).

TMEM165-CDG

This disorder was discovered in 2012 (Foulquier et al. 2012); however, the full clinical phenotype was only defined in 2013 (Zeevaert et al. 2013). Patients with this novel CDG-II have developmental and growth delay and in several cases microcephaly. The most striking phenotypic presentation is a skeletal dysplasia with metaphyseal flaring, long-bone and vertebral involvement, brachydactyly, scoliosis, and severe short stature. Patients show wrinkled skin and frequent abnormal fat distribution, which is uncommon in CDG-II except for in *ATP6V0A2*-CDG (Gardeitchik et al. 2013). Laboratory anomalies include elevated CK and liver function enzymes, abnormal coagulant, and decreased factor IX and factor XI levels. The biochemical abnormality is similar to that seen in *ATP6V0A2* and in *SLC35A1* defects; a mixed N- and O-linked glycosylation defect, with elevated tri-, di-, and monosialotransferrin; and decreased O-linked secretory glycosylation demonstrated by ApoC-III isofocusing.

TMEM165-CDG leads to a generalized trafficking defect biochemically similar to *ATP6V0A2*-CDG. In patients with *ATP6V0A2* mutations, the adenosine triphosphatase (ATPase) defect is associated with an abnormal proton gradient through the vesicular membranes, leading to abnormal maturation of secreted glycosyltransferases and abnormal glycoprotein trafficking (Huchtagowder et al. 2009). Although the exact pathomechanism in TMEM165-CDG has not been discovered, an altered pH gradient has been confirmed in the Golgi apparatus. This suggests a similar mechanism of abnormal transport and trafficking and comprehensive Golgi apparatus dysfunction as the underlying cause of hypoglycosylation in TMEM165-deficient patients.

SLC35A2-CDG

MS assay of transferrin was performed in a patient with developmental delay, hypotonia, seizures, and brain malformations (Kodera et al. 2013, Ng et al. 2013). The pattern suggested a galactose deficiency in secretory glycoproteins. Combining the results of the biochemical analysis with NGS data led to the discovery of a new galactose transporter defect resulting in a CDG. The discovery of the X-linked uridine diphosphate galactose (UDP-galactose) transporter emphasizes the power of using metabolic data through interpretation of exome sequencing results. The exciting aspect of this novel finding is the detection of de novo mutations in the X-linked *SLC35A2* gene, which is unique in CDGs. An even more striking finding is the same defect in mosaic form in two other individuals with CDG-II who had an almost identical abnormal transferrin pattern, although NGS did not reveal the underlying molecular etiology (Ng et al. 2013, Kodera et al. 2013). The discovery of mosaicism in CDGs teaches us about the importance of glycosylation for survival and offers a new explanation for phenotypic variability within a certain type of CDG. As the transferrin pattern normalized throughout the course of disease in several *SLC35A2*-CDG patients without significant clinical improvement, we emphasize again the need for molecular diagnostics in several CDG types, even in the absence of clear glycosylation abnormalities. Normal transferrin pattern has been reported in *PMM2*-CDG and *SRD5A3*-CDG patients, as well (Vermeer et al. 2007, Mohamed et al. 2011b).

STT3A-CDG and STT3B- CDG

In two unrelated consanguineous families, mutations were found in two different isoforms of the catalytic subunit of oligosaccharyltransferase (OST). *STT3* is highly conserved and transfers oligosaccharides onto the asparagine residues in nascent glycoproteins (Shrimal et al. 2013). In the first family, a brother and sister were diagnosed with CDG-I and *STT3A* mutations. Both had developmental delay, failure to thrive, hypotonia, and seizures; boy had a more severe phenotype with poor vision, intractable seizures, and an inability to sit up without assistance at the age of 13 years.

