review

Congenital disorders of glycosylation: Have you encountered them?

Vibeke Westphal, PhD, Geetha Srikrishna, PhD, and Hudson H. Freeze, PhD

Protein glycosylation creates hundreds of sugar structures that serve a spectrum of functions. So it is not surprising that one percent of transcribed genes produce or recognize sugar chains. Given this substantial investment, it is also not surprising that defects in sugar chain biosynthesis have substantial consequences for development, growth, and survival. In the past few years, mutations in seven of these genes have been shown to cause congenital disorders of glycosylation (CDG). Their pathologies, like glycosylation itself, are diverse and affect multiple systems. The CDG are almost certainly underdiagnosed in the community, so increased clinical awareness of the many presentations of CDG is important. Simple diagnostic tests, and possible dietary therapy for some of these patients, make it important to consider and rule out altered glycosylation in unexplained cases of psychomotor retardation, hypotonia, coagulopathy, hepatic fibrosis, protein-losing enteropathy, feeding difficulties, and failure to thrive.

THE SETTING

An overview of the field of glycobiology can be found in a recent comprehensive textbook.[1] Protein glycosylation can be as simple as adding a single sugar (monosaccharide). More often, it is complex. Mammalian cells select nine different monosaccharides and join them to proteins and/or to each other, forming hundreds of different structures (glycans). Branched sugar chains composed of various monosaccharides decorate cell surfaces, extracellular matrices, and secreted proteins. About 1% of the transcribed genome is devoted to the synthesis and recognition of sugar chains.[2] What are their functions? There are many, including proper nascent protein folding, imparting protease resistance, intracellular trafficking, cell-substratum and cell-cell specific interactions, leukocyte trafficking, and growth regulation, to name a few.[1,3–10] A blanket statement of glycan function is not possible, because there are so many structures. In addition, the function of a glycan varies with oligosaccharide presentation, regulated expression of proteins in specific cell types, expression and specificities of glycosidases and glycosyl transferases and microheterogeneity of glycan structures on individual proteins. In some cases, a highly specific glycan structure is essential, while in others, complete absence of any glycosylation seems inconsequential.[1,7,11,12] It is impossible to predict whether the presence of a glycan or a unique structure on a sugar chain will ultimately affect a function. Like protein phosphorylation, glycosylation is a post-translational event and is not read from the genetic template. Instead, an amino acid consensus sequence generates a potential glycosylation site. Many of them are used, but others are not. Furthermore, a single glycosylation site can house a series of structurally related sugar chains at this same location.[13,14] Glycosylation can be diverse, heterogenous, and yet specific. It is maleable, adaptable, yet indispensible for survival, from yeast to humans.[7,11,15]

Much of our understanding of glycan biosynthesis and function emerged from metabolic labeling, analysis of mutant cell lines, and effects of specific inhibitors on glycosylation.[16–19] Recently, the ability to delete genes coding for glycosyltransferases in various biosynthetic pathways of the mouse has shown unsuspected connections to signal transduction,[3] immune regulation,[20,21] development,[22,23] and human disease.[24]

Several types of linkages occur between sugars and proteins. The two major ones are called N- and O-linkages. N-linked glycans are attached through nitrogen of an asparagine and O-linked glycans through the oxygen of serine or threonine. Since the types of CDG discovered so far are primarily disorders of N-glycosylation, we will focus on that in this review. In this pathway, a 14-sugar residue oligosaccharide is first transferred from a transient lipid carrier to nascent proteins in the lumen of the endoplasmic reticulum (ER). The sugar chain is trimmed, sometimes removing most of the original glycan, and then selectively extended with different sugars in various branching patterns (Fig. 1). At least 50 known genes contribute to the synthesis of glycans typically found on plasma proteins. In the past few years, mutations in seven of these genes have been found to cause human disease. Their clinical presentations are, like glycosylation itself, diverse and heterogenous, affecting multiple functions and organ systems.

N-LINKED GLYCOSYLATION

Nearly all cell surface and secreted proteins are N-glycosylated as they pass through the ER during or shortly after they enter the ER lumen. A dolichol pyrophosphate (Dol-PP)-linked 14-sugar oligosaccharide (LLO) is transferred *en bloc* from the lipid carrier to asparagine (Asn) residues in the context Asn-Xaa-Ser or Asn-Xaa-Thr, where Xaa can be any amino acid except proline. The 14-sugar unit is a tri-branched oligosaccharide composed of 3 glucose (Glc), 9 mannose

From The Burnham Institute, La Jolla, California.

