CMLS, Cell. Mol. Life Sci. 58 (2001) 1085–1104
1420-682X/01/081085-20 $ 1.50 + 0.20/0

CMLS Cellular and Molecular Life Sciences

Review

Congenital disorders involving defective N-glycosylation of proteins

H. Schachter

Department of Structural Biology and Biochemistry, The Research Institute, The Hospital for Sick Children, 555 University Avenue, Toronto, Ontario M5G 1X8 (Canada), Fax +1 416 813 5022, e-mail: harry@sickkids.on.ca
Department of Biochemistry, University of Toronto, Toronto Ontario M5G 1X8 (Canada)

Received 10 January 2001; received after revision 5 March 2001; accepted 6 March 2001

Abstract. This review deals with several of the main autosomal recessive congenital disorders involving defective N-glycosylation of proteins (the addition of glycans linked to the polypeptide chain by a β-linkage between the anomeric carbon of N-acetylglucosamine and the amido group of L-asparagine). These congenital disorders of glycosylation (CDG, previously known as carbohydrate-deficient glycoprotein syndromes) are a group of multisystemic diseases often involving severe psychomotor retardation. Six distinct variants of CDG in group I (types Ia–If) have been described to date and the defects have been localized to deficiencies in the assembly of the dolichylpyrophosphate-linked oligosaccharide N-glycan precursor and its transfer to asparagine residues on the nascent polypeptides. Two variants of CDG group II (types IIa and IIb) have been identified as defects in the processing of protein-bound N-glycans. Hereditary erythroblastic multinuclearity with a positive acidified-serum lysis test (HEMPAS; congenital dyserythropoietic anemia type II) presents as a relatively mild dyserythropoietic anemia. The genetic defect in most cases of HEMPAS is not known, but α-3/6-mannosidase II is involved in at least some patients. Leukocyte adhesion deficiency type II (LAD II) is a rare disorder characterized by recurrent infections, persistent leukocytosis and severe mental and growth retardation. LAD II is due to lack of availability of GDP-fucose. The study of these diseases and of relevant animal models has provided strong evidence that N-glycans are essential for normal mammalian development.

Key words. Glycosylation; N-glycans; congenital disease; congenital disorders of glycosylation; congenital dyserythropoietic anemia; leukocyte adhesion deficiency; mannose metabolism; fucose metabolism.

Introduction

A glycoconjugate is a covalent complex between carbohydrate and non-carbohydrate moieties. The non-carbohydrate moiety may be a protein, peptide, amino acid, lipid or some other aglycone. This review will deal with congenital diseases involving defects in the glycosylation of proteins. Glycans can be conjugated to amino acids either by N-glycosyl or O-glycosyl linkages [1]. The only N-glycan in animals involves a beta-linkage between the anomeric carbon of N-acetylglucosamine (GlcNAc) and the amido group of L-asparagine (Asn) although other N-glycan linkages have been found in bacterial glycoproteins. Several O-glycan linkages are present in animals. The alpha-linkage between the anomeric carbon of N-acetylgalactosamine (GalNAc) and the hydroxyl group of either serine (Ser) or threonine (Thr) was first described in mucins but is also commonly found in non-mucinous glycoproteins. Other common O-glycan linkages in animals are the beta-linkage between xylose (Xyl) and the hydroxyl group of Ser (characteristic of proteoglycans) and the beta-linkage between galactose (Gal) and the hydroxyl group of hydroxylysine (characteristic of the collagens). Proteoglycans are a subclass of glycoproteins

in which the carbohydrate moieties are polysaccharides that contain amino sugars (glycosaminoglycans).
Carbohydrate macromolecules usually consist of various building blocks (monosaccharides such as mannose, galactose, GalNAc, etc.) that can be linked together in several different ways (the anomeric carbon of one sugar can be attached in alpha- or beta-linkage to one of several carbons of the linking sugar). This allows the structure to become branched. Whereas the other major biological macromolecules (proteins, nucleic acids) are linear and are synthesized by a linear template mechanism, large carbohydrates must be made on an assembly line (the endoplasmic reticulum-Golgi apparatus) along which various enzymes add and remove sugars. The sugar sequences and branching patterns are controlled not by copying a template but by the substrate specificities of these enzymes (mainly glycosyltransferases and glycosidases).
The diversity of linkages and branching patterns between monomer building blocks confers on carbohydrates the ability to carry an enormous amount of information in very compact structures [2]. The cell surface is covered with protein- and lipid-bound carbohydrate structures that vary significantly between cell types and at different stages of mammalian development. There is considerable evidence that these carbohydrates play important roles in the interaction of a cell with its cellular and fluid environment [3–5]. The essential role of glycoproteins and proteoglycans in development has been demonstrated by studies on mice with null mutations in various glycosyltransferases and glycosidases [6–14], on mutant *Drosophila melanogaster* [15] and *Caenorhabditis elegans* [16–19], and on human congenital diseases with defects in the glycosylation of proteins [20, 21].
This review will deal with several of the main autosomal recessive congenital disorders involving defects in the synthesis of Asn-linked glycans (tables 1, 2), congenital disorders of glycosylation (CDG), congenital dyserythropoietic anemia type II, and leukocyte adhesion deficiency type II. Other defects in glycosylation are listed in tables 1 and 3. Since at least 0.5–1% of the transcribed human genome is devoted to the production of proteins involved in the synthesis, degradation, and function of glycoconjugates [11], many other such congenital diseases may exist.

Congenital Disorders of Glycosylation

CDG (previously known as carbohydrate-deficient glycoprotein syndromes) are a group of congenital multisystemic diseases characterized by defective N-glycosylation. The discovery of these diseases was based on the observation by Jaeken et al. [22] of decreased serum thyroxine-binding globulin and increased arylsulfatase A activity in two patients with familial psychomotor retardation. In 1984, Jaeken et al. [23] reported sialic acid deficiency in serum and cerebrospinal fluid transferrin from identical twin sisters with demyelinating disease, demonstrating for the first time that this new syndrome was due to defective protein glycosylation. There were about 280 patients with this disease worldwide as of October 1998 [24].
In 1999, at a meeting held in Belgium, a new classification and nomenclature for CDG was proposed [25, 26]. Six distinct variants of CDG in group I have been described to date (types Ia–If; table 2) and the defects have been localized to deficiencies in the assembly of the dolichyl-pyrophosphate-linked oligosaccharide N-glycan precursor and its transfer to asparagine residues on the nascent polypeptides. Two variants of CDG group II (types IIa and IIb; table 2) have been identified as defects in the processing of protein-bound N-glycans. CDG patients in which the defect has not yet been determined (table 2) will not be discussed here.

CDG group I (CDG-I)

The following discussion is based primarily on papers published prior to the separation of CDG group I patients into separate types. Since CDG-Ia is the most common type of CDG-I, the discussion of clinical and pathological features refers primarily to CDG-Ia patients. However, the section on clinical biochemistry is relevant to all CDG-I types.

Clinical and pathological features

CDG-I occurs worldwide and affects both sexes [27–34]. The patients show moderate to severe neurological disease, a characteristic dysmorphy, and variable involvement of other organs [24, 27, 32, 35–38]. In the neonatal period, the patients may show slow head movements, and in infancy, alternating internal strabismus, abnormal eye movements, axial hypotonia and hyporeflexia. Feeding problems, vomiting and diarrhea may occur, resulting in severe developmental delay and failure to thrive [39, 40]. Infants may show a distinctive lipodystrophy (peculiar distribution of subcutaneous fat), nipple retraction, and hypogonadism. Some infants show liver failure, cardiac insufficiency, pericardial effusion [41–43], nephrotic syndrome, and multiorgan failure [43–46]. Older children usually develop cerebellar ataxia and marked psychomotor retardation [47]. Retinitis pigmentosa [48, 49], joint contractures, skeletal deformities [50], stroke-like episodes, epilepsy, and peripheral neuropathy may also develop. About 20% of the patients die within the first year. Some adults show premature aging [51]. Language and motor development are severely delayed and walking without support is rarely achieved. The IQ ranges from 40 to 60 and the children usually have an extroverted and cheerful disposition.

Table 1. Autosomal recessive congenital disorders with defective N-glycan synthesis.

Name of disease	Type of disease	Enzyme defect	Gene	OMIM*	Locus Link*
Congenital disorders of Glycosylation	severe developmental abnormalities	see table 2			
HEMPAS (congenital dyserythropoietic anemia type II, CDA II)	relatively mild disease with mild anemia and hemosiderosis	alpha-mannosidase II deficiency; other genes	*MAN2A1*	224100 154582	4124
Leukocyte adhesion deficiency type II (LAD II)	immunodeficiency and severe develop-mental abnormalities	(a) GDP-fucose synthesis (b) GDP-fucose transport		266265	
Inclusion cell disease (I-cell disease or mucolipidosis II)	lysosomal glycoprotein storage disease with severe developmental abnormalities	N-acetylglucosamine-1-phosphotransferase required for synthesis of Man-6-phosphate targeting signal	*GNPTA*	252500	2795
Pseudo-Hurler polydystrophy (mucolipidosis III)	A milder form of I-cell disease	different lesion in same gene as I-cell disease	*GNPTA*	252600	2795
Galactosemia type I	failure to thrive, liver disease, mental retardation, cataracts	galactose-1-phosphate uridyltransferase	*GALT*	230400	2592
Galactosemia type II		galactokinase 1	*GALK1*	230200 604313	2584
Galactosemia type III		UDP-Gal-4-epimerase	*GALE*	230350	2582

* OMIM, Online Mendelian Inheritance in Man; Locus Link summarizes all the known information on a particular gene. Databases can be accessed at http://www.ncbi.nlm.nih.gov/

Table 2. Congenital disorders of glycosylation (CDG).

