The Application of Clinical Genetics

Congenital protein hypoglycosylation diseases

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Abstract: Glycosylation is an essential process by which sugars are attached to proteins and lipids. Complete lack of glycosylation is not compatible with life. Because of the widespread function of glycosylation, inherited disorders of glycosylation are multisystemic. Since the identification of the first defect on N-linked glycosylation in the 1980s, there are over 40 different congenital protein hypoglycosylation diseases. This review will include defects of N-linked glycosylation, O-linked glycosylation and disorders of combined N- and O-linked glycosylation.

Keywords: congenital disorders of glycosylation, dystroglycanopathies, hypoglycosylation, glycoproteins

Introduction
Glycosylation of proteins is a post-translational process that is important for many proteins to function. The addition of carbohydrates to the proteins stabilizes the protein, is involved in cell-cell interaction and cellular signaling, and modulates protein function. Defects in glycosylation encompass abnormalities in the synthesis of N-linked glycoproteins, O-linked glycoproteins, or both, as well as a defect in lipid-glycosylation. Since the characterization of N-linked defects in the 1980s, the field of clinical glycobiology has rapidly progressed and now includes defects in glycosyltransferases for some forms of muscular dystrophy.

Protein glycosylation is defined as the synthesis of glycans and their covalent attachment to proteins. Glycans are also attached to lipids forming glycolipids (glycophospholipids, glycosphingolipids). Approximately 0.5%–1% of the transcribed human genome is responsible for the synthesis, degradation, and function of glycoconjugates.

Glycoproteins are classified by the type of linkage of the oligosaccharides to the peptide. This attachment can be either N-linked through the amide group of selected asparagine residues to an N-acetylgalactosamine (GlcNAc) residue, or O-linked through the hydroxyl group mainly of serine or threonine residues via N-acetylgalactosamine (GalNAc), mannose (Man), xylose, or other monosaccharide residues.

In general, the process of protein glycosylation occurs in fours stages that take place in the cytosol, the endoplasmic reticulum (ER), and the Golgi compartments. In the first stage, monosaccharides are activated from dietary sources as well as salvage pathways in the cell.
Activated monosaccharides are generated through a series of phosphorylation, epimerization, and acetylation reactions. These become the high-energy nucleotide sugar donors.

The next stage involves transport of the sugar donors from the cytoplasm into the lumen of the ER, by binding to dolichol phosphate, and the Golgi by specific transporters. For transport into the ER, the nucleotide sugars bind to the cytosolic side of the dolichol phosphate which is membrane bound. A “flippase” mediates the transfer of the sugar into the luminal side of the ER. Defects in either the synthesis of the dolichol phosphate [DPM1-CDG (CDG-IIe)], or in the transfer LEC35-CDG (CDG-IIf) result in clinical disease (see below). In the Golgi, there are specific nucleotide sugar transporters to transfer the activated monosaccharides to the luminal side. Abnormal glycosylation can be seen if these transporters are deficient, such as the GDP-Fucose transporter in SLC35C1-CDG (CDG-IIc).

In the third stage, specific glycosyltransferases attach the monosaccharides to the growing oligosaccharide. This occurs in a sequential and highly controlled fashion. Most of the N- and O-linked defects described to date are deficiencies of a specific glycosyltransferase.

The final stage represents Golgi trafficking and integrity mechanisms. Glycoproteins and glycosyltransferases are transported to their final destination via the cisternae and Golgi vesicles. Defects in the subunits of the conserved oligomeric Golgi (COG) complex result in defective glycosylation.