Hudson H. Freeze, PhD, The Burnham Institute, 10901 N. Torrey Pines Road, La Jolla, CA 92037.

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(Man), and 2 N-acetylglucosamine (GlcNAc) residues, annotated as $Glc_3Man_9GlcNAc_2$ (Normal Lipid-Linked Precursor, Fig. 1). It is sequentially synthesized using a specific glycosyltransferase to add each sugar. Initially, the growing sugar chain faces the cytoplasmic side of the ER where UDP-GlcNAc contributes GlcNAc-1-P, and a second GlcNAc, and GDP-Man contributes the first 5 Man units. The molecule then "flips" to the lumen of the ER where the remaining 4 Man and 3 Glc, are attached in specific linkages using hexose-phosphate-dolichol donors, Dol-P-Man and Dol-P-Glc, respectively. The oligosaccharide is then transferred to the nascent polypeptide chain by the oligosaccharyl transferase complex located in the ER membrane near the translocation complex. The GlcNAc residue that was directly linked to the Dol-PP lipid is now bound to the amide N of Asn. After the transfer, specific glycosidases trim the $Glc_3Man_9GlcNAc_2$ chain, removing Glc and some Man in the ER. Additional Man is often removed in the Golgi and further trimming of some chains is followed by the addition of 2–4 branches composed of GlcNAc, Gal, and a terminal sialic acid (Sia), to form complex-type sugar chains. The restructuring of these chains is called oligosaccharide processing. Congenital disorders of glycosylation are caused by defective monosaccharide activation or interconversion or by defective addition or removal of the sugar units to the chain. This defect leads to insufficient or abnormal *N*-linked sugar chains on some proteins and the pathology seen in CDG patients.

CONGENITAL DISORDERS OF GLYCOSYLATION

Congenital disorders of glycosylation were previously called carbohydrate deficient glycoprotein syndromes (CDGS). Types were biochemically defined by abnormal isoelectric focusing (IEF) patterns of serum transferrin (Tf).[25] The current nomenclature for CDG is based on the identification of specific gene defects.[26] Group I disorders affect the biosynthesis of the dolichol-linked precursor oligosaccharide and its transfer to proteins. Group II includes defects in N-linked oligosaccharide processing and/or the addition of other kinds of sugar chains to proteins. The type is defined within the group by lower case letters based on the chronological order of discovery of the defective gene. CDG patients with an unknown defect are designated CDG-x. This nomenclature will continue to evolve as more defects in other glycosylation biosynthetic pathways are identified.

BIOCHEMICAL OVERVIEW OF CDG

Table 1 lists the defects identified in CDG, and Figure 1 shows their position in the biosynthetic pathway. All are autosomal recessive disorders. Carriers have normal Tf patterns and are asymptomatic. CDG is divided into two major groups. In Group I disorders (CDG I-a to I-e), there is an absence of entire oligosaccharide chains on some proteins either because of an insufficient amount of precursor oligosaccharide or because it is poorly transferred to the protein by the oligosaccharyltransferase complex.

Several types of CDG specifically affect only the N-linked pathway, while others affect the synthesis of early precursors such as GDP-Man or Dol-P-Man, which are used in multiple pathways. For instance, CDG-Ia (OMIM 212066) is caused by mutations in the *PMM2* gene located on chromosome 16. The gene encodes the phosphomannomutase involved in the conversion from Man-6-P to Man-1-P. Mutations in this gene reduce the size of GDP-Man pool and produces insufficient LLO for full glycosylation.[27,28] A second PMM gene, *PMM1*, is located on chromosome 22. However, this gene encodes an enzyme with a slightly different enzymatic activity and is not involved in the disorder.[29,30] CDG-Ib (OMIM 602579)[31–33] results from mutations in the *MPI1* gene on chromosome 15, encoding phosphomannose isomerase (PMI) that converts fructose-6-P to Man-6-P. Loss of this enzyme also reduces the size of the GDP-Man pool, but the clinical pictures of CDG-Ib and CDG-Ia patients are quite different (see below). Mutations in *DPM1* (chromosome 20), the catalytic subunit of Dol-P-Man synthase, cause CDG-Ie (OMIM 603503). The truncated LLO ($Man_5GlcNAc_2$-PP-Dol) is inefficiently transferred to protein.[34,35] Effects of these mutations on other types of glycosylation have not been systematically examined.