Type	Enzyme defect	Gene	OMIM*	Locus Link*	Acronym
Ia	phosphomannomutase 2	*PMM2*	212065 601785	5373	CDG-Ia
Ib	phosphomannose isomerase	*MPI*	602579 154550	4351	CDG-Ib
Ic	dolichyl-PP-Glc:$Man_9GlcNAc_2$-PP-dolichyl alpha-1,3-glucosyltransferase	*ALG6*	603147 604566	29929	CDG-Ic
Id	dolichyl-PP-Man:$Man_5GlcNAc_2$-PP-dolichyl alpha-1,3-mannosyl-transferase	*ALG3* *NOT56L*	601110	10159	CDG-Id
Ie	dolichol-P-Man synthase 1	*DPM1*	603503	8813	CDG-Ie
If	dolichol-P-Man utilization defect 1; SL15, suppressor of Lec15 and Lec35	*MPDU1*	604041	9526	CDG-If
Ix	Genetic basis unknown		603585 212067		CDG-Ix
IIa	UDP-GlcNAc:alpha-6-D-mannoside beta-1,2-N-acetylglucosaminyltransferase II (GnT II)	*MGAT2*	212066 602616	4247	CDG-IIa
IIb	alpha-1,2-glucosidase I	*GCS1*	601336	7841	CDG-IIb

* OMIM, Online Mendelian Inheritance in Man; Locus Link summarizes all the known information on a particular gene. Both databases can be accessed at http://www.ncbi.nlm.nih.gov/
Group I: defects in N-linked protein glycosylation due to deficiencies in the assembly of the dolichylpyrophosphate linked oligosaccharide and/or its transfer to asparagine residues on the nascent polypeptides.
Group II: defects in the processing of N-glycans or addition of other glycans to proteins.

Table 3. Other diseases involving abnormal glycosylation.

Name of disease	Type of disease	Enzyme defect	Gene	OMIM*	Locus Link*
Paroxysmal nocturnal hemoglobinuria (PNH)	acquired hematologic disorder with complement-mediated red cell hemolysis	synthesis of N-acetylglucosaminyl phosphatidylinositol (GlcNAc-PI)	*PIGA*	311770	5277
Tn polyagglutinability	acquired hematologic disorder with anemia, leukopenia and thrombocytopenia	beta-1,3-D-galactosyltransferase involved in O-glycan core 1 synthesis		230430	
Ehlers-Danlos syndrome, progeroid form	congenital disease with delayed mental development, connective tissue abnormalities, loose skin, hypotonia	xylosylprotein beta-1,4-D-galactosyltransferase involved in dermatan sulfate proteoglycan synthesis	*XGPT1* *B4GALT7*	130070 604327	11285

* OMIM, Online Mendelian Inheritance in Man; Locus Link summarizes all the known information on a particular gene. Databases can be accessed at http://www.ncbi.nlm.nih.gov/

Computerized tomography (CT), nuclear magnetic resonance imaging (MRI), and postmortem studies of the brain often show olivopontocerebellar atrophy (OPCA) and a small brain stem [27, 37, 52–57]. Other postmortem findings may include pericarditis, renal cysts, fibrosis of the testes and lymph node abnormalities. Peripheral nerves may show multivacuolar myelinoid bodies in the Schwann cells. Endo- and epineurial fibroblasts may contain cytoplasmic inclusions [27, 58]. The liver may show fibrosis, intracellular lipid vacuoles, and lysosomal inclusions.

Clinical biochemistry

The basic defect in CDG-I is defective N-glycosylation of proteins throughout the body with resultant multisystemic disease. Diagnosis is made by examination of the complex N-glycans on serum transferrin, but other glycoproteins can also be used. Serum transferrin has only two asparagine N-glycosylation sites per molecule, both of which are normally occupied by complex N-glycans, primarily of the disialo-biantennary N-acetyllactosamine type with smaller amounts of monosialo-biantennary, disialo-triantennary, trisialo-triantennary, and tetrasialo-tetraantennary N-glycans [37, 59–65]. Normal serum transferrin therefore exists as a series of glycoforms containing two to seven sialic acid residues per mole of protein (designated S2–S7). Isoelectric focusing and other techniques can be used to determine the relative amounts of serum transferrin glycoforms. Most CDG-I sera show reductions in S4 and S5 and increases in S0 and S2 (table 4). The presence of appreciable amounts of normal S4 indicates that the CDG-I mutations are leaky.

The N-glycan structures on transferrin glycoforms S4, S2, and S0 from Japanese CDG-I patients have been analyzed by mass spectrometry and other methods [37, 66–69]. The only N-glycans detected on S4 and S2 were normal complex N-glycans, while S0 was completely devoid of carbohydrate. These and other studies show that each glycosylation site on transferrin is either occupied by a normal N-glycan or is not occupied (an 'all-or-none' pattern), indicating that the addition of oligosaccharide to Asn is the defective step. The serum glycoprotein glycoform patterns are normal in the CDG-I fetus and, therefore, pre-natal diagnosis is not possible by this method [70]. Abnormal glycoform patterns appear in the second to third weeks of post-natal life.

Performing a detailed glycoform analysis is important, since carbohydrate-deficient serum transferrin also occurs in other conditions such as chronic alcohol intake

Table 4. Distribution of transferrin glycoforms in normal and CDG sera.

Transferrin glycoform*	Percent distribution		
	Normal [23, 29, 66, 235–237]	CDG-I [23, 27, 29, 82, 236, 238, 239]	CDG-IIa [63, 64, 165]
S0	0	2–29	~ 0
S1		+	+
S2	1–7	14–37	95
S3	4–17	0–19	~ 4
S4	46–73	18–57	~ 0
S5	10–30	5–17	~ 0
S6	0–9	0–6	~ 0
S7	trace		

* The designations S0–S7 refer to serum transferrin glycoforms with 0–7 sialic acid residues per mole of protein, respectively.
The data are based on papers published prior to the separation of CDG group I patients into separate types. Since CDG-Ia is the most common type of CDG-I, the data refer primarily to CDG-Ia patients. However, the other CDG-I types (table 2) present with very similar transferrin glycoform patterns.

[71], galactosemia [72–74], untreated fructosemia [75, 76], and hepatic disease [71].

Due to the great variations in clinical presentations of CDG (discussed below), screening for carbohydrate-deficient transferrin glycoforms is recommended for patients not only with psychomotor retardation and hypotonia, but also in unexplained feeding problems, failure to thrive, liver fibrosis, coagulopathy, and intestinal disease, even in the absence of severe neurological disease.

Many glycoproteins not only in serum but also in cerebrospinal fluid [23, 77] and other tissues show incomplete glycosylation. The levels of various serum glycoproteins may be decreased or increased, probably secondary to damage to the liver and other organs, or to other factors. Significant abnormalities have been observed in the levels of plasma proteins which modify coagulation [78–83]. This may contribute to the hemorrhages, venous thromboses, and stroke-like episodes reported in some CDG-I patients. Abnormal plasma hormone levels have been reported [22, 84, 85]. Female patients may show a hypergonadotrophic hypogonadism associated with dysfunctional follicle-stimulating hormone bioactivity. In males, testosterone levels tend to be low with normal or slightly raised gonadotrophin values.

Hypoglycosylation of glycosyltransferases and glycosidases may affect their activity or intracellular localization, thereby producing secondary alterations in carbohydrate structure that may result in misleading glycoform patterns [86]. For this and other reasons, some CDG-I patients do not show the typical 'all-or-none' serum transferrin glycoform pattern [87, 88].

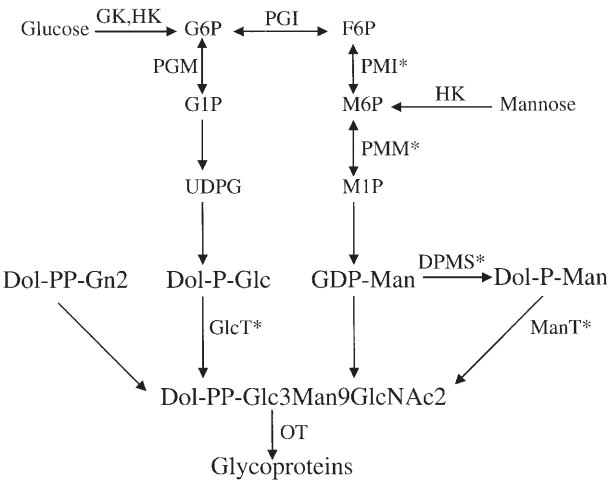

Figure 1. Metabolic pathway from glucose and mannose to dolichol pyrophosphate oligosaccharide. Enzymes: HK, hexokinase; GK, glucokinase; PGI, phosphoglucoisomerase; PGM, phosphoglucomutase; PMI, phosphomannoisomerase; PMM, phosphomannomutase; GlcT, α-1,3-glucosyltransferase; ManT, α-1,3-mannosyltransferase; DPMS, Dol-P-Man synthase; OT, oligosaccharyltransferase. Asterisks on PMM, PMI, GlcT, ManT, and DPMS denote enzyme defects in CDG types Ia, Ib, Ic, Id, and Ie respectively. Intermediates: G6P, glucose-6-phosphate; G1P, glucose-1-phosphate; F6P, fructose-6-phosphate; M6P, mannose-6-phosphate; M1P, mannose-1-phosphate; UDPG, UDP-glucose; Dol-P-Glc, dolichol phosphate glucose; Dol-P-Man, dolichol phosphate mannose; Dol-PP-Gn2, dolichol pyrophosphate N-acetylchitobiose; Dol-PP-Glc3Man9GlcNAc2, dolichol pyrophosphate $Glc_3Man_9GlcNAc_2$. Dol-PP-$Glc_3Man_9GlcNAc_2$ is assembled by the addition of Man from both GDP-Man and Dol-P-Man and Glc from Dol-P-Glc to dolichol pyrophosphate N-acetylchitobiose.