The biosynthesis of N-linked glycans occurs cotranslationally and is spread over the cytosol, the ER, and the Golgi compartments. In the cytosol and the ER, monosaccharides are attached in a stepwise fashion to form a lipid-linked oligosaccharide (LLO). The monosaccharide donors are either nucleotide- or dolichol phosphate-linked sugars synthesized in the cytosol. These are the substrates for specific glycosyltransferases, which sequentially form the progressively growing LLO in the ER. In the ER, the completed basic glycan (Glc,Man,GlcNAc₂, where Glc is glucose, Man is mannose, GlcNAc is N-acetylglucosamine) is transferred en bloc to the asparagine, which is part of the consensus sequence on the protein, Asn-X-Ser/Thr (where X is any amino acid). This transfer is catalyzed by the oligosaccharyltransferase complex. The glycosylated protein is further processed in the ER and the Golgi into a more complex structure by removal of glucose and mannose residues and the addition of GlcNAc, galactose, fucose, and sialic acid residues.

In contrast, the biosynthesis of O-linked glycans occurs post-translationally and mainly within the Golgi compartment. In addition, there is no consensus sequence to determine the O-glycosylation sites within a protein. However, since O-linked glycosylation occurs after protein folding; only exposed serine and threonine residues are available for glycosylation. There are seven different types of O-glycan attachments in humans, classified by the first sugar (GalNAc, Xyl, GlcNAc, Gal, Man, Glc, Fuc) bound to the protein. The mucin-type O-glycoprotein, which begins with the addition of a GalNAc to a serine or threonine of a fully formed protein, and the glycosaminoglycans (GAGs) where a Xyl is the first sugar, are the most common O-linked glycoproteins. Following this initial step, there is elongation and modification of the oligosaccharides. Mucin-type O-glycans can be further subdivided into eight core structures depending on the second sugar(s) and/or binding position resulting in at least 50 different types. The GAGs also occur in various different forms and contain 100 or more monosaccharides in their chain. The structures of the other 5 O-glycan types show less variability and mostly occur in one conformation.

A specific type of O-glycosylation involves the attachment of a mannose. While many brain glycoproteins contain O-linked mannose oligosaccharides, only α-dystroglycan has been well characterized. Alpha-dystroglycan is part of the dystrophin-glycoprotein complex which is important for
membrane stability and cell signaling functions in the central nervous system as well as muscle. Alpha-dystroglycan is heavily glycosylated with N-linked oligosaccharides and mucin-type O-linked oligosaccharides. In addition, it has O-linked mannosyl oligosaccharides. This glycan type is initiated in the ER by the combined action of protein-O-mannosyltransferases, POMT1/POMT2 and uses dolichol-phosphate activated mannose as a substrate. The glycan is further elongated by a specific GlcNAc-transferase (encoded by POMGnT1) in the Golgi. Additional glycosyltransferases yet to be identified extend the glycan with a galactose and a sialic acid residue. Defects in POMT1/POMT2 and POMGnT1 cause abnormal glycosylation of α-dystroglycan and result in muscular dystrophy.

Clinical diagnoses
Abnormalities in the synthesis of N-linked glycoproteins, O-linked glycoproteins or both will be reviewed. By far the most abundant and well studied of the protein hypoglycosylation defects are the group of disorders known as Congenital Disorders of Glycosylation (formerly known as carbohydrate-deficient glycoprotein syndrome-CDGS) which are defects in the elongation pathway of the biosynthesis of N-glycans that occurs in the ER (type I CDG) and defects in the processing pathway in the ER and Golgi (type II CDG). Due to the utilization of some of the enzymes in the biosynthesis of N-glycans and O-glycans, several of the subtypes of CDG affect O-glycosylation as well.

In the O-linked glycoprotein pathway, clinically deleterious defects in three particular branches have been elucidated. First, a defect in O-GalNAc glycan is responsible for familial tumoral calcinosis. Second, defects in O-mannosylation are responsible for the dystroglycanopathies which cause several types of muscular dystrophy. Hereditary inclusion body myopathy (HIBM) is caused by a defect in sialic acid biosynthesis and has also been shown to have a defect in O-mannosylation in muscle, and is discussed with this group as well. Third, O-xylosylglycan defects are responsible for a progeroid variant of Ehlers-Danlos syndrome and hereditary multiple exostoses syndrome.