CDG-Ic (OMIM 603147), -Id (OMIM 601110), -IIa (OMIM 212066), and -IIb are all specific to the N-linked glycosylation pathway. Mutations in the human homolog of *ALG6* (chromosome 1), which encodes an α-1,3-glucosyltransferase used to add the first Glc to the LLO precursor, cause CDG-Ic.[36–38] CDG-Id patients are deficient in an α-mannosyl transferase encoded by the *NOT56 l* gene (chromosome 3). Mutations in this gene affect the function of the mannosyltransferase that transfers mannose from dolichol-phosphate mannose (Dol-P-Man) on to the LLO intermediate $Man_5GlcNAc_2$-PP-Dol resulting in an accumulation of the LLO intermediate.[39] CDG-IIb results from the failure of α-glucosidase I to remove the terminal Glc from the oligosaccharide after its transfer from the lipid carrier to protein. CDG-IIa results from defects in *MGAT2* (chromosome 14), a GlcNAc-transferase required for the synthesis of complex sugar chains with two or more Sia-containing branches.[40,41] Leukocyte adhesion deficiency type II (LAD-II, OMIM 116920) results from impaired import of GDP-Fucose into the Golgi from the cytoplasm.[42] This disorder may also be caused by defects in the conversion of GDP-Man to GDP-Fuc.[43] Since the defective genes have not been identified, it is classified as "CDG-X."

Recent estimates of the frequency of CDG-Ia is 1/20,000 in the European populations. This estimation is based on the determination of the frequency of the most common mutation in *PMM2* (G422A → R141H) believed to originate from Scandinavia.[44,45] However, this mutation has never been found in a homozygous state.[44,46] The geographical origin and the frequency of the known *PMM2* mutations have been reviewed recently .[46a]

