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New Disorders in Carbohydrate Metabolism: Congenital Disorders of Glycosylation and Their Impact on the Endocrine System

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Introduction

Protein glycosylation occurs in all cells. It can be used to insure proper folding of nascent proteins, impart protease resistance, dictate intracellular trafficking, cell-substratum and cell-cell specific interactions, leukocyte trafficking, and growth regulation [1–9]. For some proteins, a highly specific sugar chain is essential for function. In others, complete absence of any glycosylation seems inconsequential [10,11]. Glycoproteins are involved in virtually every endocrine axis. Protein glycosylation can affect the stability, binding affinity and ligand specificity of polypeptide hormones, hormone carrier proteins and hormone receptors. Therefore, an abnormality of protein glycosylation would be expected to have an impact on many endocrine functions.

In this review of the Congenital Disorders of Glycosylation (CDG) we cover the basics of protein N-glycosylation and the known types of CDG. Then we focus on endocrine functions of CDG patients and attempt to describe and interpret the effect of hypoglycosylation on these complex processes. In addition, we hope to make physicians in the fields of endocrinology and metabolic diseases more aware of the broad spectrum of these disorders.

Overview of N-Linked Glycosylation

CDGs are caused by defects in the synthesis, transfer, and remodeling of a universal 14 sugar residue precursor oligosaccharide, which is first added to nascent proteins in the lumen of the endoplasmic reticulum (ER). During oligosaccharide processing many of these chains are trimmed and then extended once again with other sugars in various branching patterns (Fig. 1). More than 50 genes contribute to the synthesis of glycans found in typical plasma glycoproteins [12,13].

The dolichol pyrophosphate (Dol-PP)-linked 14-sugar oligosaccharide (LLO) is composed of 3 glucose (Glc), 9 mannose (Man) and 2 N-acetylglucosamine (GlcNAc) residues, $Glc_3Man_9GlcNAc_2$. It is transferred *en bloc* from the lipid carrier to proteins containing an available Asn-X-Thr/Ser sequence. Each sugar is added to the growing LLO in a specific order using at least 13 glycosyltransferases. GDP-Man contributes the first 5 Man units on the cytoplasmic face of the ER and then the molecule "flips" to the lumen of the ER [14] where 4 Man and 3 Glc are added via 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. After the transfer, the sugar chain is processed. Specific glycosidases trim the $Glc_3Man_9GlcNAc_2$ chain, removing all Glc and some Man in the ER. Additional Man is often trimmed in the Golgi followed by the addition of 2–4 branches composed of GlcNAc, Gal and a terminal sialic acid (Sia) to form complex-type sugar chains.

Defining Congenital Disorders of Glycosylation

Mutations have now been found in 11 genes of this pathway. Congenital disorders of glycosylation used to be called Carbohydrate Deficient Glycoprotein Syndromes (CDGS) and were biochemically defined by abnormal isoelectric focusing (IEF) patterns of serum transferrin (Tf) [15]. This is still the most widely used diagnostic test, but

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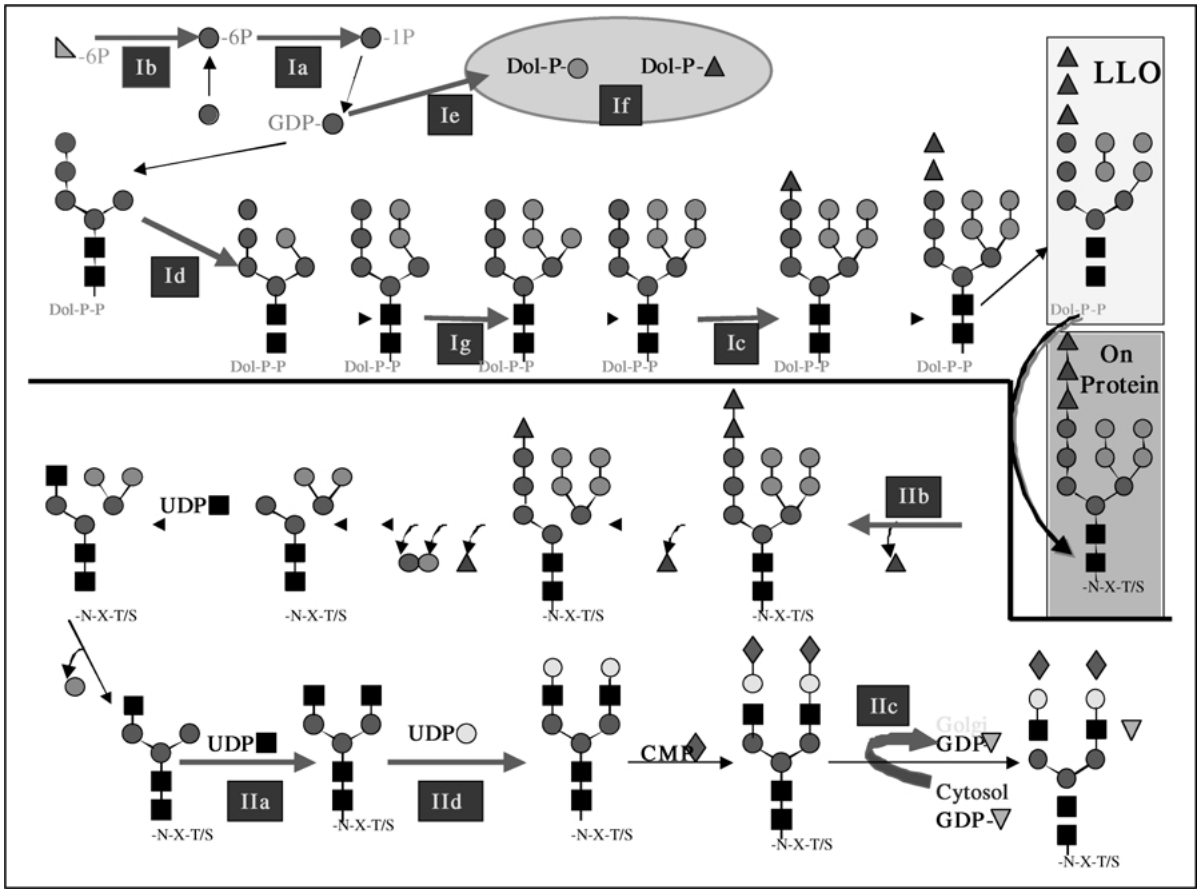

Fig. 1. *N-linked oligosaccharide biosynthesis and congenital disorders of glycosylation. Many steps of N-linked oligosaccharide biosynthesis and processing are shown. Heavy red lines indicate a defective step that causes CDG, and the specific type is shown in the box below the line. Symbols for monosaccharides: (● or ●) mannose, (◺) fructose, (B) N-acetylglucosamine, (▲) glucose, (○) galactose, (♦) sialic acid, and (▽) fucose.*