In the second family, the affected individual carried *STT3B* mutations and had congenital neurologic abnormalities, hypotonia, ID, failure to thrive, and feeding problems but only a very mildly abnormal transferrin. *STT3A* and *STT3B* are both essential subunits of the OST complex (Shrimal et al. 2013). The primary neurologic presentation and very mild glycosylation abnormality in the second family suggests that certain cases might be missed using standard diagnostic screening for ID (Shrimal et al. 2013).

SSR4-CDG

A male patient of pubertal age showed a very mild CDG-I pattern biochemically and had a history of microcephaly, excess skin around the neck and micrognathia, fat pads, mild hypospadias, and clinodactyly of the fourth and fifth toes bilaterally. This was characterized as a new glycosylation defect due to a de novo mutation in the X-linked *SSR4* gene, which encodes a protein of the heterotetrameric translocon-associated protein (TRAP) complex (Losfeld et al. 2013). The patient had a relatively stable clinical picture, with developmental delay, persistent stridor, mild epilepsy, muscle hypotonia, and gastroesophageal reflux (Losfeld et al. 2013). The TRAP complex binds to the OST complex, which is very

important in N-linked glycosylation. The novel *SSR4* gene defect was detected by NGS. SSR proteins interact with different OST complex proteins, including STT3A, DAD1, and DDOST. These have been previously implicated in CDG defects with hypoglycosylation of secretory proteins. STT3A takes part in N-glycosylation of the nascent protein. DDOST and DAD1 are modulators of OST stability. CDG-I in a patient with *SSR4* mutation is the first evidence that the TRAP complex is directly involved in N-glycosylation (Losfeld et al. 2013).

PGM3-CDG

Phosphoglucomutase 3 (PGM3) deficiency is a recently characterized autosomal recessive disorder associated with decreased PGM3 enzyme activity and decreased O- and N-linked 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 UDP-GlcNAc. This precursor is then further modified to make N-glycans, 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 et al. 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 (Zhang et al. 2014). Subsequent evaluation and whole-genome sequencing in both families identified *PGM3* as a possible candidate gene, a finding confirmed by Sanger sequencing. O-linked glycosylation was assessed in two patients from family one and three from family two. Each of these patients demonstrated increased high T-antigen/Sialyl T-antigen ratios, suggesting hyposialylation, a finding consistent with decreased UDP-GlcNAc (Zhang et al. 2014). Although serum transferrin glycosylation was normal, total N-linked glycans showed decreased galactosylation of N-linked oligosaccharides in three patients (Zhang et al. 2014). Interestingly, UDP-GlcNAc and UDP N-acetylglactosamine 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.

Newly arising treatment options in CDG

DPAGT1-CDG

DPAGT1 catalyzes the first step of N-linked protein glycosylation (Wu et al. 2003). The original clinical presentation of DPAGT1 deficiency showed a severe neurologic condition with CNS involvement and eye abnormalities (Wu et al. 2003) but also muscle weakness and progressive fatigue similar to that seen in congenital myasthenic syndromes (CMS). In CMS, impaired signal transmissions occur at the level of the neuromuscular junction. DPAGT1 is required for glycosylation of acetylcholine receptor (AChR) subunits. In

DPAGT1-associated CMS, patients have reduced AChR levels at the endplate. Whole-exome sequencing also identified *DPAGT1* as a new gene associated with CMS in unsolved myasthenic patients (Belaya et al. 2012). DPAGT1 patients are characterized by limb muscle weakness and respond to treatment with cholinesterase inhibitors. This finding demonstrates that impairment of the N-linked glycosylation pathway can lead to the development of CMS (Belaya et al. 2012). Interestingly, CMS can occur due to other CDG types as well, resulting from mutations in *ALG2* and *ALG14* (Cossins et al. 2013); not all myasthenic CDG patients respond optimally to antimyasthenic treatment.