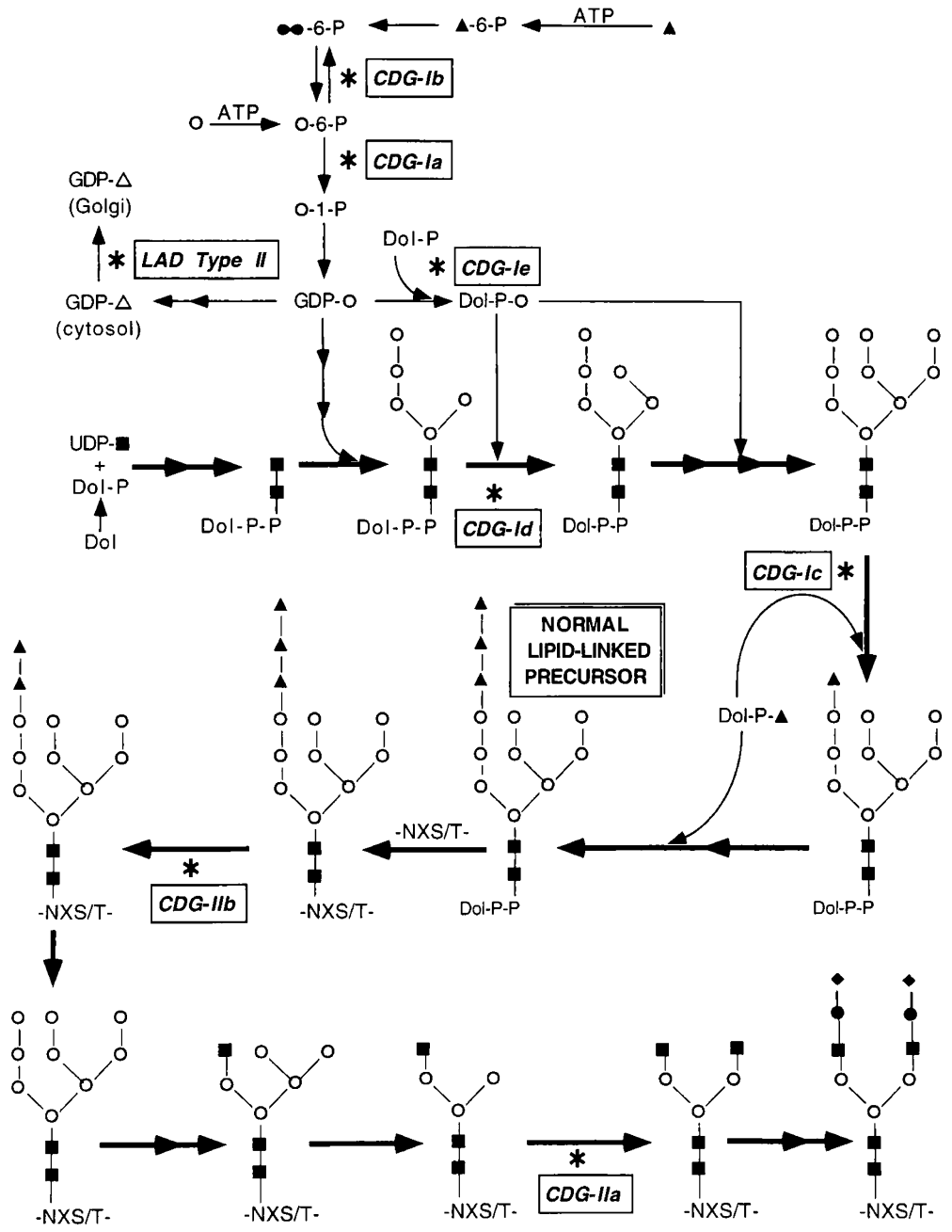

Fig. 1 N-linked oligosaccharide biosynthesis and congenital disorders of glycosylation (CDG). This condensed pathway outlines the biosynthesis and processing of N-linked oligosaccharides. Steps indicated by an asterisk are deficient in various forms of CDG. Symbols are used to denote monosaccharides: (▲) glucose, (⬬) fructose, (○) mannose, (●) galactose, (Δ) fucose, (◆) sialic acid, and (■) N-acetylglucosamine. Starting at the upper left, mannose (○) delivered through the mannose transporter can be used directly for glycoprotein synthesis by conversion to Man-6-P (○-6-P) using hexokinase and ATP. Alternatively, fructose-6-P (⬬-6-P) from glucose (▲) is converted to Man-6-P using phosphomannose isomerase (PMI, the defective enzyme in CDG-Ib). Phosphomannomutase (PMM) converts Man-6-P into Man-1-P (○-1-P). *PMM2* is defective in CDG-Ia. Man-1-P and GTP form GDP-Man (GDP-○) via GDP-Man pyrophosphorylase. GDP-Man has several fates. It can be incorporated into glycoproteins directly or converted into dolichol-P-Man (Dol-P- ○) or into GDP-Fuc (GDP-Δ). Dol-P-Man can be incorporated into glycoproteins or glycophospholipid anchors and into C-mannosylated proteins.[88,89] Dolichol, the lipid carrier for N-linked oligosaccharides is converted to dolichol-P (Dol-P) prior to addition of GlcNAc-1-P (■-1-P) from UDP-GlcNAc (UDP-■), to form Dol-P-P-GlcNAc, the first step in synthesis of the precursor for N-linked chains. A second GlcNAc residue is added to form GlcNAc-GlcNAc core. Five Man are added from GDP-Man, each one using a separate mannosyl transferase. An additional 4 α-mannosyl transferases are required to add the final 4 Man residues derived from Dol-P-Man. Mutations in the first of these four α-mannosyl transferases causes CDG-Id. Defective production of Dol-P-Man causes CDG-Ie. Three separate α-glucosyl transferases use Dol-P-Glc (Dol-P- ▲) to add three tandem Glc residues to the lipid-bound sugar chain. Addition of the first Glc residue is deficient in CDG-Ic. The normal lipid linked precursor oligosaccharide (LLO) chain composed of 2 GlcNAc, 9 Man, and 3 Glc is transferred to Asn-X-Thr/Ser (NXS/T) sequons on proteins in the endoplasmic reticulum. Processing of the high mannose-type N-linked oligosaccharide begins with the removal of all Glc by a set of two α-glucosidases. Defects in the first α-glucosidase cause CDG-IIb. This is followed by removal of a portion of the Man residues using two different α-mannosidases. A GlcNAc residue is transferred to the chain followed by removal of two more Man units and this is followed by the addition of a GlcNAc using GlcNAc transferase I. A second GlcNAc is added using GlcNAc transferase II (*MGAT2*, which is deficient in CDG-IIa). Further build-up of the chain continues by the addition of Gal (●) and finally Sia (◆) residues to generate a two-branched chain. Different branching patterns and other chain extensions can occur on other proteins, but the final structure shown is typical of plasma glycoproteins synthesized by the liver. LAD-II is caused by a defect in the import of GDP-Fuc (GDP-Δ) into the Golgi from the cytoplasm. Like GDP-Man, GDP-Δ is a donor for other types of glycosylation pathways besides the N-linked pathway.