CDG was initially described in identical twin sisters with developmental delay, decreased thyroxine-binding globulin levels and increased lysosomal enzyme activities. Further analysis using isoelectric focusing (IEF) of serum transferring demonstrated a cathodal shift of their transferring IEF profile, which is now considered pathognomonic for the diagnosis of CDG. With the exception of MPI-CDG (CDG-Ib), which primarily presents with protein-losing enteropathy, the other subtypes of N-linked glycosylation defects all present with a combination of growth and developmental delay, hypotonia, and variable involvement of multiple organ systems. Initially the nomenclature for this group of disorders was CDG followed by the type of transferrin isolectric focusing pattern (I or II) and the letter in order of the identified gene defect (ie, CDG-Ia or CDG-IIa). With the identification of many more types of glycosylation defects, this nomenclature was changed with the gene name, followed by “CDG”, and the older nomenclature in parenthesis (ie, PMM2-CDG (CDG-Ia)).

N-linked glycosylation defects
- **PMM2-CDG (CDG-Ia)**. This is the most common, with over 700 patients described worldwide, and the prototype for the group of the CDGS. It is a multisystem disorder characterized by inverted nipples, abnormal subcutaneous fat distribution, and cerebellar hypoplasia, in combination with hypotonia and developmental delay. In infancy, there is failure to thrive with feeding difficulties and gastroesophageal reflux. Serum liver transaminases are elevated and hypoalbuminemia can occur leading to edema and even anasarca. Clotting factors, particularly Factor XI, antithrombin III, and protein C, can be decreased leading to bleeding following minor trauma or surgery. Seizures are common and there can be stroke-like episodes. Pericardial effusions, renal cysts, retinitis pigmentosa, multiple infections and endocrine abnormalities have been described. The frequency of mortality in infancy is 20%–25%. Adults with PMM2-CDG (CDG-Ia) have developmental delay, ataxia, peripheral neuropathy, scoliosis, and hypergonadotropic hypogonadism. Recent reports have widened the phenotypic spectrum to include hydrpos fetalis at the severe end and a mild neurologic phenotype in adults with multisystemic involvement at the mild end. Reports indicate that the prevalence may be as high as 1:20,000 and the carrier frequency in the Danish population may be as high as 1:60–1:79. It is likely that this disorder is under recognized.

- **MPI-CDG (CDG-Ib)**. Cyclic vomiting, profound hypoglycemia, failure to thrive, liver fibrosis, and protein-losing enteropathy, occasionally associated with coagulation disturbances without neurologic involvement, are characteristic. The clinical course is variable even within families. Taken orally, the sugar mannose appears to improve the growth and gastrointestinal manifestations of this disorder. At least 20 individuals with this diagnosis have been described.
• **ALG6-CDG (CDG-Ic).** ALG6-CDG (CDG-Ic) was previously classified as carbohydrate-deficient syndrome type V\(^{16}\) and is characterized by mild-to-moderate neurologic involvement with hypotonia, poor head control, developmental delay, ataxia, strabismus, and seizures, ranging from febrile convulsions to epilepsy.\(^{17-19}\) The clinical presentation may be milder than in PMM2-CDG (CDG-Ia) and stroke-like episodes and peripheral neuropathy have not been reported. However, retinal degeneration has been reported.\(^{20}\) An adult with ALG6-CDG (CDG-Ic) was described with brachydactyly, deep vein thrombosis, pseudotumor cerebri with normal brain MRI, and endocrine abnormalities including hyperandrogenism with virilization.\(^{20}\) Pubertal abnormalities have been described in an individual with ALG6-CDG (CDG-Ic).\(^{21}\)

Over 30 patients with ALG6-CDG (CDG-Ic) have been described.

• **ALG3-CDG (CDG-Id).** There have been five children described with defects in ALG3. They all had severe psychomotor delay, microcephaly, and severe seizures. Vision impairment was described in 3 infants, 2 with optic atrophy and iris coloboma. Arthrogryposis was described in one of the children. Siblings were described where one had significant digestive issues while the other was more neurologically impaired.\(^{22-25}\)

• **DPM1-CDG (CDG-Ie).** Five individuals had severe developmental delay, microcephaly, seizures, ocular hypertelorism, a “gothic palate,” small hands with dysplastic nails, and knee contractures.\(^{26-29}\) Additionally, two sibs were described with a milder phenotype, consisting of developmental delay, microcephaly, ataxia, and peripheral neuropathy without distinctive features or severe seizures.\(^{30}\) They had nystagmus and strabismus; one had a retinopathy.