MPI-CDG

Hepatopathy and neurological involvement are the most common features in CDG. Phosphomannose isomerase deficiency (MPI-CDG) presents with a hepatointestinal phenotype but no significant CNS involvement. Recurrent episodes of vomiting, bleeding diathesis, and recurrent thrombosis and hypoglycemia are frequently treatable by high oral doses of mannose therapy. Heparin therapy has been shown to be effective for the protein-losing enteropathy (de Lonlay and Seta 2009.); however, patients might develop early cirrhosis. Janssen et al. 2014 reported the first successful liver transplantation in a patient with therapy-resistant MPI-CDG who showed mannose-therapy-associated hemolytic jaundice, progressive liver fibrosis, severe dyspnea, and exercise intolerance due to pulmonary involvement. Posttransplantation, her exercise tolerance, pulmonary functions, and metabolic parameters became fully restored, and she remained stable 2 years after transplantation.

PGM1-CDG

PGM1 is a key enzyme that functions between glycolysis and glycogenesis catalyzing the bidirectional transfer of phosphate from position 1 to position 6 on glucose, producing glucose-6-phosphate. Glucose-1-phosphate is also connected to galactose metabolism. PGM1-CDG was previously characterized as a glycogen storage disease and was then redefined as an inborn error of glycosylation as well, upon confirming the gene defect in children with multisystem features, including cleft palate or uvula, short stature, hypoglycemia, liver function and endocrine abnormalities, coagulation abnormalities, cardiomyopathy, and normal intelligence (Timal et al. 2012). One patient with PGM1 defect had malignant hyperthermia (Mohamed et al. 2011a); two patients with mostly muscle involvement showed minimal liver involvement but exercise intolerance and exercise induced hyperammonemia (Stojkovic et al. 2009). Due to PGM1 expression pattern, patients have no CNS involvement (Perez et al. 2013). Patients with PGM1 deficiency demonstrate abnormal protein glycosylation, affecting many secretory proteins similar to classic CDGs, and increased tri-, di-, mono-, and asialotransferrin (Perez et al. 2013).

Decreased transferrin galactosylation was confirmed in PGM1-deficient patients by mass spectrometry. An altered ratio of UDP-glucose and UDP-galactose was measured in patient fibroblasts, showing decreased intercellular adhesion molecule 1 (ICAM-1) immunofluorescence as a marker of decreased cell-surface glycosylation in PGM1 deficiency (Fig. 3). UDP-glucose and UDP-galactose are essential for normal protein glycosylation. The decreased galactosylation was most striking in patients on low galactose

intake (<0.35 g/kg/day) (Scherpenzeel et al. 2012). In vitro studies with galactose supplementation in fibroblast culture of patients demonstrated increased ICAM-1 expression and improved glycosylation. Oral dietary intervention with galactose supplements in an age-appropriate range of 0.5–1 g/kg/day led to improved hepatomegaly and liver function tests and prevented hypoglycemic episodes in four PGM1 patients (Tegtmeyer et al. 2014). Even abnormal coagulation normalized, and biochemical analysis showed improved transferrin glycosylation pattern throughout a year-long galactose supplementation (Scherpenzeel et al. 2012, Tegtmeyer et al. 2014). Two patients with hypogonadotropic hypogonadism reached normal puberty during galactose therapy. (Tegtmeyer et al. 2014). However, lactose supplementation at 1 g/kg/day improved growth only but was not effective in preventing hypoglycemic episodes (Misfeldt et al. 2013). A prospective trial is underway to evaluate the optimal dosage of galactose for individuals with PGM1, and possible additional adjuvant metabolic therapies.