Beginning at the upper left, Fructose-6P (◺-6P) is converted to Man-6P (●-6P), using phosphomannose isomerase (PMI, the defective enzyme in CDG-Ib). Alternatively, mannose (●) is directly phosphorylated to yield Man-6P. Phosphomannomutase (PMM) converts Man-6P into Man-1P (●-1P). PMM2 is defective in CDG-Ia. Man-1P and UTP form GDP-Man (GDP-●) via GDP-Man pyrophosphorylase. GDP-Man can be incorporated into glycoproteins directly or converted into dolichol-P-Man (Dol-P-●). Although not shown in the figure, dolichol, the lipid carrier for N-linked oligosaccharides is converted to dolichol-P (Dol-P) and GlcNAc-1-P (B-1P) is added to it forming Dol-P-P-B. Another UDP-GlcNAc donates a second B to form Dol-P-P-BB and then GDP-Man adds 5 Man units, each one using a separate mannosyl transferase forming $Man_5GlcNAc_2$-PP-Dol. As shown next, Dol-P-Man adds 4 α-mannosyl units. Mutations in the addition of the first Dol-P-Man-dependent sugar cause CDG-Id. Defective production of Dol-P-Man itself causes CDG-Ie. Improper positioning of Dol-P-Man and Dol-P-Glu due to defects in MPDU1 reduces the efficiency of adding both of these sugars to the growing chain, causing CDG-If. Defects in the addition of the 8th Man unit cause CDG-Ig. 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 shown in the box at the extreme upper right. This entire oligosaccharide is then transferred to Asn-X-Thr/Ser (NXT/S) sequons on proteins in the ER as highlighted in the box immediately below the LLO. The heavy horizontal line separates Group I and II disorders. N-linked oligosaccharide processing begins with the removal of all Glc by a set of two α-glucosidases. Defects in this first α-glucosidase cause CDG-IIb. This is followed by removal of some Man residues using at least 5 different α-mannosidases that are variably expressed in different tissues. These lead to production of chains with 5 Man units. GlcNAc transferase I, encoded by MGAT1, adds a GlcNAc residue followed by conversion to Man3 by α-mannosidase-II. GlcNAc transferase II encoded by MGAT2, now adds a second GlcNAc. This transferase is deficient in CDG-IIa. Further build up of the chain continues by the addition of Gal (○), which is deficient in CDG-IId, and finally addition of Sia (♦) residues generate a two-branched chain. Different branching patterns and other chain extensions can occur on other proteins, but this is typical of plasma glycoproteins. CDG-IIc (LAD-II) is caused by defects in the GDP-Fuc (GDP- ▽) transporter that carries this donor from the cytoplasm into the Golgi.

the specific types of CDG are now defined by the defective gene [16], since transferrin patterns do not distinguish various types. Group I disorders (Types Ia—Ig) affect the biosynthesis of the dolichol-linked precursor oligosaccharide and its transfer to proteins. This leads to insufficient or poorly transferred sugar chains and unoccupied glycosylation sites on proteins. Group II (Types IIa–IId) includes any defect in N-linked oligosaccharide processing on the protein. CDG patients with a genetically unproven defect are called CDG-x.

Table 1. *Congenital disorders of glycosylation (CDG)*

CDG type	Enzymatic or protein defect	Gene	OMIM[a]	Chromosome location	Patients	Ref.
Ia	Phosphomannomutase 2 (PMM)	*PMM2*	212065 601785	16p13	~300	[17]
Ib	Phosphomannose isomerase (PMI)	*MPI*	602579 154550	15q22-qter	~20	[18–20]
Ic	Dolichyl-P-Glc:$Man_9GlcNAc_2$-PP-dolichyl α-1,3-glucosyltransferase	*ALG6*	603147 604566	1p22.3	~30	[21,22]
Id	Dolichyl-P-Man:$Man_5GlcNAc_2$-PP-dolichyl α-1,3-mannosyltransferase	*ALG3* *NOT56L*	601110	3	2	[23]
Ie	Dolichol-P-Man synthase 1	*DPM1*	603503	20q13	4	[24,25]
If	Dolichol-P-Man utilization defect 1 (suppressor of Lec35)	*MPDU1*	604041	17p12-13	4	[26,27]
Ig	Dolichyl-P-Man:$Man_7GlcNAc_2$-PP-dolichyl α1,6mannosyltransferase	*ALG12*	–	22	5	[28]
Ix	Multiple Defects: causes unknown	–	603585 212067	–	?	
IIa	UDP-GlcNAc:α-6-D-mannoside β-1,2-N-acetylglucosaminyltransferase II (GnT II)	*MGAT2*	212066 602616	14q21	6	[29]
IIb	α-1,2-glucosidase I	*GCS1*	601336	2p12-13	1	[30]
IIc (LAD-II)	GDP-fucose transporter (cytosol → Golgi)	–	266265	11p11.2	4	[31,32]
IId	UDP-Galactose:N-acetylglucosamine β1,4GalactosyltransferaseI	*B4GALT*	–	9	1	[33,34]

[a]OMIM = Online Mendelian Inheritance in Man (http://www.ncbi.nlm.nih.gov/).

Biochemical Overview of CDG

The CDGs are autosomal recessive disorders, and the defective genes are shown in Table 1. Carriers are asymptomatic and usually have essentially normal Tf IEF patterns. By far the most common type is CDG-Ia (OMIM 212066), which is caused by mutations in the *PMM2* gene [17,35,36]. The gene encodes the phosphomannomutase involved in the conversion of Man-6-P to Man-1-P. Mutations in this gene reduce the size of GDP-Man pool and produce insufficient LLO for full glycosylation [37,38]. Some of these patients have been mistaken for having mitochondrial disorders [39]. CDG-Ib (OMIM 602579) [18–20] results from mutations in the *MPI1* gene encoding phosphomannose isomerase (PMI) (fructose-6-P → Man-6-P). The clinical pictures of CDG-Ib and CDG-Ia patients are quite different (see below). CDG-Ic (OMIM 603147) is caused by mutations in *ALG6* which encodes an α-1,3glucosyltransferase used to add the first Glc to the immature LLO precursor [21,24,40]. Most of the other types of CDG are rare with only a few patients and will not be discussed in detail. The reader is referred to more extensive reviews [41–43].

Laboratory Diagnosis of CDG

Glycosylation of serum Tf is the most commonly used biochemical indicator of CDG [15]. Normal Tf has 2 N-linked sugar chains, and each usually carries two negatively charged sialic acids (Sia). The great majority of normal serum Tf is tetrasialylated, but Tf molecules lacking one or both sugar chains, or those having incomplete chains will have fewer Sia. Those differences are easily resolved by isoelectric focusing. Electrospray ionization-mass spectrometry [44,45] is a more precise and effective method since it can differentiate molecules that lack 1 or more sugar units from those that lack entire sugar chains. This information can provide clues to the possible defect. Neither method indicates the genetic defect; since several defects all generate identical abnormal patterns. Severity of the abnormal pattern does not correlate with disease severity. The serum Tf analysis remains the best way to detect nearly all CDG cases [15,46]. False positives arise in cases of uncontrolled fructosemia, galactosemia, heavy alcohol consumption and various causes of liver disease, which need to be excluded when considering CDG [47–51]. Prospective CDG patients should be tested for altered Tf glycosylation. False negatives also occur, so a normal pattern does not exclude CDG [52–54]. Electrospray mass spectrometric analysis is available at Mayo Medical Laboratories and the University of California San Diego Biochemical Genetics Laboratory. Tf IEF can also be done at the Laboratory for Molecular Diagnosis, University Hospital Leuven-Gasthuisberg, Belgium. Adaptation of DNA sequencing technology hardware and detection offers another way to detect altered glycosylation of total serum proteins. This approach is in early

developmental stages, but is likely to become an important diagnostic tool in the future [55].

Limited Therapy Options for CDG

The only effective therapy is oral mannose for CDG-Ib. Mannose entering the cell is converted to Man-6-P via hexokinase, effectively bypassing the impaired fructose-6-P → Man-6-P pathway and replenishing the depleted GDP-Man pools [20,37]. 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 [20,56,57]. None of the adult patients with proven CDG-Ib is currently taking mannose. They appear to be doing well, suggesting that mannose therapy will not be required for life. CDG-Ib patients and families are cautioned not to substitute commercial "neutraceutical mannose" for authentic mannose.