• **MPDU1-CDG (CDG-If).** Five individuals had severe psychomotor retardation, generalized scaly, erythematous skin, and attacks of hypertension. In addition, short stature, seizures, and vision impairment have been described.\(^{31,32}\)

• **ALG12-CDG (CDG-Ig).** Seven individuals had distinctive features, generalized hypotonia, feeding difficulties, moderate to severe psychomotor retardation, progressive microcephaly, frequent upper respiratory tract infections, impaired immunity with decreased immunoglobulin levels, and decreased coagulation factors.\(^{33-39}\) Additional features included hypogonadism with or without hypospadias in the males, seizures in two individuals, and cardiac anomalies in two sibs.

• **ALG8-CDG (CDG-Ih).** A four-month-old female had moderate hepatomegaly, severe diarrhea, and hypoalbuminemia from protein-losing enteropathy, normal facial features, and normal development, similar to PMI-CDG (CDG-Ih).\(^ {40}\) She had decreased levels of factor XI, protein C, and antithrombin III. Three other affected individuals had cardiorespiratory difficulties with lung hypoplasia, a severe hepatointestinal disorder, and hypotonia.\(^ {41}\) Two additional patients were described with hypotonia, edema, seizures and psychomotor delay.\(^ {42,43}\) All five individuals had hematopoietic issues with anemia and thrombocytopenia, and early death between ages three days and 16 months. More recently, two siblings with milder disease were described with hypotonia, cognitive impairment, pseudogynecomastia, and ataxia.\(^ {44}\)

• **ALG2-CDG (CDG-II).** A six-year-old had bilateral iris colobomas, unilateral cataract, infantile spasms beginning at age four months, and severe developmental delay; coagulation factors were abnormal.\(^ {45}\)

• **DPAGT1-CDG (CDG-Ij).** The one affected individual described had hypotonia, intractable seizures, developmental delay, and microcephaly.\(^ {46}\)

• **ALG1-CDG (CDG-Ik).** Four affected individuals had severe developmental delay, hypotonia, and early-onset seizures; the latter were intractable in three. Three individuals died between ages two weeks and ten months. As in ALG3-CDG (CDG-Id) and ALG12-CDG (CDG-Ig), also caused by mannosyltransferase defects, microcephaly was rapidly progressive. Other features included severe coagulation defects, nephrotic syndrome, liver dysfunction, coagulation abnormalities, cardiomyopathy, and immunodeficiency.\(^ {47-49}\) Brain imaging showed cerebral atrophy in two individuals and was normal in a third individual. Further studies have shown that ALG1-CDG (CDG-Ik) and PMI-CDG (CDG-Ib) may be the most frequent after PMM2-CDG (CDG-Ia) and present at the severe end of the CDG I clinical spectrum.\(^ {50}\)

• **ALG9-CDG (CDG-IL).** Three children had microcephaly, hypotonia, developmental delay, seizures, and hepatomegaly.\(^ {51-53}\) Two individuals also had failure to thrive and pericardial effusions. Renal cysts and inverted nipples were also described.

• **DOLK-CDG (CDG-Im).** Four affected infants had hypotonia and ichthyosis, and died between ages four and nine months.\(^ {54}\) Additional features included seizures and progressive microcephaly in one and dilated cardiomyopathy in two sibs.
• 
  **RFT1-CDG (CDG-In).** An infant born preterm to unrelated parents had difficulty feeding as a result of an uncoordinated suck and failure to thrive. Myoclonic jerks were noted at three weeks with hypotonia and brisk reflexes progressing to a seizure disorder. Exam was also notable for roving eye movements with normal ERG and reduced VEP. At age two years the child continues to have marked developmental delay.\(^{55-57}\) Five additional affected individuals have been described.\(^{58,59}\) The common features in all six children include severe developmental delay, hypotonia, visual disturbances, seizures, feeding difficulties, and sensorineural hearing loss. Also associated are features similar to other types of CDG including inverted nipples and microcephaly.