Table 1
Types of congenital disorders of glycosylation

Name	Defective enzyme/gene	Reaction	No. of patients	Year identified	Reference
CDG-Ia	Phosphomannomutase (*PMM2*) OMIM 212066	Man-6-P ⇔ Man-1-P	~300	1995	84
CDG-Ib	Phosphomannose isomerase (*MPI1*) OMIM 602579	Fru-6-P ⇔ Man-6-P	~12	1998	31–33
CDG-Ic	α1,3 Glucosyltransferase (*ALG6*) OMIM 603147	M_9GN_2-Dol → GM_9GN_2-Dol	~14	1999	38, 85
CDG-Id	α1,3 Mannosyltransferase (*ALG3*) OMIM 601110	M_5GN_2-Dol → M_6GN_2-Dol	1	1999	39
CDG-Ie	Dol-P-Man synthase (*DPM1*) OMIM 603503	Dol-P + GDP-Man → Dol-P-Man	4	1999	34, 35
CDG-IIa	GlcNAc transferase 2 (*MGAT2*) OMIM 212066	$GN_1M_3GN_2$ → $GN_2M_3GN_2$	4	1994	40
CDG-IIb	Glucosidase I (*GLS1*)	$G_3M_9GN_2$ → $G_2M_9GN_2$	1?	2000	86
LADII (CDG-x)	GDP-fucose transporter (genetic defect unknown) OMIM 116920	GDP-Fuc transport into the Golgi	3?	1999	42
CDG-X	Genetic defect unknown	GDP-Man ⇔ GDP-Fuc	1?	1998	43, 87

LABORATORY DIAGNOSIS OF CDG

Many glycoproteins are misglycosylated in CDG patients, but serum Tf is widely used as the best biochemical indicator of CDG.[25] Normal Tf has 2 N-linked complex type chains. Each chain usually has two branches each one terminating in a negatively charged sialic acid (Sia), which contributes to the charge on the protein. The extent of sialylation determines the mobility of the protein in electrophoretic separations, such as IEF. The great majority of normal serum Tf contains four sialic acids and is said to be tetrasialylated. Tf molecules lacking one or both sugar chains, or chains with an abnormal underlying structure have fewer Sia and higher isoelectric points (Fig. 2). They are easily resolved from normally glycosylated species. Electrospray ionization-mass spectrometry[47,48] has also been used to analyze the glycosylation status of Tf. Glycosylation of α-1 antitrypsin, haptoglobin, AT-III, and many other plasma proteins is altered in CDG patients,[25,49–51] as is β-trace protein in cerebrospinal fluid.[52] The serum Tf IEF is the best way to detect nearly all CDG cases.[25] However, the abnormal forms were reported to not appear in patients until a few weeks after birth.[53] Also, Tf IEF does not indicate the specific defect. Patients with different defects and clinical features can have identical abnormal patterns. Uncontrolled fructosemia, galactosemia, and heavy alcohol consumption also produce abnormal patterns similar to those seen in CDG patients and should be excluded while making a differential diagnosis of CDG.[54–57] A few types of CDG cannot be detected by Tf IEF. Even with these limitations, any patient suspected of having a CDG should be tested for altered Tf glycosylation.

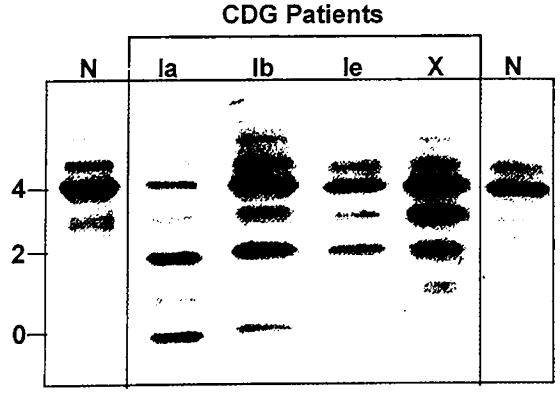

Fig. 2 Examples of transferrin isoelectric focusing patterns in controls and congenital disorder of glycosylation (CDG) patients. The isoelectric focusing (IEF) patterns of transferrin are currently the best way to biochemically confirm CDG. Each transferrin molecule has two N-glycosylation sites, and each one is normally occupied by a sugar chain containing two negatively charged sialic acids to yield "tetrasialotransferrin." This is marked "4" in the figure. Mutations that lead to the absence of one or both chains generate molecules with two or zero sialic acids, respectively. These correspond to di- and asialo forms of transferrin and have different isoelectric points (2 and 0 in the figure). Even when both chains are added, defects in assembly or processing of the chain can also affect the final addition of sialic acids and the isoelectric point.