CDG type Ia (CDG-Ia)

The biochemical defect in CDG-Ia

As described above, the 'all-or-none' serum transferrin glycoform pattern in CDG-I suggests that the addition of oligosaccharide to Asn is the defective step. This step is catalyzed by an oligosaccharyltransferase which transfers $Glc_3Man_9GlcNAc_2$ from dolichol-pyrophosphate-$Glc_3Man_9GlcNAc_2$ (Dol-PP-$Glc_3Man_9GlcNAc_2$) to the asparagine residue of the protein [89, 90]. Oligosaccharyltransferase activity was shown to be normal in fibroblasts from four CDG-Ia patients [91] suggesting a defect in the synthesis of Dol-PP-oligosaccharide [92] (fig. 1).

Earlier studies showing that CDG-Ia fibroblasts are impaired in their ability to incorporate exogenous mannose into both protein- and dolichol-linked oligosaccharides [87, 93–96] led to the eventual demonstration that CDG-Ia cells and tissues are markedly deficient ($\leq 10\%$ of the control activity) in phosphomannomutase (PMM) activity (fig. 1) [97–99]. PMM deficiency results in reduced synthesis of Man-1-phosphate, GDP-Man, Dol-P-Man, and truncated dolichol-linked oligosaccharides (fig. 1). The oligosaccharyltransferase that transfers $Glc_3Man_9GlcNAc_2$ from Dol-PP-$Glc_3Man_9GlcNAc_2$ to the asparagine residue of the protein can also use shorter lipid-linked oligosaccharides as donor substrates but does so very inefficiently [89, 95, 100]. The lowered rate of oligosaccharyltransferase activity in PMM-deficient CDG-Ia cells leaves some Asn-X-Ser/Thr sequons unglycosylated. Any truncated oligosaccharides that are transferred to protein will be processed to normal N-glycan structures either by the usual pathway or by an 'alternate pathway' independent of α-mannosidase II [101] resulting in the 'all-or-none" pattern of transferrin glycosylation described above.

Intracellular GDP-Man levels are low in CDG-Ia and Ib fibroblasts, and the addition of exogenous Man to the cells can correct this deficiency [102]. Addition of exogenous Man, but not Glc, to CDG-Ia fibroblasts also corrects both the low amount and small size of lipid-linked oligosaccharides and normalizes protein N-glycosylation [94, 103, 104]. Although Man can utilize the Glc transporter, a distinct Man-specific Glc-independent transporter has been demonstrated in mammalian cells [105]. When fibroblasts are provided with physiological con-

centrations of mannose, they use the mannose rather than glucose to supply most of the mannose needed for glycoprotein synthesis [106, 107]. Serum from CDG-Ia patients has considerably reduced mannose levels (5–40 mM) compared to normal controls (40–80 mM) [103, 108]. Ingested mannose is efficiently absorbed and increases blood mannose levels in both normal subjects and CDG patients [109]. However, oral or intravenous mannose administration has not been successful in reversing either the clinical or biochemical signs of CDG-Ia [110–113].

The genetic defect in CDG-Ia

CDG-Ia is inherited in an autosomal recessive manner. Linkage and haplotype analyses of DNA from 86 CDG-Ia pedigrees of Scandinavian, German, Belgian, and other origins have localized the gene to chromosome 16p13.3–p13.12 [114–116]. There are two human PMM isozymes (PMM1 and PMM2) encoded by two genes, *PMM1* on chromosome 22q13 [117–119] and *PMM2* on chromosome 16p13 [120, 121], respectively. The identification of 26 different *PMM2* mutations in 74 CDG-Ia patients from different geographical origins and with a documented PMM deficiency proves that PMM2 deficiency is the basis for CDG-Ia [99, 120–124]. Mutation and linkage analyses with polymorphic markers have been used to perform prenatal diagnoses for CDG-Ia [125–127]. The number of molecularly characterized cases is steadily increasing. A recent collation of data on *PMM2* mutations from six research and diagnostic laboratories lists 58 different mutations in 249 patients from 23 countries [128]. Demographic data and the number of deceased patients have been recorded (data available at http://www.kuleuven.ac.be/med/cdg).

The most frequent mutation (R141H) never occurs in homozygous alleles, whereas patients homozygous for other mutations have been found [122, 124, 129]. Seven mutant forms of human PMM2 have been produced in *Escherichia coli* and purified [130]. R141H was the least active protein, indicating that R141H homozygosity and a total lack of PMM2 are incompatible with life. Only limited correlations have to date been possible between genotype, PMM activities, and the clinical severity of the disease [88].

The carrier frequency of R141H in two normal Dutch and Danish populations was found to be 1/60, a figure in disequilibrium with the estimated CDG-Ia frequency of 1/80,000–1/20,000 in these populations [129]. Haplotype analysis of 43 patients with the R141H mutation of different geographic origins indicated that it is an old mutation in the Caucasian population. The data indicate that CDG-Ia is probably underdiagnosed.

PMM2 converts mannose 1-phosphate into mannose 6-phosphate about 20 times more rapidly than glucose 1-phosphate to glucose 6-phosphate, whereas PMM1 displays identical V_{max} values with both substrates [131]. PMM2 is the only detectable isozyme in most rat tissues except brain and lung, where PMM1 accounts for about 66 and 13% of the total activities, respectively.

Diagnosis of CDG-Ia

Once a diagnosis of CDG-I has been confirmed by demonstration of the typical transferrin glycoform pattern (table 4), PMM assays should be carried out on cultured fibroblast or lymphoblast extracts, fresh leukocytes, or liver biopsies [97, 99]. PMM activities in CDG-Ia patients may vary from less than 5 to 30% of normal values [122]. Diagnoses can also be made by PMM assays on parental leukocytes [126]. The diagnosis should be completed by mutational analysis.

Prenatal testing should be limited to families with a documented PMM deficiency and a known *PMM2* mutation [126]. PMM can be measured in chorionic villus samples, cultured chorionic villus cells, and cultured amniocytes [127].

CDG type Ib (CDG-Ib)

About 20% of patients with a type I transferrin glycoform pattern do not show PMM deficiency (table 2) [132]. CDG-Ib is due to defective phosphomannoisomerase (PMI; fig. 1) [133–136]. The clinical presentations vary but all show a type I transferrin glycoform pattern without neurological disease. Niehues et al. [133] reported a 6-year-old boy who appeared normal at birth and first developed signs at about 1 year of age, i.e., failure to thrive, recurrent and severe hypoglycemia, thrombotic episodes, protein-losing enteropathy, severe vomiting, and diarrhea. Serum albumin and antithrombin III were persistently low, first suggesting CDG. There were no signs of developmental delay, neuropathy, dysmorphy, or abnormal skeletal features. Anti-coagulant therapy to reduce his thrombotic episodes led to life-threatening intestinal bleeding. Oral mannose therapy was initiated and proved to be effective. The patient has been on oral mannose (0.1–0.15 g/kg) three to five times a day for several years. His symptoms have reversed and all his biochemical abnormalities, including his abnormal transferrin pattern, returned to normal within 11 months. Assays of fibroblast and leukocyte lysates showed an 85–95% deficiency in PMI. Heterozygous relatives showed PMI levels at 34–65% of normal. The genetic lesion in the *PMI* gene was heterozygous, a point mutation (R219Q) on one allele (from the father) and an unidentified mutation from the mother. Transfection of the mutant gene into COS cells showed no enzyme activity. Other CDG-Ib patients have presented with different *PMI* mutations [134, 135]

and similar, but not identical, signs and symptoms. Three siblings were found to have congenital hepatic fibrosis associated with a deficiency of the enzyme PMI and recurrent attacks of persistent vomiting with diarrhea and mild hepatomegaly [137].

The phosphomannoisomerase gene (*MPI*) is composed of eight exons and spans only 5 kb [136]. Eight different mutations were found in seven patients with a confirmed PMI deficiency.

PMM (CDG-Ia) and PMI (CDG-Ib) both affect the synthesis of Dol-P-Man but have very different clinical presentations. Conversion of free Man to Dol-P-Man depends on PMM but not on PMI (fig. 1) and this pathway supplies most of the Man for N-glycosylation of proteins. The clinical differences between CDG-Ia and Ib may be related to the fact that both the Glc and Man paths to Dol-P-Man are defective in CDG-Ia but not in Ib.

Since CDG-Ib is potentially lethal and mannose therapy appears to be curative, patients with idiopathic congenital hepatic fibrosis [137, 138], hypoglycemia [139, 140], coagulopathies, and failure to thrive should be tested for CDG with a serum transferrin glycoform analysis.

CDG type Ic (CDG-Ic)

CDG-Ic patients have a defect in the Dol-P-Glc : Dol-PP-$Man_9GlcNAc_2$ α1,3-glucosyltransferase (α1,3-Glc-T) that converts Dol-PP-$Man_9GlcNAc_2$ to Dol-PP-$Glc_1Man_9GlcNAc_2$ (fig. 1). Burda et al. [86] presented biochemical data on four related patients. The clinical presentation is similar to CDG-Ia but milder. Fibroblasts from all four patients showed a specific deficiency in α1,3-Glc-T. The resulting non-glucosylated dolichol-linked oligosaccharides are suboptimal substrates for the oligosaccharyltransferase (fig. 1). The limited availability of GDP-Man in PMM-deficient CDG-Ia cells decreases not only the synthesis of N-glycans but also the synthesis of GDP-Fuc, the glycosylphosphatidylinositol anchors [141], and O-mannosylated [142, 143] and C-mannosylated [144] proteins. The fact that the α1,3-Glc-T defect in CDG-Ic does not affect synthesis of these components may account for the milder clinical presentation in this subtype.