Short [58,59] and longer term [60] mannose therapy studies on CDG-Ia patients showed no verifiable improvement in the clinical condition or biochemical markers. Another therapeutic alternative in early exploratory stages is the creation of a hydrophobic "prodrug" derivative of Man-1-P that can freely enter cells, where it meets cytosolic esterases that generate the active compound.

One CDG-IIc patient responded to fucose therapy [61] and immediately normalized neutrophil levels, and over time partially regained some 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.

Common Clinical Features of CDG

The most common clinical features in various types of CDG [41,43,62–64] are shown in Table 2. There is considerable clinical heterogeneity [65–68]. Psychomotor retardation ranging from mild to severe and hypotonia are consistent features in all patients except Type-Ib. 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). Sometimes the cognitive deficiency can be mild [66,67]. Nearly all patients have feeding problems and fail to thrive. Strabismus is common and cortical blindness, retinal degeneration and reduction in retinal vascularization are seen. Cutaneous and subcutaneous abnormalities include peau d'orange of the skin, abnormal fat distribution, and retracted nipples.

Mortality in CDG-Ia children is about 20% during the first few years [63]. Throughout childhood CDG-Ia patients may be severely delayed in achieving developmental milestones, but then stabilize after childhood. Adolescents and adults have stable intellectual status [69,70], but there is progressive motor neuron weakness and atrophy of lower limbs. Since CDG-Ia patients tend to survive and stabilize after childhood, it is very likely that many adult CDG patients remain undiagnosed.

Table 2. *Clinical features seen in different types of CDG*

Type	
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, cryptorchidism, hypoglycemia, hypogonadism, hypocholesterolemia, growth retardation, abnormal thyroid function, tests ± hypothyroidism, osteopenia
Ib	*Normal* development, hypoglycemia, coagulopathy, hepatomegaly, protein-losing enteropathy, hepatic fibrosis, cyclic vomiting, diarrhea, growth retardation
Ic	Hypotonia, psychomotor retardation, internal strabismus, feeding problems, coagulopathy, seizures, normal cerebellar development, hypocortisolism
Id	Hypotonia, intractable seizures, severe psychomotor retardation, microcephaly, reduced responsiveness, optic atrophy, adducted thumbs, high-arched palate
Ie	Hypotonia, intractable seizures, delayed myelination, cortical blindness, severe psychomotor retardation, high-arched palate
If	Hypotonia, frequent seizures, blindness, dry skin, severe psychomotor retardation, severe failure to thrive, decreased food intake, frequent vomiting
Ig	Hypotonia, severe psychomotor retardation, feeding difficulties, facial dysmorphy, coagulopathy
IIa	Hypotonia, severe psychomotor retardation, frequent infections, normal cerebellum, coarse facies, widely spaced nipples, low set ears, ventricular septal defect
IIb	Hypotonia, generalized edema, hypoventilation, apnea, hepatomegaly, demyelinating polyneuropathy
IIc	Hypotonia, elevated peripheral leukocytes, failure to thrive, psychomotor retardation, short arms and legs
IId	Hypotonia, hydrocephalus, Dandy-Walker malformation, myopathy, coagulation abnormalities

Specific Endocrine Dysfunctions in CDG

Endocrine effects of CDG on sexual development

Hypergonadotropic hypogonadism. The original twin girls with CDG described by Dr. Jaeken had fluctuating levels of FSH and failed to have pubertal development [71]. Most other females with CDG of pubertal age have been described as having an absence of secondary sexual characteristics [70]. Studies of these children have demonstrated hypergonadotropic hypogonadism with elevated FSH and LH and low estradiol [72,73]. Normal pubertal development has been described in four women, one with CDG 1b [74], one with CDG 1f [64] and two with untyped CDG [75,76]. The young woman with CDG1b delivered three healthy children after uncomplicated pregnancies [74]. Males with CDG have been described to have normal or delayed puberty with normal virilization, small or normal testes, and testosterone values in the low to normal range [72,73].

The lack of pubertal development and primary ovarian failure in females with CDG resembles the phenotype in galactosemia [77]. In galactosemia, males have normal pubertal development and sexual function. Females have a high rate of pubertal delay or failure and primary or secondary amenorrhea with elevated gonadotropin levels suggestive of primary ovarian failure. The bioactivity of urinary gonadotropins from female galactosemics was normal suggesting ovarian resistance. However, there is some evidence in galactosemic women for residual ovarian function. In one of two patients, Kaufman et al. found a normal estrogen response to short term therapy with human menopausal gonadotropin (HMG, mostly FSH) [78]. A similar response to HMG has been found in patients with CDG [72,79]. However, the biological activity of FSH in CDG women was in the low normal range using a rat granulosa aromatase bioassay [72]. In addition, the half-life of FSH was low to normal in CDG consistent with previous data showing increased clearance of desialylated FSH [80,81]. The electrophoretic mobility of FSH in females with CDG was more consistent with that obtained from menopausal women and did not resemble the FSH pattern characteristic of the follicular phase of the menstrual cycle [73]. These findings suggest that reduced FSH bioactivity and bioavailability, due to inappropriate or insufficient glycosylation, is responsible for delayed or absent sexual maturation in girls with CDG.

Hypogonadism in CDG also resembles the phenotype of an inherited FSH receptor defect. In this condition, a mutation occurs in a region of the FSH receptor gene adjacent to a glycosylation site necessary for FSH binding. Males homozygous for this mutation undergo normal puberty followed by hypogonadism in adulthood characterized by low testosterone levels, small testicles and elevated FSH levels [82]. In the female, there is primary amenorrhea and variable degrees of pubertal development [83]. The glycosylation status of the FSH receptor has been shown to affect it's production and may affect it's ligand binding affinity [84]. In contrast, the unglycosylated LH receptor had normal ligand binding and signal transduction activity [85]. It is likely that sexual development in children with CDG is affected by hypoglycosylation of both FSH and its receptor.

Another potential negative effect of hypogonadism is incomplete bone mineralization. The sex steroids play a very important role in promoting bone mineralization. Inadequate levels of testosterone and estradiol in patients with hypogonadism could lead to low peak bone mineral density. Osteopenia has been reported in multiple patients with CDG [86,87]. This effect on bone mineralization could lead to increased fractures over the long-term.

Cryptorchidism. Cryptorchidism is present at birth in 3% of full-term and 17% of premature males and less than 1% of males by 9 months of age [88]. The incidence of cryptorchidism is elevated in a number of genetic diseases [89]. It is also an early manifestation of hypogonadotropic hypogonadism. Numerous males with CDG and cryptorchidism have been previously reported [90] [91–93]. A retrospective review of patients with CDG 1a enrolled in a recent U.S. open-label study of oral mannose therapy revealed that cryptorchidism was present in at least 56% of participating males (unpublished results). This would suggest that cryptorchidism should be one of the defining features of this condition and along with other features of CDG should warrant diagnostic evaluation.

Finally, as with many processes in children with CDG, it is likely that hypoglycosylation affects hormonal regulation at multiple steps along the pathway. Sexual development in children with CDG is likely affected by hypoglycosylation of both FSH and its receptor. The lack of affect of hypoglycosylation on LH receptor function may help to explain the near normal sexual development in CDG males. It is the combination of these effects that leads to the ultimate phenotypes of sexual development in males and females with CDG.