The hypoglycosylation of Tf observed in the CDG patients can be caused by reduced activity of various enzymes. To test, the specific defect cells from the patient are needed. Fibroblasts or leukocytes can easily be tested for reduced PMM or PMI activity, and fibroblasts are useful for studying the composition of the LLO by metabolic labeling.

Most plasma proteins contain complex-type oligosaccharide chains, and AT-III, protein C, protein S, and Factor XI are frequently underglycosylated in CDG patients leading to coagulopathy, which possibly contributes to the stroke-like episodes in some patients.[58] Coagulation tests such as prothrombin time and prolonged partial thromboplastin time as well as analysis of individual coagulation factors, therefore, are recommended especially if liver biopsies and other invasive procedures are to be considered. Prenatal diagnosis of CDGIa have been possible using chorionic villus samples and cultured amniocytes,[59,60] but prenatal transferrin IEF is not a reliable test.[53,61]

CLINICAL FEATURES OF CDG

Table 2 lists the most common clinical features in various types of CDG.[62,63] Many organ systems are affected to variable degrees, but it is important to stress the clinical heterogeneity even within a single known defect.[64,65] Psychomotor retarda-

Table 2
Clinical features of the congenital disorders of glycosylation (CDG) spectrum

Type	Features
CDG-Ia	Variable psychomotor retardation, hypotonia, peripheral neuropathy, stroke-like episodes, seizures, cerebellar hypoplasia, internal strabismus, abnormal eye movements, subcutaneous fat distribution, inverted nipples, cardiomyopathy, proteinuria
CDG-Ib	Normal development, hypoglycemia, coagulopathy, hepatomegaly, protein-losing enteropathy, hepatic fibrosis, cyclic vomiting, diarrhea
CDG-Ic	Hypotonia, psychomotor retardation, internal strabismus, feeding problems, coagulopathy, seizures, normal cerebellar development
CDG-Id	Hypotonia, intractable seizures, severe psychomotor retardation, microcephaly, reduced responsiveness, optic atrophy, adducted thumbs, high-arched palate
CDG-Ie	Hypotonia, intractable seizures, delayed myelination, cortical blindness, severe psychomotor retardation, high-arched palate
CDG-IIa	Hypotonia, severe psychomotor retardation, frequent infections, normal cerebellum, coarse facies, widely spaced nipples, low set ears, ventricular septal defect
CDG-IIb	Hypotonia, generalized edema, hypoventilation, apnea, hepatomegaly, demyelinating polyneuropathy
LAD-II	Elevated peripheral leukocytes, absence of CD15, Bombay blood group phenotype, failure to thrive, hypotonia, psychomotor retardation, short arms and legs, simian crease

tion ranging from mild to severe (reported in Ia, Ic, Id, Ie, IIa, and LADII patients) and hypotonia (Ia, Ic, Id, IIa, and IIb) are the most consistent features of CDG. Other neurological findings include ataxia (Ia and Ic), seizures, and stroke-like episodes. CT and MRI are recommended in patients with psychomotor retardation since they have revealed cerebellar hypoplasia (Ia, Ic), delayed myelination (Ie, IIa, IIb), microcephaly, and atrophy of the cerebrum (Ia, Ic, Id, Ie). The cognitive deficiency can be mild.[65] Nearly all patients have feeding problems, and fail to thrive, probably reflecting the great demand for cellular proliferation and synthesis of the abundant glycoconjugates.[66] Coagulopathies are frequently seen, and strabismus is also present in most patients. Other ocular findings include cortical blindness, retinal degeneration and reduction in retinal vascularization. Cutaneous and subcutaneous abnormalities include peau d'orange of the skin, abnormal fat distribution, and retracted nipples. Dysmorphic features such as adducted thumbs, dysplastic ears, highly arched palate, and thoracic deformities have also been reported (Id, Ie, IIa).

Hypothyroidism is seen in a majority of Ia patients.[67] Thus, idiopathic neonatal hypothyroidism or euthyroid status with low thyroxine-binding globulin call for investigation of CDG. Male patients with CDG-Ia undergo puberty and develop secondary sexual characteristics, but female patients generally do not.[68] Hypocortisolism has also been observed in CDG-Ic.

The presentations of CDG-Ib patients are dramatically different from CDG-Ia patients, even though their Tf IEF patterns are identical(Fig. 2), and their defects are separated by a single arrow on the metabolic chart (Fig. 1). The CDG-Ib patients do not have mental or psychomotor retardation or neuropathy, but exhibit coagulopathy (e.g., low AT-III), protein losing enteropathy, hypoglycemia, hepatic fibrosis, cyclic vomiting, and diarrhea. Many of these symptoms are treatable as discussed below.