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  **ALG11-CDG (CDG-Ip).** A single infant presented with distinctive features (microcephaly, high forehead, and low posterior hairline), hypotonia, and failure to thrive. She had severe neurologic impairment with frequent and difficult to treat seizures. She developed an unusual fat pattern around 6 months of age. She had persistent vomiting and stomach bleeding and passed away at 2 years of age.\(^{60}\)

• 
  **MAGT1-CDG.** This is a defect in a subunit of the oligosaccharyltransferase complex. Reported in a family with two girls with mild cognitive impairment and two boys with more severe cognitive involvement. The mother is reported to have mild cognitive impairment.\(^{61}\)

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  **N33/TUSC3-CDG.** Two different kindreds of siblings with non-syndromic intellectual disability were described to have a defect in this subunit of the oligosaccharyltransferase complex.\(^{62}\)

• 
  **SRD5A3-CDG.** Individuals from seven families were identified with common features including congenital eye malformations (ocular colobomas, optic disc hypoplasia, and variable degree of visual loss), nystagmus, hypotonia, and developmental delay/intellectual disability. Dermatologic complications or congenital cardiac defects were identified in some individuals.\(^{63}\) An additional 12 individuals from nine families were described with cerebellar ataxia and congenital eye malformations.\(^{64}\) Additional mutations in \textit{SRD5A3} have been identified in people with Kahrizi syndrome, which consists of coloboma, cataract, kyphosis, and intellectual disability.\(^{65}\)

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  **MGAT2-CDG (CDG-IIa).** Individuals have facial dysmorphism, stereotypic hand movements, seizures, and varying degrees of psychomotor retardation, but no peripheral neuropathy or cerebellar hypoplasia. A bleeding disorder is caused by diminished platelet aggregation.\(^{66}\) Scoliosis and respiratory compromise can also occur.\(^{67}\)

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  **GCS1-CDG (CDG-IIb) (also known as MOGS-CDG (CDG-IIb)).** An infant with generalized hypotonia, craniofacial dysmorphism, hypoplastic genitalia, seizures, feeding difficulties, hypoventilation, and generalized edema died at age 2.5 months.\(^{68}\)

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  **B4GALT1-CDG (CDG-IIId).** Mild psychomotor retardation, Dandy-Walker malformation, progressive hydrocephalus, coagulation abnormalities, and elevated serum creatine kinase concentration have been observed.\(^{69}\) An additional patient was described with normal development, mild hepatic involvement, and coagulation abnormalities.\(^{70}\)

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  **SLC35A1-CDG (CDG-IIf).** One affected infant presented at age four months with macrothrombocytopenia, neutopenia, and immunodeficiency, and died at age 37 months of complications from bone-marrow transplantation.\(^{71}\)

**O-glycosylation defects**

**O-mannosylglycan defects also known as the dystroglycanopathies**

Defective glycosylation of \(\alpha\)-dystroglycan, a component of the dystrophin-glycoprotein (DAG) complex, has been implicated in several subtypes of muscular dystrophy, known as the dystroglycanopathies. The DAG complex is a multicomponent complex linking the intracellular cytoskeleton with the extracellular matrix in muscle. Alpha-dystroglycan also plays a role in neuronal migration, this in addition to muscular dystrophy; patients with severe defective glycosylation of \(\alpha\)-dystroglycan also demonstrate central nervous system abnormalities. The dystroglycanopathies include the severe congenital muscular dystrophies, Walker-Warburg syndrome (WWS), muscle-eye-brain disease (MEB), and Fukuyama congenital muscular dystrophies (FCMD), as well as non-syndromic congenital muscular dystrophies, subtype IC and ID, and several subtypes of limb-girdle muscular dystrophy (LGMD), specifically, LGMD2I, LGMD2K, LGMD2M, LGMD2N, and LGMD2O.