Körner et al. [145] described a form of CDG, originally designated type V, which clinically resembles CDG-Ia but with normal PMM and a defect in α1,3-Glc-T. The defect is leaky and allows for residual synthesis of small amounts of normally glucosylated lipid-linked oligosaccharides. The patient, a 7-year-old girl, had been noted in her first year to have a convergent squint, recurrent edema of the upper eyelids, and recurrent infections. Muscular hypotonia, ataxia, and mental and motor developmental retardation were present at the age of 6 months. Beginning at the age of 11 months, seizures occurred during infections. Nerve conduction velocity was always normal. A slight general atrophy of the cerebrum and cerebellum was noted at the age of 4 years. Sequencing of the α1,3-Glc-T gene (see below) from this patient revealed an in-frame deletion of three nucleotides leading to the loss of isoleucine 299 [146].

Imbach et al. [147] cloned the human orthologs of the yeast genes encoding Dol-P-Glc synthase (*ALG5*) and the α1,3-Glc-T (*ALG6*) [148]. No mutations were found in the Dol-P-Glc synthase gene of the four CDG-Ic patients studied by Burda et al. [86] but a point mutation (A333V) was found in the α1,3-Glc-T gene of all four patients. Normal human α1,3-Glc-T cDNA can partially complement a yeast strain with a deletion of α1,3-Glc-T (*ALG6*) but the CDG-Ic α1,3-Glc-T cDNA was unable to do so, indicating that the CDG-Ic mutation inactivates the gene. Imbach et al. [149] recently described seven additional cases of CDG-Ic among a group of 35 untyped CDG patients. Analysis of lipid-linked oligosaccharides in patient fibroblasts showed accumulation of Dol-PP-$Man_9GlcNAc_2$. Three additional mutations in the α1,3-Glc-T gene were detected. The detrimental effect of these mutations on α1,3-Glc-T activity was confirmed by complementation of *alg6* yeast mutants. Haplotype analysis of CDG-Ic patients revealed a founder effect for the *ALG6* allele bearing the A333V mutation.

The clinical presentation of eight CDG-Ic patients homozygous for an A333V mutation [150] was mainly neurological with developmental retardation, muscular hypotonia, and epilepsy. Several signs commonly seen in CDG-Ia such as inverted nipples, abnormal fat distribution and cerebellar hypoplasia were not observed. Although the serum transferrin glycoform pattern was the same in CDG-Ia and Ic sera, beta-trace protein in cerebrospinal fluid showed a less pronounced hypoglycosylation pattern in CDG-Ic patients than in CDG-Ia patients.

Although more than 80% of CDG are type Ia, CDG-Ic may be the second-most common form of the disease.

CDG type Id (CDG-Id)

In 1995, Stibler et al. [151] presented two unrelated infants with a clinically and biochemically novel form of CDG. The first case was a German boy and the second a Turkish girl born to first-cousin parents. Both children were microcephalic and developed intractable seizures. The boy had optic atrophy and a coloboma of the iris. Both children had abnormalities of the uvula and high-arched palates. The girl had hypoplasia of the cerebellum, as is seen in CDG-Ia.

Körner et al. [152] demonstrated that the biochemical defect in these patients is a deficiency in Dol-P-Man : Dol-PP-$Man_5GlcNAc_2$ α-1,3-mannosyltransferase (α-1,3-Man-T) which transfers Man from Dol-P-Man to Dol-PP-

$Man_5GlcNAc_2$. The defect is leaky, since there is formation of some full-length Dol-PP-oligosaccharide.
The yeast *alg3* gene product, the functional ortholog of human α-1,3-Man-T, shares 30% sequence homology with the human Not56-like protein (NOT56L). NOT56L complements the glycosylation defect in a yeast strain with a disrupted *alg3* gene, showing that NOT56L is the human ortholog of yeast ALG3. Sequencing of the gene encoding NOT56L from the male CDG-Id patient showed a missense mutation (G118D). The mutation reduces but does not abolish α-1,3-Man-T activity. The patient was homozygous for the G118D mutation and his parents were heterozygous.

CDG type Ie (CDG-Ie)

Kim et al. [153] reported that fibroblasts from two patients with clinical and laboratory findings indicative of type I CDG accumulated Dol-PP-$Man_5GlcNAc_2$. Addition of 0.25 mM Man to the culture medium corrected the size of the truncated oligosaccharide. Microsomes from fibroblasts of these patients were approximately 95% deficient in Dol-P-Man synthase (fig. 1) with an apparent K_m for GDP-Man approximately sixfold higher than normal. The *DPM1* gene, coding for the catalytic subunit of Dol-P-Man synthase, was altered in both patients. One patient was homozygous for the R92G mutation. This patient was seen at age 10 months because of developmental delay, hypotonia, seizures, and acquired microcephaly. Telangiectases on the eyelids and hemangiomas of the occiput and sacrum were observed. The other patient also had the R92G mutation but was a compound heterozygote for a 13-bp deletion in exon 4 that presumably resulted in an unstable transcript. This patient was born at 29 weeks of gestation. The post-natal course was complicated by hydrops, respiratory distress, apnea, patent ductus arteriosus and transient hypertension. The infant later developed generalized intractable seizures. At 3 years of age, the child had no speech, was cortically blind, had strabismus, and swallowed poorly.
Human and mouse DPM1 cDNAs were first cloned by homology screening using the sequence of the yeast *dpm1* gene [154]. The human protein shares approximately 30% identity with yeast DPM1. Mammalian cells require DPM1 and an additional protein (DPM2) for synthesis of Dol-P-Man. DPM1 is the catalytic subunit and DPM2 is the membrane-anchored subunit. Colussi et al. [155] have cloned human, rat, nematode, and *Schizosaccharomyces pombe* DPM1 cDNAs.
Imbach et al. [156] reported a brother and sister who were hospitalized at ages 3 years and 19 months, respectively, after repeated seizure episodes. Microcephaly developed in early childhood. Hypertelorism, gothic palate, small hands with dysplastic nails and knee contractures were observed. Nipples were not inverted. Seizures began in the girl at the age of 5 weeks and in her brother at the age of 6 months. Both children were hypotonic and showed severe global developmental delay. There was no visual fixation, and they were unable to interact socially.
Biochemical analysis showed hypoglycosylation of serum transferrin and cerebrospinal fluid beta-trace protein and an accumulation of Dol-PP-$Man_5GlcNAc_2$ in their fibroblasts. The fibroblasts showed reduced Dol-P-Man synthase activity. Both siblings were found to be compound heterozygotes for two mutations in the *DPM1* gene, the R92G mutation identified by Kim et al. [153] and a 628delC deletion which caused a premature stop of translation. Complementation analysis using DPM1-null murine cells confirmed the detrimental effect of both mutations on enzymatic activity. Mannose supplementation failed to improve the glycosylation status of DPM1-deficient fibroblasts, thus precluding a possible therapeutic application of mannose in the patients [102].

CDG type If (CDG-If)

Lec15 and Lec35 are recessive mutant Chinese hamster ovary (CHO) cell lines characterized, respectively, by defects in the synthesis and utilization of Dol-P-Man. Lec35 cells accumulate Dol-PP-$Man_5GlcNAc_2$ due to their inability to utilize Dol-P-Man (fig. 1). Lec15 mutant CHO cells, which lack the DPM2 subunit of Dol-P-Man synthase but express a functional Lec35 gene, also accumulate Dol-PP-$Man_5GlcNAc_2$ but can utilize Dol-P-Glc to add some glucose to the terminal Man-α1,2 residue of the lipid-linked oligosaccharide [157]. This indicated that the Lec35 gene is required for both Dol-P-Man and Dol-P-Glc utilization. Lec35 mutant cells are indeed defective in both Dol-P-Man-dependent synthesis of lipid-linked oligosaccharides, glycosylphosphatidylinositols, and C-linked mannosylation of tryptophan, and Dol-P-Glc-dependent glucosylation of lipid-linked oligosaccharides [158, 159].
Ware and Lehrman [160] cloned a suppressor (SL15) of the Lec15 and Lec35 mutations from a CHO cDNA library. SL15 has two potential membrane-spanning regions and a likely C-terminal endoplasmic reticulum retention signal. SL15 corrects both the defective mannosylation and glucosylation in Lec35 cells but exhibits a preference for Dol-P-Man over Dol-P-Glc. SL15 is neither Dol-P-Man synthase nor an inhibitor of mannosyltransferases [158]. The function of the protein is not known but it is probably required for Dol-P-Man transport. The gene for the human homolog of SL15 has been mapped to chromosome 17p13.1–p12 [161].
Schenk et al. [162] recently reported three unrelated patients with a novel CDG type (CDG-If). The patients showed a typical type I serum transferrin glycoform pattern and normal PMM and PMI activities. Patient fibro-

blasts accumulated truncated dolichol-pyrophosphate-linked oligosaccharides (predominantly $Man_5GlcNAc_2$ and $Man_9GlcNAc_2$ with smaller amounts of the glucosylated forms of these oligosaccharides). Sequence analysis of cDNA encoding the human homolog of the CHO Lec35 protein (SL15) showed distinct mutations in all three patients. The accumulation of Dol-PP-$Man_9GlcNAc_2$ and its glucosylated form in patient fibroblasts indicates that the Lec35 mutations are leaky.

CDG type IIa (CDG-IIa)

Two CDG patients have been described who differ from the classic picture of CDG-I described above, an Iranian girl [163] and a Belgian boy [164, 165]. Both patients, designated as CDG-IIa, have inactivating point mutations in the gene encoding UDP-GlcNAc:α-6-D-mannoside β-1,2-N-acetylglucosaminyltransferase II (GnT II; fig. 2) [64, 166–169]. The first committed step in the conversion of oligomannose to complex and hybrid N-glycans is catalyzed by GnT I (fig. 2). Processing of the product of GnT I by α3/6-mannosidase II (MII; fig. 2) to form the substrate for GnT II is the first committed step in the conversion of hybrid to complex N-glycans [101, 170–173]. GnT II is a typical type II transmembrane protein [174, 175], the gene for which has been mapped to human chromosome 14q21.