LADII patients are mentally retarded, have failure to thrive, and suffer from recurrent serious infections. In these patients, neutrophil extravasation is greatly reduced due to the absence of fucosylated oligosaccharides, which are critical for neutrophil rolling prior to extravasation.[43]

The constellation of clinical manifestations associated with the disease calls for the attention and care of a multidisciplinary team of pediatricians and pediatric neurologists, hematologists, gastroenterologists, and endocrinologists.

An informal survey of 60 parents of CDG children in the United States conducted by our lab indicated that about 25% of children are correctly diagnosed early on, but most were initially considered to have a "metabolic defect," "multisystemic genetic disorder," or "cerebral palsy." The heterogeneity of patients with these disorders underscores the need to test more broadly than is currently being done. The disorder has almost certainly been underdiagnosed so far. Increased awareness of CDG is evidenced by the growing number of CDG papers and recognition of specific defects (Fig. 3).

The majority of CDG patients diagnosed so far are type Ia (Table 1). The mortality rate in CDG-Ia children is about 20% during the first few years.[62] Throughout childhood CDG-Ia patients may be severely delayed in achieving developmental milestones, but they tend to stabilize after childhood and have stable intellectual status[69] in adolescence and adulthood.[70] Adolescence and adulthood are characterized by progressive motor neuron weakness associated with atrophy of lower limbs. Adolescent CDG patients are extrovert, and communicate well despite dysarthria. Few CDG adults are known and some mentally retarded adults have been diagnosed in their second decade. Some adult patients will do well in assisted living environments. Since CDG-Ia patients tend to survive and stabilize

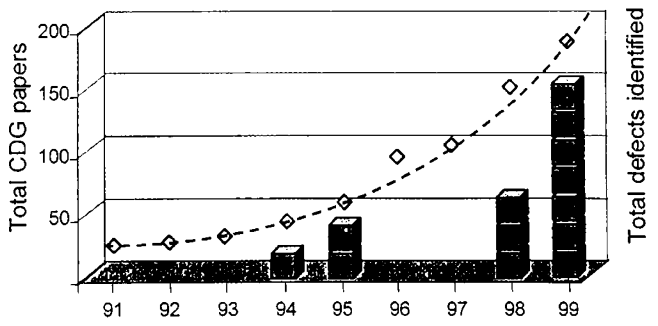

Fig. 3 Congenital disorder of glycosylation (CDG) publications and identification of specific defects. In the past two years, the number of identified CDG defects has increased sharply along with published papers in the field. The trend is likely to continue as additional defects are being uncovered by laboratories that interface glycobiology and clinical medicine. Expanded awareness of CDG will deepen and broaden the appreciation of many roles glycosylation plays in clinical medicine.

after childhood, it is very likely that many adult CDG patients remain undiagnosed.

Only a few cases of CDG-Ib, -Id, -Ie, -IIa, and -IIb are currently known (Table 1). CDG-Ic was discovered 2 years ago, and may be more common than the number in Table 1 indicates, since 7 mutations have already been found in the defective gene encoding an α-1,3 glucosyltransferase.[37,70a,71,72]

THERAPY

Most CDG patients significantly benefit from typical palliative therapies. The only effective treatment therapy is oral mannose for CDG-Ib. This therapy works because mannose can be transported into cells by a mannose-specific transporter and then converted to Man-6-P via hexokinase, effectively bypassing the impaired fructose-6-P 224 Man-6-P pathway and replenishing the depleted GDP-Man pools.[27,33] Four doses of 0.1–0.15 g/kg per day mannose reverses hypoglycemia and deficient AT-III within a few weeks, and within 1–2 months plasma protein levels are normal and protein-losing enteropathy disappears.[33,73,74] A few patients have now been on mannose supplemented diets for several years with only minor side-effects (bloating, loose stools). Excessive dosing can increase HbA1c levels, which return to normal when mannose intake is reduced.[75]

Few foods contain free mannose, except for milk (50–100 μM), although plant and animal glycoproteins contain available mannose. Plant products such as locust bean gum are composed of galactomannan,[76] but most of the mannose is probably unavailable because it occurs in a beta-linkage, rather than the alpha linkage seen in most oligosaccharides. Carob powder, which is made from this gum contains a small amount of free mannose, but it should not be considered as a therapeutic mannose substitute.