Endocrine effects of CDG on adrenal function

Serum cortisol levels in CDG have been found to be low or normal [72,94]. A patient with CDG 1c developed hypocortisolism requiring cortisol replacement in the setting of severe hypoproteinemia secondary to a protein-losing enteropathy [94]. Twin sisters with CDG 1a were found to have normal free cortisol levels with low transcortin values [72]. Cortisol is heavily protein bound

in serum, chiefly to albumin and transcortin. Studies of transcortin suggest that glycosylation is required for its normal intra-cellular processing and secretion, but not for its ability to bind cortisol [95,96]. These findings suggest that the hypocortisolism seen in the patient with CDG 1c and a protein-losing enteropathy is likely due to both the combination of low transcortin and generalized hypoproteinemia. Review of the literature shows few cases of hypocortisolism in the setting of hypoproteinemia. One involved congenital nephrotic syndrome and associated hypothyroidism [97]. Another was an 8 week old infant with untyped CDG found to have a low random cortisol in the setting of nephrotic syndrome [98].

Endocrine effects of CDG on thyroid function

Thyroid function tests are frequently abnormal in children with CDG. Thyrotropin (TSH) is a glycoprotein secreted by the pituitary which regulates production of thyroxine (T4) and triiodothyronine (T3) by the thyroid. The effects of glycosylation on function of TSH and its receptor were recently reviewed [99]. Glycosylation has been shown to affect TSH bioactivity and receptor affinity [100–102]. Thyroid binding globulin (TBG) is the primary serum carrier glycoprotein for T4 and T3. The most common laboratory abnormality in thyroid function of CDG patients is a low total T4 reflective of partial TBG deficiency.

The initial characterization of CDG in identical twins included partial deficiency of thyroid binding globulin (TBG) which is present in approximately 75% of patients with CDG [62,103]. Patients with CDG showed an increase in hypoglycosylated TBG [15]. TBG hypoglycosylation reduces its half-life by 15% [62,103]. However, TBG isolated from patients with CDG has normal immunoreactivity and affinity for T4, T3, and rT3 [104]. Abnormalities in thyroid binding capacity have been found in CDG 1a, 1b and 1x (unpublished data) and CDG II [105]. Since the degree of TBG deficiency does not appear to be sufficient to explain the reduction of thyroid binding capacity, Macchia et al. speculated the presence of an interfering substance [104]. In general, the partial TBG deficiency does not appear to affect thyroidfunction.

A diagnosis of chemical hypothyroidism is made on the basis of low free thyroxine and elevated TSH. TSH values have previously been shown to be age-dependent with a slight elevation in infancy normalizing in late childhood [72]. However, TSH in CDG contain an abundance of poorly processed carbohydrates [91]. The analysis of free thyroxine has been in the low to normal range [72]. Free thyroxine analyzed by equilibrium dialysis, the most accurate method, has been reported as normal in a group of seven patients with CDG [104]. Diagnosis of hypothyroidism and L-thyroxine supplementation should be reserved for those children with elevated TSH and low free thyroxine measured by equilibrium dialysis. In general, children with CDG are chemically euthyroid.

Endocrine effects of CDG on growth

Children with CDG exhibit growth failure that may be nutritional and/or a result of dysregulated hormone signaling. Somatic growth in children is regulated by growth hormone. Growth hormone exerts its effects on somatic growth by activation of the insulin-like growth factor cascade. Growth Hormone Binding Protein, a soluble form of the external domain of growth hormone receptor, is decreased in poor nutrition and in some patients with primary growth hormone resistance and poor nutrition. Circulating Insulin-like Growth Factors (IGFs) are produced primarily in the liver in a growth hormone-dependent fashion [106]. Free IGF acts through cell-surface receptors to stimulate growth in numerous end organs. The levels of free IGF in tissues and serum are regulated by the formation of complexes with IGF binding proteins, IGFBPs [107]. In the serum, greater than 90% of IGF is found in a ternary complex comprised of IGF, IGFBP-3 and an acid-labile subunit (ALS) [108]. The formation of the ternary complex is important in determining the stability of IGFs in serum [109] and their delivery to the target tissues. The degree of ALS glycosylation has been shown to affect its ability to form a complex with IGFBP-3 [110,111]. Glycosylation of IGFBP-3 does not affect its ability to bind IGF, but may increase its susceptibility to proteolysis [112,113].

Previous studies of the GH/IGF cascade in children with CDG have shown that random levels of GH are low in males and high to very high in females. Serum IGF-1 concentrations were low in infants and children and low to normal in adolescents independent of gender [72] GHBP, IGFBP-3 and ALS values have not been reported in children with both CDG and failure to thrive. IGFBP-3 and ALS values are lower in children with CDG than in age-matched controls [114]. There has been one report of a young lady with normal levels of IGF-1, IGFBP-3 and GHBP [75]. However, except for patients with CDG 1b and CDG 1f, this young woman is the only reported patient with CDG 1 to have experienced normal puberty. The patient with CDG 1f was investigated because of growth retardation at 2 years and found to have growth hormone deficiency and dwarfism as an adult [64]. One girl with an untyped CDG-x had documented growth hormone deficiency found in the setting of hypoglycemic episodes and proven by growth hormone stimulation testing with insulin-induced hypoglycemia and arginine [91]. She had also been diagnosed with hypothyroidism nine months earlier and had been receiving L-thyroxine supplementation. The combination of L-thyroxine and growth hormone therapy resulted in a significant growth

response with improvement from <3 percentile to the 50th percentile.

The importance of normal glycosylation to the growth of children, and potentially to the components of the GH/IGF cascade, is illustrated by the normalization of growth seen in a girl with CDG1b following institution of mannose therapy. At 30 months of age, she was <3 percentile for height and >97 percentile for weight. At 3 years, following 6 months of mannose therapy, her height was 43 percentile and weight was unchanged but now 89 percentile. This represents an extraordinary annualized height velocity of 23 cm/yr with normal for her age being 8 cm/yr. Subsequently, she continued to grow along the 40 percentile (unpublished results of patient in [57]). This extraordinary growth response to mannose in a child who was either normal or overweight suggests that glycosylation of growth factors, carrier proteins and receptors played an important role in the growth. In addition, this finding downplays the role of poor nutrition as a factor in the short stature of children with CDG.

It is likely that the growth of children with CDG is affected both by nutritional factors and by the effects of hypoglycosylation on the function and stability of the components of the GH/IGF cascade. Further studies are necessary to determine the extent to which protein hypoglycosylation negatively impacts growth in children with CDG.

Endocrine effects of CDG on glucose metabolism

The association of hypoglycemia with CDG was first described in patients with CDG 1b [19,57,115]. Hyperinsulinism was documented in three cases and the patients responded to treatment with diazoxide. In one report, initiation of mannose therapy allowed rapid tapering of diazoxide therapy without recurrent hypoglycemia [115]. In another patient, hyperinsulinism was not proven as the cause of hypoglycemia [57]. The patient was treated with a combination of diazoxide and frequent meals. Initiation of mannose therapy rapidly improved fasting tolerance.

There have been several cases of hypoglycemia in patients with other types of CDG. In one report, a girl with untyped CDG had frequent episodes of hypoglycemia associated with growth retardation at 2 years of age. Investigations demonstrated growth hormone deficiency and the patient received growth hormone with resulting improvement in growth and hypoglycemia [91]. In a recent case series of patients with CDG 1a, three patients had episodes of hypoglycemia. One was reported as having hyperinsulinemic hypoglycemia [92].

The mechanism by which hypoglycosylation can cause hyperinsulinism in patients with CDG is unknown. However, the rapid resolution of hypoglycemia following initiation of mannose in two patients with CDG 1b, suggests a role of glycosylation in maintenance of normoglycemia. Due to the presence of documented hyperinsulinism in several patients, one could speculate that glycosylation is important in the regulation of insulin secretion, possibly of the sulfonylurea receptor.