Clinical features and clinical biochemistry of CDG-IIa

The CDG-IIa patients had severe psychomotor retardation but there was no peripheral neuropathy and the cerebellum was normal on nuclear MRI. Both patients had a similar dysmorphy particularly of the face (coarse features, low-set ears), widely spaced nipples, a ventricular septal defect, a striking stereotypic behaviour (tongue thrusting, hand washing and other movements), generalized hypotonia and limb weakness, and normal deep-tendon reflexes. The girl was lost from follow-up after the age of 3. The boy's speech was limited to a few monotonous sounds at 10 years. He suffered from frequent infections in infancy and epilepsy developed at 6 years. The diagnosis of CDG was not made until he was 9.5 years old when an investigation of bleeding gums revealed a typical CDG coagulopathy.

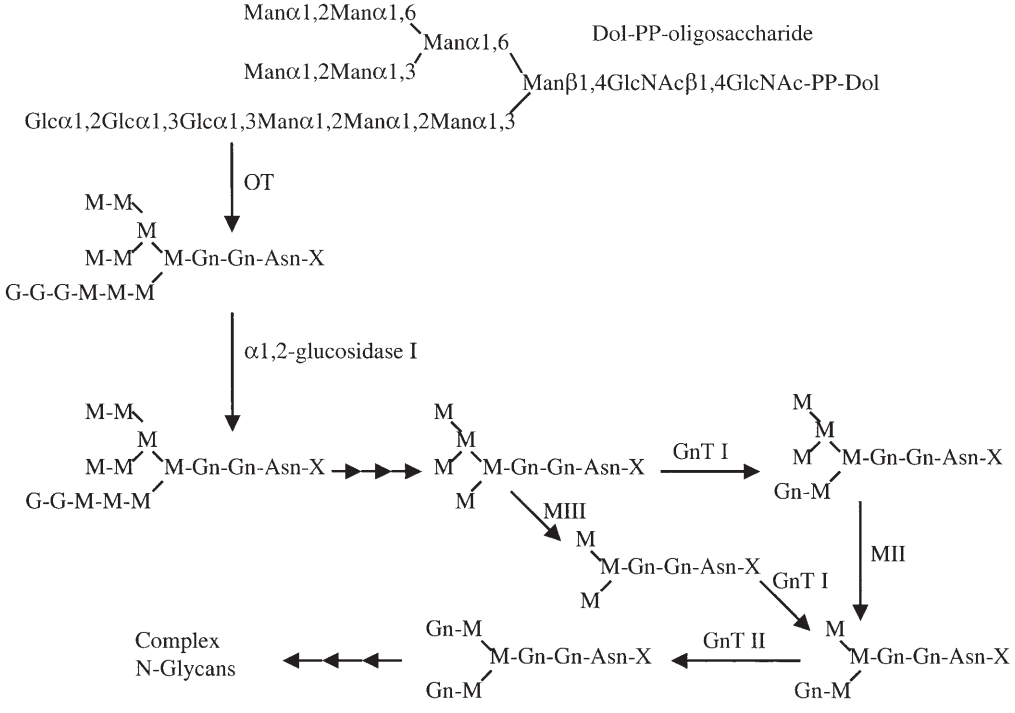

Figure 2. Pathway from dolichol pyrophosphate oligosaccharide to complex N-glycans. Oligosaccharyltransferase (OT) transfers $Glc_3Man_9GlcNAc_2$ from dolichol pyrophosphate oligosaccharide to an Asn residue in a nascent protein chain in the rough endoplasmic reticulum (RER). The glycosylated protein is released from the ribosome into the RER lumen where α1,2-glucosidase I and α1,3-glucosidase II remove the three Glc residues. ER α1,2-mannosidase, and Golgi α1,2-mannosidases I remove four Man residues to produce $Man_5GlcNAc_2$-protein; α1,3-glucosidase II, ER α1,2-mannosidase, and Golgi α1,2-mannosidases I are represented by three arrows together. In the medial Golgi compartment, the successive actions of GnT I, α3,6-mannosidase II (MII), and GnT II convert $Man_5GlcNAc_2$-protein to the biantennary complex N-glycan $GlcNAc_2Man_3GlcNAc_2$-protein. HEMPAS, CDG-IIa, and CDG-IIb are due, respectively, to defects in Golgi MII (at least in some cases), Golgi GnT II, and endoplasmic reticulum α1,2-glucosidase I. MIII is a GnT I-independent α3/6-mannosidase which provides an alternate pathway that does not require MII.

Biochemical differences from CDG-I are the absence of proteinuria, a deficiency of clotting factors IX and XII, decreased β-glucuronidase, and normal serum glutamic pyruvic transaminase activity, albumin, cholesterol, and arylsulfatase A. As occurs in CDG-I, the CDG-IIa patient showed decreased levels of many serum glycoproteins (thyroxine-binding globulin, clotting factor XI, antithrombin III, proteins C and S, heparin cofactor II, β-galactosidase, α1-anti-trypsin, apolipoprotein B, haptoglobin). Serum IgM and cerebrospinal fluid protein were elevated. Radiological examination showed osteopenia and other skeletal abnormalities. MRI revealed no cerebellar atrophy but delayed myelination and global cortical atrophy.

The CDG-IIa serum transferrin isoelectric focusing pattern differs markedly from that in CDG-I (table 4). Electrospray-mass spectrometry of CDG-IIa serum transferrin showed no or minimal amounts of asialo-, tetrasialo-, pentasialo- and hexasialotransferrins, low amounts of monosialotransferrin, a moderate amount (about 4%) of trisialotransferrin and a large amount (95%) of disialotransferrin [63, 64, 165]. Carbohydrate analysis and high-resolution proton nuclear magnetic resonance spectroscopy of CDG-IIa serum transferrin [63] showed that the major N-glycan is Manα1-6[NeuNAcα2-6Galβ1-4GlcNAcβ1-2Manα1-3]Man-R where R is -β1-4GlcNAcβ1-4GlcNAc-Asn-X. Each glycosylation site of the major disialotransferrin (S2) component is occupied by this truncated monosialo-monoantennary N-glycan. The presence of truncated N-glycans clearly differentiates the CDG-IIa transferrin glycoform pattern from the 'all-or-none' model described above for CDG-I (table 4). The parents of the CDG-IIa patients showed a normal pattern.

The large amount of normal transferrin glycoform S4 in CDG-I serum indicates that this mutation is leaky. In contrast, the mass spectrometry data [63] indicate that there is little, if any, normal S4 in CDG-IIa serum. Thus the mutation in CDG-IIa appears to be stringent.

The biochemical and genetic defects in CDG-IIa

The truncated monosialo-monoantennary N-glycan described above suggested that CDG-IIa patients may have a defect in GnT II. Indeed, GnT II activity was reduced by over 98% in fibroblast extracts from the two unrelated CDG-IIa patients [165] and there was no detectable GnT II activity in mononuclear cell extracts from the male patient [166]. There were two different point mutations in the catalytic domain of GnT II, S290F and H262R, in the girl and boy, respectively [167]. Both mutations occur in the C-terminal catalytic domain [172, 176] at locations which are conserved between rat and human GnT II [175]. Both patients are homozygous for their respective mutations and have therefore inherited the same allele from each parent. The father, mother, and brother of the boy show one normal allele and one allele with the CDG-IIa mutation.

Both mutant forms of GnT II were expressed in the baculovirus/Sf9 system [167] and showed less than 1% of the enzyme activity of normal GnT II. Thirteen blood relatives of the patient were identified as heterozygotes and showed a significant reduction (33–68%) in mononuclear cell GnT II activity. CDG-IIa is a recessive autosomal disease located at chromosome 14q21.

Over 60% of mouse embryos lacking the GnT II gene develop fully but 99% of newborns die during the first week of post-natal development [6]. The few surviving mice are runted and exhibit facial dysmorphia, kyphoscoliosis, muscular atrophy, tremors, and osteopenia. Patho-histologic findings indicate reduced ossification at the growth plates, increased prevalence of skeletal cartilage, and a calcified bone density that is reduced by over 30%. This appears with a significant increase in the frequency of osteoclasts. Although the surviving female 'nul' mice are fertile, surviving males are infertile with a block in spermatogenesis. Some serum coagulation factors are reduced. The production of T and B lymphocytes is reduced, hypocalcinemia is observed, and a ventral septal defect of the heart was noted in one of the surviving mice. The majority of the 'null' mice die with gastrointestinal blockage. Gastrointestinal hemorrhages and alterations in microbial colonization of the gastrointestinal tract were observed in some animals. Like the GnT II-null mice, the majority of humans with CDG-IIa probably die in gestation or shortly after birth, and the surviving GnT II-null mouse is likely very similar to the human CDG-IIa patient in many pathologic and biochemical features. Although a third case of CDG-IIa has been described recently [177], it is a very rare disease.

CDG type IIb (CDG-IIb)

Glucosidase I is an endoplasmic reticulum N-glycan-processing enzyme which removes the distal Glc-α1,2 residue from the $Glc_3Man_9GlcNAc2$-Asn-protein after oligosaccharyltransferase-catalyzed transfer of oligosaccharide from Dol-PP-oligosaccharide to an Asn residue (fig. 2). A glucosidase I defect has recently been described in a neonate with severe generalized hypotonia and dysmorphic features [178]. The clinical course was progressive and characterized by the occurrence of hepatomegaly, hypoventilation, feeding problems, seizures, and fatal outcome at 74 days after birth. An accumulation of Glcα1-2Glcα1-3Glcα1-3Man in the patient's urine first indicated a possible glycosylation disorder. Studies on liver tissue and cultured skin fibroblasts revealed a severe glucosidase I deficiency (< 3% of control values). Glucosidase I activities in cultured skin fibroblasts from both pa-

rents were found to be 50% of control values. Tissues from the patient subjected to Western blot analysis revealed strongly decreased amounts of glucosidase I protein in the homogenate of the liver, and a less-severe decrease in cultured skin fibroblasts. The patient was a compound heterozygote for two missense mutations in the glucosidase I gene, R486T and F652L. The mother and father were heterozygous for the R486T and F652L mutations, respectively.