Diagnostic flowchart

The biochemical finding of a type 1 pattern (CDG-I) by isoelectric-focusing capillary electrophoresis is suggestive of a glycan assembly or transfer defect in the cytosol- or ER-localized dolichol-linked glycan synthesis. PMM enzyme activity measurement should be performed in fibroblasts or leukocytes to rule out the most common PMM2-CDG defect. In patients with gastrointestinal involvement and bleeding diathesis, MPI should be assayed to exclude or confirm MPI-CDG. Abnormal lipid-linked oligosaccharides in fibroblasts or MS might indicate a dolichol-linked oligosaccharide synthesis or assembly defect. Several CDG-I defects are recognizable syndromes (see previous), which can initiate direct gene sequencing (Lefeber et al. 2011). The biochemical finding of a type 2 pattern (CDG-II) suggests abnormal glycan processing in the pathway between the step when the glycan is transferred onto the protein and the final secretion step from the Golgi apparatus. MS/MS of isolated serum N-glycans can identify isolated N-glycosylation defects such as B4GALT1-CDG, PGM1-CDG, or MAN1B1-CDG in addition to their secondary causes, but several CDG-IIs have nonspecific glycan profiles (Guillard et al. 2011, Lefeber et al. 2011, Scherpenzeel et al. 2012, Tegtmeyer et al. 2014). Combined glycosylation defects, including the N- and O-linked secretory pathway, frequently lead to CDG-II with abnormal mucin type O-glycosylation. Examples include different conserved oligomeric Golgi (COG) complex-related CDGs, leading to global Golgi trafficking failure, or other combined defects such as ATP6V0A2-CDG, TMEM165-CDG, or SLC35A1-CDG. These defects are associated with abnormal secretory pathway function, abnormal retrograde trafficking or abnormal acidification and could be investigated by serum ApoC-III isoelectric focusing (Lefeber et al. 2011).

NGS techniques in glycosylation disorders are much more powerful in the presence of biochemical glycosylation abnormalities (Losfeld et al. 2013). However, even in classic secretory glycosylation defects such as PMM2-CDG or SRD5A3-CDG, transferrin screening can be fully normal (Supplementary Fig. 2 and Table 1).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

ApoC-III	Apoprotein C-III
CDG	Congenital disorders of glycosylation
CK	Creatine kinase
CNS	Central nervous system
CMS	Congenital myasthenic syndromes
DPM	Dolichol phosphomannose
ER	Endoplasmic reticulum
GDP	Guanosine diphosphate
GPI	Glycophosphatidylinositol
ID	Intellectual disability
MS	Mass spectrometry
MS/MS	Tandem mass spectrometry
NGS	Next-generation sequencing
OST	Oligosaccharyltransferase
PMI	Phosphomannose isomerase
TIEF	Transferrin isoelectric focusing
TRAP	Translocon-associated protein
UDP	Uridine diphosphate

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Fig. 1. Recognizable clinical features in different N-linked glycosylation defects. **a** Abnormal fat distribution in phosphomannomutase 2 (PMM2)-CDG (CDG-Ia). **b** Liver cirrhosis in phosphomannose isomerase (MPI)-CDG (CDG-Ib). **c** Distal phalangeal aplasia in ALG6-CDG (CDG-Ic). **d** Ichthyosis and iridial and retinal coloboma are characteristic for SRD5A3-CDG. **e** Distal arthrogryposis in ALG8-CDG (CDG-Ih). **f** Myasthenic face and ptosis are common in DPAGT1-CDG (CDG-Ij). **g** Venous thrombosis leads to asymmetry in limb circumference in ALG1-CDG (CDG-Ik)

Fig. 2. Recognizable clinical features in N-glycosylation defects affecting the Golgi system. **a** Cutis laxa and abnormal fat distribution in ATP6V0A2-CDG. **b** Adducted thumbs and **c** microcephaly in COG7-CDG. **d** Abnormal abdominal fat distribution, high forehead, deep-set eyes, and prominent eyebrows in MAN1B1-CDG

Fig. 3. Immunohistochemistry of intercellular adhesion molecule 1 (ICAM-1) (green fluorescence) in **a** healthy control fibroblasts and in **b** phosphoglucomutase-1 (PGM1)-deficient patient fibroblasts. ICAM-1 antibodies bind to membrane-associated glycoproteins on the cell surface. Nucleus: 4'-6'-diamidino-2-phenylindole (DAPI) staining (*blue*). Patients with PGM1 congenital disorders of glycosylation (CDG) show little or no fluorescence label on fibroblast cell surface