Endocrine effects of CDG on lipid metabolism

Hypocholesterolemia has been described in a number of patients with CDG [87]. It is well known that glycation of LDL in the setting of hyperglycemia due to diabetes mellitus leads to impaired uptake of LDL by the liver LDL receptor resulting in elevated LDL and triglycerides. It has been speculated that underglycosylation of LDL improves uptake of the lipid rich particles by the liver leading to hypocholesterolemia. In addition, stability of the apolipoproteins may be affected by their degree of glycosylation.

Summary of endocrine and metabolic effects in CDG

The endocrine system involves glycoproteins at many steps in multiple different hormonal pathways, including polypeptide hormones, hormone transport proteins, and hormone receptors. There is a significant amount of literature devoted to the analysis of the effects of the glycosylation status of these components of the hormonal cascades *in vitro*. The hormonal abnormalities in patients with CDG give us a glimpse of the impact of altered glycosylation on the endocrine system *in vivo*. Further studies of the hormonal milieu in patients with CDG will help us to better understand both CDG and the endocrine system.

Identification of More Patients and New Defects

Estimates suggest that 100–200 CDG-Ia patients are born every year in the United States and a similar or higher number in Europe [116]. This means that the expanding spectrum of these disorders is quite underdiagnosed. Heightened awareness of glycosylation as a cause of metabolic and endocrine malfunctions, appreciation of the diversity of CDG patients, and broader-based testing for these disorders will probably lead to the identification of new and previously overlooked patients.

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References

1. Granovsky M, Fata J, Pawling J, Muller WJ, Khokha R, Dennis JW. Suppression of tumor growth and metastasis in Mgat5-deficient mice. *Nat Med* 2000;6:306–312.
2. Ellgaard L, Molinari M, Helenius A. Setting the standards: Quality control in the secretory pathway. *Science* 1999;286:1882–1888.
3. Varki A, Cummings R, Esko J, Freeze H, Hart G, Marth J, eds. *Essentials of Glycobiology*. 1st ed. New York: Spring Harbor Laboratory Press, 1999.
4. Thor G, Brian AA. Glycosylation variants of murine interleukin-4: Evidence for different functional properties. *Immunology* 1992;75:143–149.
5. Kornfeld S. Structure and function of the mannose 6-phosphate/insulinlike growth factor II receptors. *Annu Rev Biochem* 1992;61:307–330.
6. Varki A. Biological roles of oligosaccharides: All of the theories are correct. *Glycobiology* 1993;3:97–130.
7. Rudd PM, Wormald MR, Stanfield RL, Huang M, Mattsson N, Speir JA, DiGennaro JA, Fetrow JS, Dwek RA, Wilson IA. Roles for glycosylation of cell surface receptors involved in cellular immune recognition. *J Mol Biol* 1999;293:351–366.
8. Gahmberg CG, Tolvanen M. Why mammalian cell surface proteins are glycoproteins. *Trends Biochem Sci* 1996;21:308–311.
9. Springer T. Traffic signals on endothelium for lymphocyte recirculation and leukocyte emmigration. In: Paul L, Issekutz T, eds. *Adhesion Molecules in Health and Disease*. New York: Marcel Dekker, 1997:1–54.
10. Gagneux P, Varki A. Evolutionary considerations in relating oligosaccharide diversity to biological function. *Glycobiology* 1999;9:747–755.
11. Dwek RA. Biological importance of glycosylation. *Dev Biol Stand* 1998;96:43–47.
12. Freeze H. Update and perspectives on congenital disorders of glycosylation. *Glycobiology* 2001;11:129R–143R.
13. Freeze HH, Westphal V. Balancing N-linked glycosylation to avoid disease. *Biochimie* 2001;83:791–799.
14. Helenius J, Ng DT, Marolda CL, Walter P, Valvano MA, Aebi M. Translocation of lipid-linked oligosaccharides across the ER membrane requires Rft1 protein. *Nature* 2002;415:447–450.
15. Stibler H, Holzbach U, Kristiansson B. Isoforms and levels of transferrin, antithrombin, alpha(1)-antitrypsin and thyroxine-binding globulin in 48 patients with carbohydrate-deficient glycoprotein syndrome type I. *Scand J Clin Lab Invest* 1998;58:55–61.
16. Aebi M, Helenius A, Schenk B, Barone R, Fiumara A, Berger EG, Hennet T, Imbach T, Stutz A, Bjursell C and others. Carbohydrate-deficient glycoprotein syndromes become congenital disorders of glycosylation: An updated nomenclature for CDG. First International Workshop on CDGS. *Glycoconj J* 1999;16:669–671.
17. Van Schaftingen E, Jaeken J. Phosphomannomutase deficiency is a cause of carbohydrate-deficient glycoprotein syndrome type I. *FEBS Lett* 1995;377:318–320.
18. de Koning TJ, Dorland L, van Diggelen OP, Boonman AM, de Jong GJ, van Noort WL, De Schryver J, Duran M, van den Berg IE, Gerwig GJ and others. A novel disorder of N-glycosylation due to phosphomannose isomerase deficiency. *Biochem Biophys Res Commun* 1998;245:38–42.
19. Jaeken J, Matthijs G, Saudubray JM, Dionisi-Vici C, Bertini E, de Lonlay P, Henri H, Carchon H, Schollen E, Van Schaftingen E. Phosphomannose isomerase deficiency: A carbohydrate-deficient glycoprotein syndrome with hepatic-intestinal presentation. *Am J Hum Genet* 1998;62:1535–1539.
20. Niehues R, Hasilik M, Alton G, Körner C, Schiebe-Sukumar M, Koch HG, Zimmer KP, Wu R, Harms E, Reiter K and others. Carbohydrate-deficient glycoprotein syndrome type Ib. Phosphomannose isomerase deficiency and mannose therapy. *J Clin Invest* 1998;101:1414–1420.
21. Imbach T, Burda P, Kuhnert P, Wevers RA, Aebi M, Berger EG, Hennet T. A mutation in the human ortholog of the *Saccharomyces cerevisiae* ALG6 gene causes carbohydrate-deficient glycoprotein syndrome type-Ic. *Proc Natl Acad Sci USA* 1999;96:6982–6987.
22. Burda P, Borsig L, de Rijk-van Andel J, Wevers R, Jaeken J, Carchon H, Berger EG, Aebi M. A novel carbohydrate-deficient glycoprotein syndrome characterized by a deficiency in glucosylation of the dolichol-linked oligosaccharide. *J Clin Invest* 1998;102:647–652.
23. Korner C, Knauer R, Stephani U, Marquardt T, Lehle L, von Figura K. Carbohydrate deficient glycoprotein syndrome type IV: Deficiency of dolichyl-P-Man:Man(5)GlcNAc(2)-PP-dolichyl mannosyltransferase. *Embo J* 1999;18:6816–6822.
24. Imbach T, Grünewald S, Schenk B, Burda P, Schollen E, Wevers RA, Jaeken J, de Klerk JB, Berger EG, Matthijs G and others. Multi-allelic origin of congenital disorder of glycosylation (CDG)-Ic. *Hum Genet* 2000;106:538–545.
25. Kim S, Westphal V, Srikrishna G, Mehta DP, Peterson S, Filiano J, Karnes PS, Patterson MC, Freeze HH. Dolichol phosphate mannose synthase (DPM1) mutations define congenital disorder of glycosylation Ie (CDG-Ie). *J Clin Invest* 2000;105:191–198.
26. Schenk B, Imbach T, Frank CG, Grubenmann CE, Raymond GV, Hurvitz H, Raas-Rotschild A, Luder AS, Jaeken J, Berger EG and others. MPDU1 mutations underlie a novel human congenital disorder of glycosylation, designated type If. *Journal of Clinical Investigation* 2001;108:1687–1695.
27. Kranz C, Denecke J, Lehrman MA, Ray S, Kienz P, Kreissel G, Sagi D, Peter-Katalinic J, Freeze HH, Schmid T and others. A mutation in the human *MPDU1* gene causes congenital disorder of glycosylation type If (CDG-If). *Journal of Clinical Investigation* 2001;108:1613–1619.
28. Chantret I, Dupre T, Delenda C, Bucher S, Dancourt J, Barnier A, Charollais A, Heron D, Bader-Meunier B, Danos O and others. Congenital disorders of glycosylation type Ig is defined by a deficiency in dolichyl-P-mannose: Man7GlcNAc2-PP-dolichyl mannosyltransferase. *J Biol Chem* 2002;30:30.
29. Jaeken J, Schachter H, Carchon H, De Cock P, Coddeville B, Spik G. Carbohydrate deficient glycoprotein syndrome type II: A deficiency in Golgi localised N-acetyl-glucosaminyltransferase II. *Arch Dis Child* 1994;71:123–127.
30. de Praeter CM, Gerwig GJ, Bause E, Nuytinck LK, Vliegenthart JF, Breuer W, Kamerling JP, Espeel MF, Martin JJ, de Paepe AM and others. A novel disorder caused by defective biosynthesis of N-linked oligosaccharides due to glucosidase I deficiency. *Am J Hum Genet* 2000;66:1744–1756.
31. Lühn K, Wild MK, Eckhardt M, Gerardy-Schahn R, Vestweber D. The gene defective in leukocyte adhesion deficiency II encodes a putative GDP-fucose transporter. *Nat Genet* 2001;28:69–72.
32. Lübke T, Marquardt T, Etzioni A, Hartmann E, von Figura K, Korner C. Complementation cloning identifies CDG-IIc, a new type of congenital disorders of glycosylation, as a GDP-fucose transporter deficiency. *Nat Genet* 2001;28:73–76.
33. Hansske B, Thiel C, Lubke T, Hasilik M, Honing S, Peters V, Heidemann PH, Hoffmann GF, Berger EG, von Figura K and others. Deficiency of UDP-galactose: N-acetylglucosamine beta-1,4-galactosyltransferase I causes the congenital disorder of glycosylation type IId. *J Clin Invest* 2002;109:725–733.