Surprisingly, the serum transferrin isoelectric focusing pattern was reported to be normal. The action of Golgi endo-α1,2-mannosidase [179, 180] accounts for the presence of Glcα1-2Glcα1-3Glcα1-3Man in the patient's urine and provides a by-pass alternate pathway towards complex N-glycans. However, the severity of the clinical picture indicates severe problems in complex N-glycan formation in many organs of the infant's body; the normal serum transferrin glycoform pattern is thus difficult to explain.

Hereditary erythroblastic multinuclearity with a positive acidified-serum lysis test; congenital dyserythropoietic anemia type II (HEMPAS)

Clinical features

Ineffective and morphologically abnormal erythropoiesis is called dyserythropoiesis and results in anemia if the output of erythrocytes by the bone marrow is significantly reduced. In 1966 in Toronto, Crookston et al. [181] reported a young man (MF) with a life-long history of dyserythropoietic anemia. His bone marrow showed marked hyperplasia and multinuclear erythroblasts. His erythrocytes were lysed by acidified sera from other donors but not by his own serum. The disorder was later found in several other patients, including two sisters (CL, LF) and was determined to be a congenital dyserythropoietic anemia (CDA). The disease was named hereditary erythroblastic multinuclearity with a positive acidified-serum lysis test (HEMPAS) [182]. Three major types of CDA have been classified [183]. HEMPAS is type II CDA and is distinguished from types I and III CDA primarily on the basis of multinuclear erythroblasts and a positive serum lysis test.

In 1987, there were over 120 known cases of HEMPAS [184], but there are probably about 300 reported cases to date [185, 186]. HEMPAS consists of a group of autosomal recessive disorders, the age of diagnosis varies from infancy to old age, and the anemia varies from slight to severe. Jaundice, hepatosplenomegaly, diabetes, and gallstones are common. The body iron stores in HEMPAS are usually increased; hepatic hemosiderosis is common and hepatic cirrhosis has been reported. Electron microscopy shows that the red cell membrane is 'duplicated' and covered with pits and plaques [187, 188]. HEMPAS manifests itself primarily as a disease of erythroblasts and erythrocytes although some patients express biochemical defects in leukocytes and other organs [189].

Structural studies on HEMPAS erythrocyte glycans

About 30% of normal individuals have in their serum an antibody which when acidified can lyse HEMPAS red cells. HEMPAS serum does not contain this antibody. HEMPAS red cells also react strongly with antibodies against poly-N-acetyllactosamines [182, 184, 187, 188] suggesting that the abnormal antigen may be carbohydrate in nature. Several laboratories have reported decreased erythrocyte membrane protein glycosylation [190–193].

Whereas normal red cells have bands 3 and 4.5 that are rich in poly-N-acetyllactosamines, Fukuda et al. [194] found that there was no detectable poly-N-acetyllactosamine on bands 3 and 4.5 of HEMPAS erythrocytes. HEMPAS red cells, however, show the concomitant appearance of large amounts of a poly-N-acetyllactosamine-bearing compound identified as a macroglycolipid [191, 194–196]; normal red cells have a negligible quantity of macroglycolipid.

Fukuda et al. [197] reported varying amounts of the following protein-bound carbohydrate structures on erythrocytes from four HEMPAS patients: NeuNAcα2-6Galβ1-4GlcNAcβ1-2Manα1-3[Manα1-6]Man-R (R is -β1-4GlcNAcβ1-4GlcNAc-Asn-X; glycopeptide 1), suggesting a defect in GnT II (fig. 2), and NeuNAcα2-6Galβ1-4GlcNAcβ1-2Manα1-3[Manα1-6(Manα1-3)Manα1-6]Man-R (glycopeptide 2), suggesting a defect in α3,6-mannosidase II (MII; fig. 2). Glycopeptides 1 and 2 were major constituents in patients TO and GC, respectively [197, 198].

Analysis of HEMPAS lymphocytes and lymphoblasts

Lymphocytes from HEMPAS patient TO showed GnT II levels 10% of normal [197], and patient GC lymphoblasts had almost no detectable MII activity [198], findings compatible with the accumulation of glycopeptides 1 and 2, respectively. Northern blot analysis of mRNA from cultured transformed lymphoblasts using a probe for human MII showed that GC had levels of message less than 10% of normal [198]. These results suggest that GC cells contain a mutation in the *MII* gene that results in inefficient expression of MII mRNA, either through reduced transcription or message instability. Recently, a second human *MII*-like gene called *MIIx* has been described, but its role in HEMPAS remains to be elucidated [199].

The genetic defect in HEMPAS

Normal adult human erythrocyte band 3 carries a single biantennary N-glycan with poly-N-acetyllactosamine

chains on both antennae [200], whereas band 3 from all HEMPAS patients studied to date lacks poly-N-acetyllactosamine chains [194, 197, 198, 201–203]. Since there are poly-N-acetyllactosamine chains on the Manα1-3 arm of the normal band 3 N-glycan, a defect in either GnT II or MII should not result in complete absence of poly-N-acetyllactosamine chains. Indeed, band 3 from CDG-IIa patients (lacking GnT II, see above) retains about 50% of its poly-N-acetyllactosamine content [166], consistent with the fact that there is no preferential attachment of poly-N-acetyllactosamine chains to either of the arms of a biantennary N-glycan although preferential attachment to the Manα1-6 arm has been reported for tri- and tetra-antennary N-glycans [3, 204]. The relatively benign clinical picture of HEMPAS is completely different from the severe abnormalities found in CDG-IIa. Therefore, lack of GnT II is not likely to be the primary defect in HEMPAS.

Linkage analysis and cytogenetic studies in 12 southern Italian families [205, 206] located the CDA II gene (*CDAN2*) to chromosome 20q11.2 and excluded the genes encoding GnT II (chromosome 14q21 [175]), MIIx (chromosome 15q25 [199]) and MII (chromosome 5q21-q22 [199]). Northern analyses of mRNA from several Italian HEMPAS patients showed a reduction of MII and GnT II mRNAs suggesting a secondary effect, such as defective transcription or mRNA maturation involving both the genes for GnT II and MII [185, 206]. The authors suggest that the HEMPAS mutation may alter the properties of a tissue-specific cellular factor which specifies the utilization of distinct polyadenylation signals within the 3′ untranslated region of both mRNAs. However, Fukuda [186, 203] did not find a reduction in GnT II or MII transcripts in southern Italian HEMPAS cases and suggested that a defect exists in a Golgi or cytoplasmic protein regulating membrane trafficking, and that this defect affects glycosylation.

Genetic heterogeneity in CDA II was demonstrated by Iolascon et al. [207] who found two unrelated families from southern Italy in which CDA II was not linked to the *CDAN2* locus. The HEMPAS gene of patient GC (described above), with over 90% decrease in MII expression [198], is probably also not linked to the *CDAN2* locus. The mutation responsible for the lowered MII mRNA levels in patient GC has not yet been determined. Fukuda [186, 203] has reported preliminary evidence, published only in reviews, that another HEMPAS case (LF) may also have a defect in MII. LF is a compound heterozygote with a deletion in one allele and an insertion in the other. GC and LF are descended from British and Irish families, respectively. There are probably HEMPAS variants which involve neither the *CDAN2* locus nor *MII* [207–210].

Since the *MII* gene is normally expressed in a variety of cells and tissues, the HEMPAS defect is not restricted to erythroid cells. However, there are cells and tissues that are not affected by the HEMPAS genetic defect, suggesting that there may be other tissue-specific MII isozymes. Another possibility is the presence of alternate processing pathways which are independent of MII action: (i) lack of Dol-P-Man synthase [101, 211], glucose starvation [212], or energy deprivation [101] all cause defective Dol-P-Man synthesis which leads to the synthesis of truncated oligosaccharides that do not require MII action; (ii) rat brain and liver have a membrane-bound α3/6-mannosidase which can cleave protein-bound $Man_5GlcNAc_2$ to $Man_3GlcNAc_2$ [213–216] without the need for GnT I or MII.

As explained above, lack of MII should not result in complete absence of poly-N-acetyllactosamine chains from erythrocyte band 3. It is possible that poly-N-acetyllactosamine chains can be added to protein-bound $Man_3GlcNAc_2$ but not to protein-bound $Man_5GlcNAc_2$.

Data from the MII-null mouse [12] support the hypothesis that MII lack can cause HEMPAS. The mice developed a dyserythropoietic anemia, splenomegaly, reticulocytosis, and other features similar to HEMPAS, concurrent with loss of erythrocyte complex N-glycans. Non-erythroid cell types continued to produce complex N-glycans by an alternate pathway comprising a distinct α3/6-mannosidase (*MIII*, fig. 2) which converts protein-bound $Man_5GlcNAc_2$ to $Man_3GlcNAc_2$ [215, 216]. The MII mutant mice, unlike HEMPAS patients, do not show multinucleated erythroblasts, hepatomegaly, liver cirrhosis, or hemosiderosis. Neither normal nor mutant mouse erythrocytes have protein-bound poly-N-acetyllactosamine chains.

Leukocyte Adhesion Deficiency Type II (LAD II)

Leukocyte adhesion deficiency type II (LAD II) is a rare disorder characterized by recurrent infections, persistent leukocytosis, and severe mental and growth retardation [217]. In 1992, two supposedly unrelated Arab Moslem boys (3 and 5 years old) were reported with a distinctive syndrome (Rambam-Hasharon syndrome) comprising unusual facial appearance, severe mental retardation, microcephaly, cortical atrophy, seizures, hypotonia, dwarfism, and recurrent infections with a high leukocyte count and without the formation of pus [218, 219]. Autosomal recessive inheritance is strongly suggested by the fact that both of the patients were the offspring of consanguineous parents.