WWS is the most severe of the dystroglycanopathies and is typically associated with death of affected individuals by the age of 2 years. The diagnostic hallmark is the combination of severe brain malformations, typically described as the Cobblestone complex, muscular dystrophy, and structural eye abnormalities, particularly microophthalmia and can include cataracts. The Cobblestone complex is a combination of type II lissencephaly, agenesis of the corpus callosum, cerebellar hypoplasia, and enlarged ventricles.\(^{72,73}\)
With a slightly milder phenotype than WWS, MEB is characterized by muscular dystrophy, eye involvement (congenital myopia and glaucoma, retinal hypoplasia without congenital cataracts), developmental delay, and structural brain defects ranging from microcephaly to the Cobblestone complex.72,73

FCMD is seen almost exclusively in Japan. Like WWS and MEB, FCMD is also characterized by muscle and brain involvement, however, only occasionally are eyes involved.72,74

Congenital muscular dystrophy type 1C and type 1D are caused by defects in the genes FKRP and LARGE respectively (see below). Like the other congenital muscular dystrophies, there is congenital weakness and developmental delay along with varying degrees of brain malformations,72,75–79 and phenotypes as severe as WWS have been described.80,81

- **POMT1-CDG.** POMT1 encodes the protein-O-mannosyltransferase 1 that catalyzes the first step in O-mannosyl glycan synthesis.82 Mutations in POMT1 have been identified in patients with WWS,83 a milder congenital muscular dystrophy with calf hypertrophy, microcephaly, and cognitive impairment,84 and in patients with LGMD2K.85,86

- **POMT2-CDG.** POMT2 is a second O-mannosyltransferase which complexes with POMT1 for the O-mannosyltransferase activity.87,88 Mutations in POMT2 have been described in patients with WWS,89 a MEB-like phenotype,90 a milder congenital muscular dystrophy phenotype,91 and LGMD2N.92

- **POMGnT1-CDG.** Mutations in the POMGnT1 gene which encodes the glycosyltransferase, O-mannose β-1, 2-N-acetylgalcosaminyltransferase were identified in patients with MEB.93,94 Subsequently, mutations in POMGnT1 were identified in patients with WWS95–98 and LGMD2O.99 POMGnT1 catalyzes the transfer of N-acetylgalactosamine to the O-linked mannose of glycoproteins including α-dystroglycan.

- **FKTN-CDG.** The fukutin (FKTN) gene was initially identified to cause FCMD.100–102 FKTN mutations have been identified in patients with the more severe phenotype, WWS97,103–105 as well as milder non-WWS congenital muscular dystrophy106,107 and LGMD2M.108,109 Mutations in FKTN have also been identified in patients with isolated hyper-CKaemia110 and cardiomyopathy.111,112 The fukutin protein shares sequence homology to a bacterial glycosyltransferase, but its precise function is unknown.113 It has been reported that there is colocalization and molecular interaction of fukutin with POMGnT1, suggesting that fukutin may form a complex with POMGnT1 and modulate its enzymatic activity.114

- **FKRP-CDG.** FKRP encodes the fukutin-related protein. Mutations in this gene cause the widest variability in clinical phenotypes, ranging from in utero, severe WWS to very mild LGMD21. Like fukutin, the function of FKRP is unknown, although it shares homology with FKTN and other glycosyltransferases.