Early studies in cultured fibroblasts from CDG-Ia patients showed that adding 200–500 μM mannose to the growth medium corrects inadequate glycosylation[77] by replenishing the deficient GDP-Man pool. Mannose is efficiently absorbed from the gut and multiple oral doses can maintain plasma levels at several fold higher than the normal average of 55 μM, although CDG-Ia patients have lower steady state levels (5–40 μM).[78] Short-[79,80] and longer-term[81] therapy studies to date have not shown verifiable improvement in the clinical condition or biochemical markers for the CDG-Ia patients. Some mannose therapy studies are still under way. Another therapeutic alternative would be a hydrophobic "prodrug" derivative of Man-1-P that could enter cells and generate the parent compound using resident cytosolic esterases. This approach is being explored. Gene replacement therapy has not been attempted.

One LAD-II patient has also shown a positive response to monosaccharide therapy.[82] Fucose therapy immediately normalized the neutrophil levels, and over time partially restored synthesis of the fucosylated oligosaccharides. Fevers and recurrent infections disappeared, prophylactic antibiotics were discontinued, and the patient gained weight. Significant psychomotor improvement was also observed in this child, probably due to the more stable clinical status.

TESTING FOR CDG

When is it appropriate to test for CDG? Certainly patients showing variable psychomotor retardation, hypotonia, and the classic dysmorphic features of inverted nipples, fat pads, strabismus, and decreased AT-III are likely candidates. However, unexplained feeding problems, failure to thrive, hepatic fibrosis, gastrointestinal abnormalities, coagulopathy, cerebellar ataxia, hypothyroidism and hypoglycemia could also be due to CDG. Even in the absence of psychomotor retardation, severe neurological disease, and dysmorphism, CDG should be ruled out. Again, Tf IEF will detect most of these cases. Since CDG-Ib is treatable, Tf IEF is imperative in cases of unexplained hepatic dysfunction, protein-losing enteropathy, coagulopathy, and hypoglycemia.

Neither LAD-II nor CDG-IIb, the α1,2glucosidase deficiency, can be detected by the Tf IEF test. The latter was detected by the accumulation of a tetrasaccharide in the urine. This molecule resulted from an endo-α-mannosidase cleavage of the sugar chains shortly after their addition to protein. LAD-II was identified by the absence of fucosylated glycan antigen CD15, and presence of Bombay blood group phenotype, which indicates the absence of the fucosylated H-antigen.[83]

Tf IEF analysis to identify CDG patients is available at several locations including Mayo Medical Laboratories, University of California San Diego Biochemical Genetics Laboratory, and at Laboratory for Molecular Diagnosis, University Hospital Leuven-Gasthuisberg, Belgium. Enzymatic analysis for Types-Ia and I-b are available in San Diego and Leuven. Additional molecular analysis and/or identification of other CDG defects are also available at research laboratories affiliated with these centers.

In 1996, parents of CDG children in the United States founded The CDG Family Network, Inc. By publishing a newsletter, arranging annual family conferences, through the Internet page *www.cdgs.com*, and email correspondence *cdgforum@ultranet.com*, this nonprofit organization provides information and support for the CDG community. Similar groups in Scandinavia (*http://www.emu.lu.se/cdg/indexeng.shtml*) and Germany (*http://www.cdg-syndrom.de/*) provide information in Europe.

IDENTIFICATION OF MORE PATIENTS AND NEW DEFECTS

Based on current population and birth rates, Matthijs estimates that over 100 CDG-Ia patients are born each year in the United States and a similar or higher number in Europe (Matthijs, personal communication). Because <100 CDG patients of all types have been identified in the United States, the current spectrum of CDG is highly underdiagnosed. Additional defects are being identified in the N-linked pathway and many of them affect the addition of glycan chains to proteins and

should be apparent from Tf IEF analysis. However, it is important to point out that CDG-IIb and LAD-II Tf patterns are normal; therefore, other methods to detect altered glycosylation, e.g., lectins or carbohydrate specific antibodies need to be developed. Mice ablated in specific glycosyltransferases show altered lectin binding patterns in blood,[21,24] but animal experiments often strive for complete loss of function and give a clearly altered lectin binding picture. CDG mutations that are compatible with life appear to be leaky and lectin or antibody probes may show less dramatic changes than those seen in knockout mice.

Furthermore, genetic background and environmental stresses, such as infections, fever, and nutritional state could be important determinants in the severity of CDG expression. Heightened awareness of glycosylation as a cause of inherited disease, appreciation of the diversity of CDG patients, and broader-based testing for these disorders will all contribute to the diagnosis of new and previously overlooked patients, the discovery of new types of CDG, and the development of potential therapies for them.

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