Both patients manifested the Bombay (*hh*) phenotype caused by a lack of H blood group α1,2-fucosyltransferase (*FUT1*, [220]) action. Both children were also nonsecretors (*sese*, due to lack of activity of the secretor α1,2-fucosyltransferase, *FUT2* [221]), and Lewis-negative (*lele*, due to lack of activity of the Lewis blood group α1,3/4-fucosyltransferase, *FUT3* [222]). However, the

above fucosyltransferase activities were expressed at normal levels. A general defect in fucose metabolism was therefore suggested [218, 223, 224].

The immune defect in the patients is due to the absence of fucosylated selectin ligands [225] with a resultant defect in the adhesion of leukocytes to endothelia and lack of recruitment of neutrophils to sites of inflammation [see ref. 4 for a review of leukocyte adhesion]. LAD II neutrophils are deficient in expression of selectin ligand activity and exhibit a correspondingly diminished ability to roll on endothelium and to traffic to inflammatory sites in vivo. Neutrophils from LAD II patients bind minimally or not at all to E- or P-selectin [225]. Cell surface expression of fucosylated glycoconjugates was induced by exposure of lymphoblastoid cell lines, human umbilical vein endothelial cells, and fibroblasts from LAD II patients to exogenous fucose [226]. GDP-mannose-4,6-dehydratase, the first of two enzymes in the de novo GDP-L-fucose biosynthesis pathway, was reported to show altered kinetics in cell lysates from a LAD II patient compared with controls [227], but no mutations were identified in patient cDNA encoding the dehydratase. Furthermore, the levels of immunoreactive enzyme in cell lysates were comparable in the patient and controls, suggesting that the LAD II defect was due to a mutation affecting an unidentified dehydratase-regulating protein.

Lübke et al. [228] recently reported a patient of Turkish origin with a new type of LAD II due to decreased import of GDP-fucose into the Golgi with resultant decreased fucosylation of glycoproteins. In vitro assays of cytosolic extracts from leukocytes and fibroblasts of the patient demonstrated a normal GDP-L-fucose biosynthesis from GDP-D-mannose [229]. Analysis of the two enzymes involved in the pathway, GDP-mannose 4,6-dehydratase and FX protein, revealed normal numbers of transcripts without any detectable mutations within the coding regions of either gene. However, the saturable, high-affinity import of GDP-fucose into Golgi-enriched vesicles was deficient in the patient, while import of UDP-Gal and the activity of GDPase (which generates the nucleoside phosphate GMP required for antiport of GDP-fucose) were normal [228]. Addition of L-fucose to the medium of fibroblasts restored the fucosylation of glycoproteins. The rat liver Golgi membrane GDP-fucose transporter has been identified and purified [230]. Transport was stimulated two to three-fold after pre-loading proteoliposomes with GMP, the putative antiporter.

Sialyl-Lewis X was absent from the surface of polymorphonuclear neutrophils from this patient and cell binding to E- and P-selectins was severely impaired, causing an immunodeficiency [231]. Elevation of peripheral neutrophil counts occurred within several days after birth. A severe hypofucosylation of glycoconjugates was present in all cell types investigated. Since the fucosylation defect in the patient's fibroblasts can be corrected by addition of L-fucose to the culture medium [228], oral fucose therapy of the patient was attempted [232, 233]. This resulted in dramatic improvement in fucosylation of the selectin ligands and other glycoproteins and an overall improvement in clinical condition. There was no hemolysis (a possibility due to the presence of antibodies against blood group H in the sera of LAD II patients) or other side effects. During 9 months of treatment, infections and fever disappeared, elevated neutrophil counts returned to normal and psychomotor capabilities improved.

A recent review [234] reported that five LAD II patients have been reported to date, four of Arabic origin living in Israel (which include the original two patients described above) and one of Turkish origin (also described above). A defect in the transport of GDP-Fuc into Golgi vesicles was observed in one of the Arab patients [234] but the kinetics were different from those observed in the Turkish patient. While oral fucose was an effective treatment for the Turkish patient, it was ineffective in the Arab patient.

1 Vliegenthart J. F. G. and Montreuil J. (1995) Primary structure of glycoprotein glycans. In: New Comprehensive Biochemistry, vol. 29a, Glycoproteins, pp. 13–28, Montreuil J., Vliegenthart J. F. G. and Schachter H. L. (eds), Elsevier, Amsterdam

2 Laine R. A. (1994) A calculation of all possible oligosaccharide isomers both branched and linear yields 1.05×10^{12} structures for a reducing hexasaccharide: the isomer barrier to development of single-method saccharide sequencing or synthesis systems. Glycobiology. **4:** 759–767.

3 Fukuda M. (2000) Cell surface carbohydrates: cell type-specific expression. In: Frontiers in Molecular Biology, vol. 30, Molecular and Cellular Glycobiology, pp. 1–61, Fukuda M. and Hindsgaul O. (eds), Oxford University Press, Oxford

4 Homeister J. W. and Lowe J. B. (2000) Carbohydrate recognition in leukocyte-endothelial cell interactions. In: Frontiers in Molecular Biology, vol. 30, Molecular and Cellular Glycobiology, pp. 62–115, Fukuda M and Hindsgaul O. (eds), Oxford University Press, Oxford

5 Brusés J. L. and Rutishauer U. (2000) Polysialic acid in neural cell development: roles, regulation and mechanism. In: Frontiers in Molecular Biology, vol. 30, Molecular and Cellular Glycobiology, pp. 116–132, Fukuda M. and Hindsgaul O. (eds.), Oxford University Press, Oxford

6 Wang Y., Tan J., Campbell R. M., Ditto D., Le D., Schachter H. et al. (2000) Investigating a model of human CDG type II reveals a heterogenic disease with cell type-specific defects and frequent peri-natal lethality. Glycobiology. **10:** 1131–1132

7 Metzler M., Gertz A., Sarkar M., Schachter H., Schrader J. W. and Marth J. D. (1994) Complex asparagine-linked oligosaccharides are required for morphogenic events during post-implantation development. EMBO J. **13:** 2056–2065

8 Ioffe E. and Stanley P. (1994) Mice lacking N-acetylglucosaminyltransferase I activity die at mid-gestation, revealing an essential role for complex or hybrid N-linked carbohydrates. Proc. Natl. Acad. Sci. USA. **91:** 728–732

9 Stanley P. (2000) Functions of carbohydrates revealed by transgenic technology. In: Frontiers in Molecular Biology, vol. 30, Molecular and Cellular Glycobiology, pp. 169–198, Fukuda M. and Hindsgaul O. (eds), Oxford University Press, Oxford

10 Granovsky M., Fata J., Pawling J., Muller W. J., Khokha R. and Dennis J. W. (2000) Suppression of tumor growth and metastasis in Mgat5-deficient mice. Nat. Med. **6:** 306–312.