- **LARGE-CDG.** The LARGE gene is the 5th largest gene in the human genome, spanning 664 kb of genomic DNA on chromosome 22q12.3-q13.1, and has homology to the glycosyltransferase gene family,115 and may have bifunctional activity.116 Mutations in LARGE were originally described in MDC1D,17,76 however, mutations in LARGE have also been described in patients with the severe congenital muscular dystrophy, WWS.90

- **GNE-CDG.** GNE encodes a bifunctional enzyme (UDP-N-acetylgalcosamine 2-epimerase/N-acetylmannosamine kinase) in the biosynthetic pathway of sialic acid, and was identified by linkage analysis to be mutated in hereditary inclusion body myopathy (HIBM).117,118 Mutations in GNE cause mostly a distal myopathy, that begins with juvenile onset of muscle weakness which spares the quadriceps and on muscle pathology demonstrates rimmed vacuoles and cytoplasmic and intranuclear inclusions.119,120 GNE mutations were also described in another distal myopathy with rimmed vacuoles, Nonaka myopathy.121,122

### O-xylosylglycan defects

- **4GalT7-CDG.** This defect causes progeroid variant of Ehlers-Danlos syndrome. One patient has been described with premature ageing with loose, hyperelastic skin and joint hyperlaxity. In addition, he had developmental delay, macrocephaly, hypotonia, short stature and osteopenia.2

- **EXT1/EXT2-CDG.** Defects in the Golgi-localized EXT1/EXT2 complex which has both glucuronyltransferase and N-acetyl-D-hexosaminyltransferase activities, cause hereditary multiple exostoses syndrome. EXT1/EXT2-CDG is the most frequent glycosylation defect with an incidence of 1/50,000–100,000 and is the only disorder inherited in an autosomal dominant fashion. It is characterized by benign osteochondromas on the ends of long bones. These lesions are often present at birth, but not usually diagnosed until childhood, progressively grow through adolescence and stop during adulthood. The risk of progression into sarcoma is about 3%.2
O-fucosylglycan defects

- B3GALT1-CDG. Also known as Peters Plus syndrome, this disorder is characterized by abnormalities of the anterior chamber of the eye (of which Peters anomaly is the most common), in combination with other systemic symptoms. Included features can be short stature, developmental delay, craniofacial abnormalities including cleft lip with or without cleft palate.123

O-GalNAc defects

- GALNT3-CDG. Defects in the GALNT3 gene cause familial tumoral calcinosis which is a severe disorder involving phosphatemia and massive calcium deposits in the skin and subcutaneous tissue.124

Combined N- and O-linked defects

Many of the glycosylation pathways, specifically the sugar transporters and the Golgi trafficking, are common to both N- and O-linked glycoprotein synthesis, thus defects in these mechanisms affect both pathways.

- DPM3-CDG (CDG-Io). A single described individual diagnosed with CDG at 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 followed by a stroke-like episode at age 21.125 In addition to abnormal N-linked glycosylation, there was abnormal O-linked mannosylation of α-dystroglycan (similar to the other dystroglycanopathies).

- SLC35C1-CDG (CDG-Ile). Severe growth and psychomotor retardation, microcephaly, hypotonia, craniofacial dysmorphism, and recurrent bacterial infections with persistent, highly elevated peripheral blood leukocyte count are characteristic.126

- COG7-CDG (CDG-Ile). Six affected infants had distinctive features with a small mouth (although one had full lips), microretrognathia, short neck, wrinkled and loose skin, adducted thumbs, and overlapping long fingers; hypotonia; skeletal abnormalities; hepatosplenomegaly; progressive jaundice; seizures; and early death.127–130

- COG1-CDG (CDG-Ilg). An affected infant presented in the first month of life with feeding difficulties, failure to thrive, and hypotonia. She had mild psychomotor delays, rhizomelic short stature, and progressive microcephaly with slight cerebral and cerebellar atrophy on brain MRI, as well as cardiac abnormalities and hepatosplenomegaly.131

- COG8-CDG (CDG-Ilh). Two affected infants were reported who had severe psychomotor delay, hypotonia, seizures, esotropia, failure to thrive, and progressive microcephaly.132,133

- COG4-CDG (CDG-IIj). The first child described presented at 4 months with complex seizure that was treated with Phenobarbital. At age 3 years, additional findings included hypotonia, microcephaly, ataxia, brisk uncoordinated movements, absent speech, motor delays and recurrent respiratory infections.134 A second child presented with failure to thrive, frequent and recurrent infections, diarrhea, hypotonia, hepatosplenomegaly, seizures, elevated liver transaminases, and diffuse cerebral atrophy on brain MRI.135,136