34. Peters V, Penzien JM, Reiter G, Korner C, Hackler R, Assmann B, Fang J, Schaefer JR, Hoffmann GF, Heidemann PH. Congenital disorder of glycosylation IId (CDG-IId)—A new entity: Clinical presentation with Dandy-Walker malformation and myopathy. *Neuropediatrics* 2002;33:27–32.
35. Matthijs G, Schollen E, Bjursell C, Erlandson A, Freeze H, Imtiaz F, Kjaergaard S, Martinsson T, Schwartz M, Seta N and others. Mutations in PMM2 that cause congenital disorders of glycosylation, type Ia (CDG-Ia). *Hum Mutat* 2000;16:386–394.
36. Matthijs G, Schollen E, Pardon E, Veiga-Da-Cunha M, Jaeken J, Cassiman JJ, Van Schaftingen E. Mutations in PMM2, a phosphomannomutase gene on chromosome 16p13, in carbohydrate-deficient glycoprotein type I syndrome (Jaeken syndrome). *Nat Genet* 1997;16:88–92.
37. Rush JS, Panneerselvam K, Waechter CJ, Freeze HH. Mannose supplementation corrects GDP-mannose deficiency in cultured fibroblasts from some patients with congenital disorders of glycosylation (CDG). *Glycobiology* 2000;10:829–835.
38. Krasnewich DM, Holt GD, Brantly M, Skovby F, Redwine J, Gahl WA. Abnormal synthesis of dolichol-linked oligosaccharides in carbohydrate-deficient glycoprotein syndrome. *Glycobiology* 1995;5:503–510.
39. Briones P, Vilaseca MA, Garcia-Silva MT, Pineda M, Colomer J, Ferrer I, Artigas J, Jaeken J, Chabas A. Congenital disorders of glycosylation (CDG) may be underdiagnosed when mimicking mitochondrial disease. *Europ J Paediatr Neurol* 2001;5:127–131.
40. Korner C, Knauer R, Holzbach U, Hanefeld F, Lehle L, von Figura K. Carbohydrate-deficient glycoprotein syndrome type V: Deficiency of dolichyl-P-Glc:Man9GlcNAc2-PP-dolichyl glucosyltransferase. *Proc Natl Acad Sci USA* 1998;95:13200–13205.
41. Schachter H. Congenital disorders involving defective N-glycosylation of proteins. *Cell Mol Life Sci* 2001;58:1085–1104.
42. Jaeken J, Matthijs G, Carchon H, Schaftingen EV. Defects of N-Glycan Synthesis. In: Scriver CR, Beaudet AL, Sly WS, Valle D, eds. *The Metabolic and Molecular Bases of Inherited Diseases*, 8th ed., Vol. 1. New York: McGraw-Hill, Medical Publishing Division, 2001: 1601–1622.
43. Freeze HH. Congenital disorders of glycosylation and the pediatric liver. *Semin Liver Dis* 2001;21:501–616.
44. Bergen HR, Lacey JM, O'Brien JF, Naylor S. Online single-step analysis of blood proteins: The transferrin story. *Anal Biochem* 2001;296:122–129.
45. Lacey JM, Bergen HR, Magera MJ, Naylor S, O'Brien JF. Rapid determination of transferrin isoforms by immunoaffinity liquid chromatography and electrospray mass spectrometry. *Clin Chem* 2001;47:513–518.
46. Clayton P, Winchester B, Di Tomaso E, Young E, Keir G, Rodeck C. Carbohydrate-deficient glycoprotein syndrome: Normal glycosylation in the fetus. *Lancet* 1993;341:956.
47. DiMartini A, Day N, Lane T, Beisler AT, Dew MA, Anton R. Carbohydrate deficient transferrin in abstaining patients with end-stage liver disease. *Alcohol Clin Exp Res* 2001;25:1729–1733.
48. Charlwood J, Clayton P, Keir G, Mian N, Young E, Winchester B. Prenatal diagnosis of the carbohydrate-deficient glycoprotein syndrome type 1A (CDG1A) by a combination of enzymology and genetic linkage analysis after amniocentesis or chorionic villus sampling. *Prenat Diagn* 1998;18:693–699.
49. Stibler H, von Dobeln U, Kristiansson B, Guthenberg C. Carbohydrate-deficient transferrin in galactosaemia. *Acta Paediatr* 1997;86:1377–1378.
50. Martensson O, Harlin A, Brandt R, Seppa K, Sillanaukee P. Transferrin isoform distribution: Gender and alcohol consumption. *Alcohol Clin Exp Res* 1997;21:1710–1715.
51. Adamowicz M, Pronicka E. Carbohydrate deficient glycoprotein syndrome-like transferrin isoelectric focusing pattern in untreated fructosaemia. *Eur J Pediatr* 1996;155:347–348.
52. Mader I, Dobler-Neumann M, Kuker W, Stibler H, Krageloh-Mann I. Congenital disorder of glycosylation type Ia: Benign clinical course in a new genetic variant. *Childs Nerv Syst* 2002;18:77–80.
53. Dupre T, Cuer M, Barrot S, Barnier A, Cormier-Daire V, Munnich A, Durand G, Seta N. Congenital disorder of glycosylation Ia with deficient phosphomannomutase activity but normal plasma glycoprotein pattern. *Clin Chem* 2001;47:132–134.
54. Fletcher JM, Matthijs G, Jaeken J, Van Schaftingen E, Nelson PV. Carbohydrate-deficient glycoprotein syndrome: Beyond the screen. *J Inherit Metab Dis* 2000;23:396–398.
55. Callewaert N, Geysens S, Molemans F, Contreras R. Ultrasensitive profiling and sequencing of N-linked oligosaccharides using standard DNA-sequencing equipment. *Glycobiology* 2001;11:275–281.
56. de Lonlay P, Cormier-Daire V, Vuillaumier-Barrot S, Cuer M, Durand G, Munnich A, Saudubray JM, Seta N. Carbohydrate-deficient blood glycoprotein syndrome. *Arch Pediatr* 2000;7:173–184.
57. Babovic-Vuksanovic D, Patterson MC, Schwenk WF, O'Brien JF, Vockley J, Freeze HH, Mehta DP, Michels VV. Severe hypoglycemia as a presenting symptom of carbohydrate-deficient glycoprotein syndrome. *J Pediatr* 1999;135:775–781.
58. Kjaergaard S, Kristiansson B, Stibler H, Freeze HH, Schwartz M, Martinsson T, Skovby F. Failure of short-term mannose therapy of patients with carbohydrate-deficient glycoprotein syndrome type 1A. *Acta Paediatr* 1998;87:884–888.
59. Mayatepek E, Schröder M, Kohlmuller D, Bieger WP, Nutzenadel W. Continuous mannose infusion in carbohydrate-deficient glycoprotein syndrome type I. *Acta Paediatr* 1997;86:1138–1140.
60. Marquardt T, Hasilik M, Niehues R, Herting M, Muntau A, Holzbach U, Hanefeld F, Freeze H, Harms E. Mannose therapy in carbohydrate-deficient glycoprotein syndrome type I—first results of the German multicenter study. *Amino Acids* 1997;12:389.
61. Marquardt T, Luhn K, Srikrishna G, Freeze HH, Harms E, Vestweber D. Correction of leukocyte adhesion deficiency type II with oral fucose. *Blood* 1999;94:3976–3985.
62. Jaeken J, Stibler H, Hagberg B. The carbohydrate-deficient glycoprotein syndrome. A new inherited multisystemic disease with severe nervous system involvement. *Acta Paediatr Scand Suppl* 1991;375:1–71.
63. Krasnewich D, Gahl WA. Carbohydrate-deficient glycoprotein syndrome. *Adv Pediatr* 1997;44:109–140.
64. Jaeken J, Carchon H. Congenital disorders of glycosylation: The rapidly growing tip of the iceberg. *Curr Opin Neurol* 2001;14:811–815.
65. Grünewald S, Schollen E, Van Schaftingen E, Jaeken J, Matthijs G. High residual activity of PMM2 in patients' fibroblasts: Possible pitfall in the diagnosis of CDG-Ia (phosphomannomutase deficiency). *Am J Hum Genet* 2001;68:347–354.
66. Westphal V, Peterson S, Patterson M, Tournay M, Blumenthal A, Treacy E, Freeze H. Functional significance of PMM2 mutations in mildly affected patients with congenital disorders of glycosylation Ia. *Genetics in Medicine* 2001;3:393–398.
67. van Ommen CH, Peters M, Barth PG, Vreken P, Wanders RJ, Jaeken J. Carbohydrate-deficient glycoprotein syndrome type 1a: A variant phenotype with borderline cognitive dysfunction, cerebellar hypoplasia, and coagulation disturbances. *J Pediatr* 2000;136:400–403.
68. Grünewald S, Imbach T, Huijben K, Rubio-Gozalbo ME, Verrips A, de Klerk JB, Stroink H, de Rijk-van Andel JF, Van Hove JL, Wendel U and others. Clinical and biochemical characteristics of

congenital disorder of glycosylation type Ic, the first recognized endoplasmic reticulum defect in N-glycan synthesis. *Ann Neurol* 2000;47:776–781.
69. Barone R, Pavone L, Fiumara A, Bianchini R, Jaeken J. Developmental patterns and neuropsychological assessment in patients with carbohydrate-deficient glycoconjugate syndrome type IA (phosphomannomutase deficiency). *Brain Dev* 1999;21:260–263.
70. Stibler H, Blennow G, Kristiansson B, Lindehammer H, Hagberg B. Carbohydrate-deficient glycoprotein syndrome: Clinical expression in adults with a new metabolic disease. *J Neurol Neurosurg Psychiatry* 1994;57:552–556.
71. Jaeken J, Vanderschueren-Lodewyckx M, Caeaer P, Snoeck L, Corbeel L, Eggermont E. Familial psychomotor retardation with markedly fluctuating serum prolactin, FSH and GH levels, partial TBG deficiency, increased serum arysulphatase A and increased CSF protein: A new syndrome? *Pediatric Res* 1980;14:179.
72. de Zegher F, Jaeken J. Endocrinology of the carbohydrate-deficient glycoprotein syndrome type 1 from birth through adolescence. *Pediatr Res* 1995;37:395–401.
73. Kristiansson B, Stibler H, Wide L. Gonadal function and glycoprotein hormones in the carbohydrate-deficient glycoprotein (CDG) syndrome. *Acta Paediatr* 1995;84:655–659.
74. Westphal V, Kjaergaard S, Davis JA, Peterson SM, Skovby F, Freeze HH. Genetic and metabolic analysis of the first adult with congenital disorder of glycosylation type Ib: Long-term outcome and effects of mannose supplementation. *Mol Genet Metab* 2001;73:77–85.
75. Pineda M, Pavia C, Vilaseca MA, Ferrer I, Temudo T, Chabas A, Stibler H, Jaeken J. Normal pubertal development in a female with carbohydrate deficient glycoprotein syndrome. *Arch Dis Child* 1996;74:242–243.
76. Artigas J, Cardo E, Pineda M, Nosas R, Jaeken J. Phosphomannomutase deficiency and normal pubertal development. *J Inherit Metab Dis* 1998;21:78–79.
77. McDowell G, Gahl WA. Inherited disorders of glycoprotein synthesis: Cell biological insights. *Proc Soc Exp Biol Med* 1997;215:145–157.
78. Kaufman FR, Kogut MD, Donnell GN, Goebelsmann U, March C, Koch R. Hypergonadotropic hypogonadism in female patients with galactosemia. *N Engl J Med* 1981;304:994–998.
79. Ohzeki T, Motozumi H, Hanaki K, Ohtahara H, Urashima H, Tsukuda T, Kobayashi S, Shiraki K, Ohno K. Carbohydrate-deficient glycoprotein syndrome in a girl with hypogonadism due to inactive follicle stimulating hormone. *Horm Metab Res* 1993;25:646–648.
80. Sairam MR, Bhargavi GN. A role for glycosylation of the alpha subunit in transduction of biological signal in glycoprotein hormones. *Science* 1985;229:65–67.
81. Ulloa-Aguirre A, Maldonado A, Damian-Matsumura P, Timossi C. Endocrine regulation of gonadotropin glycosylation. *Arch Med Res* 2001;32:520–532.
82. Tapanainen JS, Aittomaki K, Min J, Vaskivuo T, Huhtaniemi IT. Men homozygous for an inactivating mutation of the follicle-stimulating hormone (FSH) receptor gene present variable suppression of spermatogenesis and fertility. *Nat Genet* 1997;15:205–206.
83. Aittomaki K, Herva R, Stenman UH, Juntunen K, Ylostalo P, Hovatta O, de la Chapelle A. Clinical features of primary ovarian failure caused by a point mutation in the follicle-stimulating hormone receptor gene. *J Clin Endocrinol Metab* 1996;81:3722–3726.
84. Davis D, Liu X, Segaloff DL. Identification of the sites of N-linked glycosylation on the follicle-stimulating hormone (FSH) receptor and assessment of their role in FSH receptor function. *Mol Endocrinol* 1995;9:159–170.
85. Davis DP, Rozell TG, Liu X, Segaloff DL. The six N-linked carbohydrates of the lutropin/choriogonadotropin receptor are not absolutely required for correct folding, cell surface expression, hormone binding, or signal transduction. *Mol Endocrinol* 1997;11:550–562.
86. Kristiansson B, Andersson M, Tonnby B, Hagberg B. Disialotransferrin developmental deficiency syndrome. *Arch Dis Child* 1989;64:71–76.
87. Hagberg BA, Blennow G, Kristiansson B, Stibler H. Carbohydrate-deficient glycoprotein syndromes: Peculiar group of new disorders. *Pediatr Neurol* 1993;9:255–262.
88. Berkowitz GS, Lapinski RH, Dolgin SE, Gazella JG, Bodian CA, Holzman IR. Prevalence and natural history of cryptorchidism. *Pediatrics* 1993;92:44–49.
89. Behrman R. *Nelson Textbook of Pediatrics*. Philadelphia: W.B. Saunders Company, 1997.
90. Veneselli E, Biancheri R, Di Rocco M, Tortorelli S. Neurophysiological findings in a case of carbohydrate-deficient glycoprotein (CDG) syndrome type I with phosphomannomutase deficiency. *Europ J Paediatr Neurol* 1998;2:239–244.
91. Ferrari MC, Parini R, Di Rocco MD, Radetti G, Beck-Peccoz P, Persani L. Lectin analyses of glycoprotein hormones in patients with congenital disorders of glycosylation. *Eur J Endocrinol* 2001;144:409–416.
92. Enns GM, Steiner RD, Buist N, Cowan C, Leppig KA, McCracken MF, Westphal V, Freeze HH, O'Brien JF, Jaeken J, Behera S, Hudgins L. Clinical and molecular features in North American congenital disorder of glycosylation type I patients with diverse ethnic origins. *J Pediatrics* 2002;141:695–700.
93. Acarregui MJ, George TN, Rhead WJ. Carbohydrate-deficient glycoprotein syndrome type 1 with profound thrombocytopenia and normal phosphomannomutase and phosphomannose isomerase activities. *J Pediatr* 1998;133:697–700.
94. Westphal V, Murch S, Kim S, Srikrishna G, Winchester B, Day R, Freeze HH. Reduced heparan sulfate accumulation in enterocytes contributes to protein-losing enteropathy in a congenital disorder of glycosylation. *Am J Pathol* 2000;157:1917–1925.
95. Avvakumov GV, Warmels-Rodenhiser S, Hammond GL. Glycosylation of human corticosteroid-binding globulin at aspargine 238 is necessary for steroid binding. *J Biol Chem* 1993;268:862–866.
96. Avvakumov GV. Structure and function of corticosteroid-binding globulin: Role of carbohydrates. *J Steroid Biochem Mol Biol* 1995;53:515–522.
97. Warady BA, Howard CP, Hellerstein S, Alon U, Grunt JA. Congenital nephrosis in association with hypothyroidism and hypoadrenocorticism. *Pediatr Nephrol* 1993;7:79–80.
98. Hutchesson AC, Gray RG, Spencer DA, Keir G. Carbohydrate deficient glycoprotein syndrome: Multiple abnormalities and diagnostic delay. *Arch Dis Child* 1995;72:445–446.
99. Rose SR. Disorders of thyrotropin synthesis, secretion, and function. *Curr Opin Pediatr* 2000;12:375–381.
100. Thotakura NR, Desai RK, Szkudlinski MW, Weintraub BD. The role of the oligosaccharide chains of thyrotropin alpha- and beta-subunits in hormone action. *Endocrinology* 1992;131:82–88.
101. Graves P, Pritsker A, Davies TF. Post-translational processing of the natural human thyrotropin receptor: Demonstration of more than two cleavage sites. *J Clin Endocrinol Metab* 1999;84:2177–2181.
102. Oda Y, Sanders J, Roberts S, Maruyama M, Kiddie A, Furmaniak J, Smith BR. Analysis of carbohydrate residues on recombinant human thyrotropin receptor. *J Clin Endocrinol Metab* 1999;84:2119–2125.
103. Jaeken J, Carchon H. The carbohydrate-deficient glycoprotein syndromes: An overview. *J Inherit Metab Dis* 1993;16:813–820.