- COG5-CDG (CDG-IIk). A single individual was described with mild developmental delay in motor and language.137

- COG6-CDG (CDG-III). A single infant presented with severe neurologic disease including vitamin K deficiency, intracranial bleeding, vomiting, intractable seizures and early infant death.138

- ATP6V0A2-CDG and other cutis laxa. Multiple patients with autosomal recessive cutis laxa have subsequently been found to have abnormal transferring isoelectric focusing and mutations in the ATP6V0A2 gene.139–141

Diagnostic testing

Since the identification of a protein glycosylation defect in 1984, isoelectric focusing of serum transferrin is the diagnostic screening test of choice.142 This method relies on the amount of negative charge on the transferrin related to the number of terminal sialic residues. Type I pattern is characterized by a decrease in the mature tetrasialo-transferrin and an increase of disialo- and asialo-transferrins due to defective assembly of the N-linked glycans in the ER. A type II pattern is characterized by increases of the trisialo- and monosialo-transferrins due to a defect in processing.143 Since that time, other testing modalities have been employed to recognize deficient glycosylation of serum transferring including capillary electrophoresis,144 high-pressure liquid chromatography (HPLC145). The utilization of multiple mass spectrometry methods has expanded the diagnostics and characterization of glycosylation. This allows both determination of the number of glycosylated sites and the variety of N-linked glycan structures. These methods include electrospray mass spectrometry (ESI-MS146), liquid chromatography (LC) coupled with ESI-MS,147,148 and matrix assisted laser desorption ionization (MALDI)-MS.149,150
Isoelectric focusing of serum apolipoprotein C-III (APOC3) can be done to investigate abnormal mucin type O-glycosylation, which can be seen in some of the combined N- and O-linked glycosylation defects. Immunohistochemistry using antibodies to the glycosylated epitope of alpha-dystroglycan (IIH-6 or VIA4-1) can be utilized to evaluate for dystroglycanopathies.

Once a defect is suspected, confirmation of the particular gene involved requires molecular analysis. Emerging next-generation sequencing of whole exome and whole genome sequencing will aid in identifying defects.

**Management/therapy**

Infants and children with all types of protein hypoglycosylation defects require a multidisciplinary approach. Treatment should be tailored to the individual’s complications. Nutrition support providing maximal caloric intake and/or nasogastric or gastrostomy tube feedings may be necessary. A comprehensive multisystemic evaluation should be done and support given as necessary. Therapeutic adjuncts including physical, occupational and speech therapy should be utilized to aid in the medical management. Antiepileptic therapy should be used for seizures.

PMM-CDG (CDG-Ib), which is characterized by hepatic-intestinal disease, is the only type of hypoglycosylation defect where a specific therapy exists. Mannose given as 1 gram per kg body weight per day divided into five oral doses normalized hypoproteinemia and coagulation defects and rapidly improves the hypoglycemia and protein-losing enteropathy. In some patients with PMI-CDG (CDG-Ib), heparin therapy can be an alternative to mannose in the treatment of the enteropathy.

There has been a report of correction of the infections and improved neutrophil count with fucose treatment in a patient with SLC35C1-CDG (CDG-IIc), a defect in the fucose transporter.

For the dystroglycanopathies, overexpression of LARGE and galgt2 has been shown to improve glycosylation of alpha-dystroglycan in cells from patients and animal models of muscular dystrophy. In addition, patients with dystroglycanopathies have responded to oral steroids with improvement of muscle function.

**Conclusion**

The field of glycosylation defects has been rapidly expanding due to improved clinical awareness and biochemical diagnostic techniques. The clinical spectrum for defects in both N- and O-linked glycosylation is extremely broad, challenging clinicians to screen for these defects in a variety of settings and disciplines. With some 500 genes involved in the synthesis and function of glycoproteins, it is likely that many more defects have yet to be identified.

**Disclosure**

Dr Sparks receives grant funding from NIH/NIAMS and MDA.

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