104. Macchia PE, Harrison HH, Scherberg NH, Sunthornthepfvarakul T, Jaeken J, Refetoff S. Thyroid function tests and characterization of thyroxine-binding globulin in the carbohydrate-deficient glycoprotein syndrome type I. *J Clin Endocrinol Metab* 1995;80:3744–3749.
105. Ramaekers VT, Stibler H, Kint J, Jaeken J. A new variant of the carbohydrate deficient glycoproteins syndrome. *J Inherit Metab Dis* 1991;14:385–388.
106. Butler AA, LeRoith D. Minireview: Tissue-specific versus generalized gene targeting of the igf1 and igf1r genes and their roles in insulin-like growth factor physiology. *Endocrinology* 2001;142:1685–1688.
107. Wetterau LA, Moore MG, Lee KW, Shim ML, Cohen P. Novel aspects of the insulin-like growth factor binding proteins. *Mol Genet Metab* 1999;68:161–181.
108. Baxter RC. Circulating levels and molecular distribution of the acid-labile (alpha) subunit of the high molecular weight insulin-like growth factor-binding protein complex. *J Clin Endocrinol Metab* 1990;70:1347–1353.
109. Guler HP, Zapf J, Schmid C, Froesch ER. Insulin-like growth factors I and II in healthy man. Estimations of half-lives and production rates. *Acta Endocrinol (Copenh)* 1989;121:753–758.
110. Janosi JB, Firth SM, Bond JJ, Baxter RC, Delhanty PJ. N-Linked glycosylation and sialylation of the acid-labile subunit. Role in complex formation with insulin-like growth factor (IGF)-binding protein-3 and the IGFs. *J Biol Chem* 1999;274:5292–5298.
111. Boisclair YR, Rhoads RP, Ueki I, Wang J, Ooi GT. The acid-labile subunit (ALS) of the 150 kDa IGF-binding protein complex: An important but forgotten component of the circulating IGF system. *J Endocrinol* 2001;170:63–70.
112. Sommer A, Spratt SK, Tatsuno GP, Tressel T, Lee R, Maack CA. Properties of glycosylated and non-glycosylated human recombinant IGF binding protein-3 (IGFBP-3). *Growth Regul* 1993;3:46–49.
113. Firth SM, Baxter RC. Characterisation of recombinant glycosylation variants of insulin-like growth factor binding protein-3. *J Endocrinol* 1999;160:379–387.
114. Miller B, Khosravi M, Zimmerman D, Patterson M, Connover C. The IGF cascade in children with CDG 1A. *Glycoconjugate Journal* 2001;18:C8.8,57.
115. de Lonlay P, Cuer M, Vuillaumier-Barrot S, Beaune G, Castelnau P, Kretz M, Durand G, Saudubray JM, Seta N. Hyperinsulinemic hypoglycemia as a presenting sign in phosphomannose isomerase deficiency: A new manifestation of carbohydrate-deficient glycoprotein syndrome treatable with mannose. *J Pediatr* 1999;135:379–383.
116. Schollen E, Kjaergaard S, Legius E, Schwartz M, Matthijs G. Lack of Hardy-Weinberg equilibrium for the most prevalent PMM2 mutation in CDG-Ia (congenital disorders of glycosylation type Ia). *Eur J Hum Genet* 2000;8:367–371.