11 Varki A. and Marth J. (1995) Oligosaccharides in vertebrate development. Semin. Dev. Biol. **6:** 127–138
12 Chui D., OhEda M., Liao Y. F., Panneerselvam K., Lal A., Marek K. W. et al. (1997) Alpha-mannosidase-II deficiency results in dyserythropoiesis and unveils an alternate pathway in oligosaccharide biosynthesis. Cell **90:** 157–167
13 Marek K. W., Vijay I. K. and Marth J. D. (1999) A recessive deletion in the GlcNAc-1-phosphotransferase gene results in peri-implantation embryonic lethality. Glycobiology **9:** 1263–1271
14 Shafi R., Iyer S. P., Ellies L. G., O'Donnell N., Marek K. W., Chui D. et al. (2000) The O-GlcNAc transferase gene resides on the X chromosome and is essential for embryonic stem cell viability and mouse ontogeny. Proc. Natl. Acad. Sci. USA **97:** 5735–5739
15 Selleck S. B. (2000) Proteoglycans and pattern formation: sugar chemistry meets developmental genetics. Trends Genet. **16:** 206–212
16 Herman T. and Horvitz H. R. (1999) Three proteins involved in *Caenorhabditis elegans* vulval invagination are similar to components of a glycosylation pathway. Proc. Natl. Acad. Sci. USA **96:** 974–979
17 Bulik D. A., Wei G., Toyoda H., Kinoshita-Toyoda A., Waldrip W. R., Esko J. D. et al. (2000) *sqv-3, -7*, and *-8*, a set of genes affecting morphogenesis in *Caenorhabditis elegans*, encode enzymes required for glycosaminoglycan biosynthesis. Proc. Natl. Acad. Sci. USA **97:** 10838–10843
18 Okajima T., Yoshida K., Kondo T. and Furukawa K. (1999) Human homolog of *Caenorhabditis elegans sqv-3* gene is galactosyltransferase I involved in the biosynthesis of the glycosaminoglycan-protein linkage region of proteoglycans. J. Biol. Chem. **274:** 22915–22918
19 Culetto E. and Sattelle D. B. (2000) A role for *Caenorhabditis elegans* in understanding the function and interactions of human disease genes. Hum. Mol. Genet. **9:** 869–877
20 Schachter H. (1999) Molecular Basis of Glycoconjugate Disease, Biochim. Biophys. Acta, vol. 1455, Elsevier, Amsterdam
21 Montreuil J., Vliegenthart J. F. G. and Schachter H. (1996) New comprehensive biochemistry, vol. 30, Glycoproteins and Disease, Elsevier, Amsterdam
22 Jaeken J., Vanderschueren-Lodeweyckx M., Casaer P., Snoeck L., Corbeel L., Eggermont E. et al. (1980) Familial psychomotor retardation with markedly fluctuating serum prolactin, FSH and GH levels, partial TBG deficiency, increased serum arylsulphatase A and increased CSF protein: a new syndrome? Pediat. Res. **14:** 179
23 Jaeken J., van Eijk H. G., van der Heul C., Corbeel L., Eeckels R. and Eggermont E. (1984) Sialic acid-deficient serum and cerebrospinal fluid transferrin in a newly recognized genetic syndrome. Clin. Chim. Acta. **144:** 245–247
24 Carchon H., Van Schaftingen E., Matthijs G. and Jaeken J. (1999) Carbohydrate-deficient glycoconjugate syndrome type IA (phosphomannomutase deficiency). Biochim. Biophys. Acta. **1455:** 155–165
25 Aebi M. and others. (2000) Letter to the Glyco-Forum: carbohydrate-deficient glycoprotein syndromes become congenital disorders of glycosylation, an updated nomenclature for CDG. Glycobiology **10:** iii–v
26 Orlean P. (2000) Congenital disorders of glycosylation caused by defects in mannose addition during N-linked oligosaccharide assembly. J. Clin. Invest. **105:** 131–132
27 Jaeken J., Carchon H. and Stibler H. (1993) The carbohydrate-deficient glycoprotein syndromes – Pre-Golgi and Golgi disorders? Glycobiology **3:** 423–428
28 Jaeken J., Matthijs G., Barone R. and Carchon H. (1997) Carbohydrate deficient glycoprotein (CDG) syndrome type I. J. Med. Genet. **34:** 73–76
29 Stibler H. and Jaeken J. (1990) Carbohydrate deficient serum transferrin in a new systemic hereditary syndrome. Arch. Dis. Child. **65:** 107–111
30 Stibler H., Blennow G., Kristiansson B., Lindehammer A. and Hagberg B. (1994) Carbohydrate-deficient glycoprotein syndrome: clinical expression in adults with a new metabolic disease. J. Neurol. Neurosurg. Psychiatry **57:** 552–556
31 Eeg-Olofsson K. E. and Wahlström J. (1991) Genetic and epidemiological aspects of the carbohydrate-deficient glycoprotein syndrome. Acta Paediatr. Scand. **80:** 63–65
32 Winchester B., Clayton P., Mian N., Di-Tomaso E., Dell A., Reason A. et al. (1995) The carbohydrate-deficient glycoprotein syndrome: an experiment of nature in glycosylation. Biochem. Soc. Trans. **23:** 185–188
33 Gahl W. A. (1997) Carbohydrate-deficient glycoprotein syndrome: hidden treasures. J. Lab. Clin. Med. **129**: 394–395
34 McDowell G. and Gahl W. A. (1997) Inherited disorders of glycoprotein synthesis: cell biological insights. Proc. Soc. Exp. Biol. Med. **215:** 145–157
35 Ohno K., Yuasa I., Akaboshi S., Itoh M., Yoshida K., Ehara H. et al. (1992) The carbohydrate deficient glycoprotein syndrome in three Japanese children. Brain Dev. **14:** 30–35
36 Hagberg B. A., Blennow G., Kristiansson B. and Stibler H. (1993) Carbohydrate-deficient glycoprotein syndromes – peculiar group of new disorders. Pediatr. Neurol. **9:** 255–262
37 Yamashita K. and Ohno K. (1996) Carbohydrate-deficient glycoprotein syndrome type I. In: New Comprehensive Biochemistry, vol. 30, Glycoproteins and Disease, pp. 445–455, Montreuil J., Vliegenthart J. F. G. and Schachter H. (eds), Elsevier, Amsterdam
38 Krasnewich D. and Gahl W. A. (1997) Carbohydrate-deficient glycoprotein syndrome. Adv. Pediatr. **44:** 109–140
39 Petersen M. B., Brostrom K., Stibler H. and Skovby F. (1993) Early manifestations of the carbohydrate-deficient glycoprotein syndrome. J. Pediatr. **122:** 66–70
40 Kristiansson B., Borulf S., Conradi N., Erlanson Albertsson C., Ryd W. and Stibler H. (1998) Intestinal, pancreatic and hepatic involvement in carbohydrate-deficient glycoprotein syndrome type I. J. Pediatr. Gastroenterol. Nutr. **27:** 23–29
41 Kristiansson B., Stibler H., Conradi N., Eriksson B. O. and Ryd W. (1998) The heart and pericardial effusions in CDGS-I (carbohydrate-deficient glycoprotein syndrome type I). J. Inher. Metab. Dis. **21:** 112–124
42 Garcia-Silva M. T., de Castro J., Stibler H., Simon R., Chasco-Yrigoyen A., Mateos F. et al. (1996) Prenatal hypertrophic cardiomyopathy and pericardial effusion in carbohydrate-deficient glycoprotein syndrome. J. Inher. Metab. Dis. **19:** 257–259
43 Clayton P. T., Winchester B. G. and Keir G. (1992) Hypertrophic obstructive cardiomyopathy in a neonate with the carbohydrate-deficient glycoprotein syndrome. J. Inher. Metab. Dis. **15:** 857–861
44 Jaeken J. and Carchon H. (1993) The carbohydrate-deficient glycoprotein syndromes: recent developments. Int. Pediatr. **8:** 60–62
45 Hutchesson A. C. J., Gray R. G. F., Spencer D. A. and Keir G. (1995) Carbohydrate deficient glycoprotein syndrome: multiple abnormalities and diagnostic delay. Arch. Dis. Child. **72:** 445–446
46 van der Knaap M. S., Wevers R. A., Monnens L., Jakobs C., Jaeken J. and van Wijk J. A. (1996) Congenital nephrotic syndrome: a novel phenotype of type I carbohydrate-deficient glycoprotein syndrome. J. Inher. Metab. Dis. **19:** 787–791
47 Jaeken J., Stibler H. and Hagberg B. (1991) The carbohydrate-deficient glycoprotein syndrome: a new inherited multisystemic disease with severe nervous system involvement. Acta Paediatr. Scand. Suppl. **375:** 1–71
48 Fiumara A., Barone R., Buttitta P., Dipietro M., Scuderi A., Nigro F. et al. (1994) Carbohydrate deficient glycoprotein syndrome type I: ophthalmic aspects in four Sicilian patients. Br. J. Ophthalmol. **78:** 845–846

49 Casteels I., Spileers W., Leys A., Lagae L. and Jaeken J. (1996) Evolution of ophthalmic and electrophysiological findings in identical twin sisters with the carbohydrate deficient glycoprotein syndrome type 1 over a period of 14 years. Br. J. Ophthalmol. **80:** 900–902

50 Garel C., Baumann C., Besnard M., Ogier H., Jaeken J. and Hassan M. (1998) Carbohydrate-deficient glycoprotein syndrome type I: a new cause of dysostosis multiplex. Skel. Radiol. **27:** 43–45

51 Jaeken J., Hagberg B. and Stromme P. (1991) Clinical presentation and natural course of the carbohydrate-deficient glycoprotein syndrome. Acta Paediatr. Scand. **80:** 6–13

52 Pavone L., Fiumara A., Barone R., Rizzo R., Buttitta P., Dobyns W. B. et al. (1996) Olivopontocerebellar atrophy leading to recognition of carbohydrate-deficient glycoprotein syndrome type I. J. Neurol. **243:** 700–705

53 Holzbach U., Hanefeld F., Helms G., Hanicke W. and Frahm J. (1995) Localized proton magnetic resonance spectroscopy of cerebral abnormalities in children with carbohydrate-deficient glycoprotein syndrome. Acta Paediatr. **84:** 781–786

54 Jensen P. R., Hansen F. J. and Skovby F. (1995) Cerebellar hypoplasia in children with the carbohydrate-deficient glycoprotein syndrome. Neuroradiology **37:** 328–330

55 Akaboshi S., Ohno K. and Takeshita K. (1995) Neuroradiological findings in the carbohydrate-deficient glycoprotein syndrome. Neuroradiology **37:** 491–495

56 Stromme P., Maehlen J., Strom E. H. and Torvik A. (1991) Postmortem findings in two patients with the carbohydrate-deficient glycoprotein syndrome. Acta Paediatr. Scand. **80:** 55–62

57 Horslen S. P., Clayton P. T., Harding B. N., Hall N. A., Keir G. and Winchester B. (1991) Olivopontocerebellar atrophy of neonatal onset and disialotransferrin developmental deficiency syndrome. Arch. Dis. Child **66:** 1027–1032

58 Nordborg C., Hagberg B. and Kristiansson B. (1991) Sural nerve pathology in the carbohydrate-deficient glycoprotein syndrome. Acta Paediatr. Scand. **80:** 39–49

59 März L., Hatton M. W., Berry L. R. and Regoeczi E. (1982) The structural heterogeneity of the carbohydrate moiety of desialylated human transferrin. Can. J. Biochem. **60:** 624–630

60 Spik G., Bayard B., Fournet B., Strecker G., Bouquelet S. and Montreuil J. (1975) Studies on glycoconjugates. LXIV. Complete structure of two carbohydrate units of human serotransferrin. FEBS Lett. **50:** 296–299

61 Spik G., Debruyne V., Montreuil J., van Halbeek H. and Vliegenthart J. F. G. (1985) Primary structure of two sialylated triantennary glycans from human serotransferrin. FEBS Lett. **183:** 65–69

62 Fu D. and Van Halbeek H. (1992) N-glycosylation site mapping of human serotransferrin by serial lectin affinity chromatography, fast atom bombardment-mass spectrometry, and 1H nuclear magnetic resonance spectroscopy. Anal. Biochem. **206:** 53–63

63 Coddeville B., Carchon H., Jaeken J., Briand G. and Spik G. (1998) Determination of glycan structures and molecular masses of the glycovariants of serum transferrin from a patient with carbohydrate deficient syndrome type II. Glycoconj. J. **15:** 265–273

64 Jaeken J., Spik G. and Schachter H. (1996) Carbohydrate-deficient glycoprotein syndrome type II: an autosomal recessive disease due to mutations in the N-acetylglucosaminyltransferase II gene. In: New Comprehensive Biochemistry vol. 30, Glycoproteins and Disease, pp. 457–467, Montreuil J., Vliegenthart J. F. G. and Schachter H. (eds) Elsevier, Amsterdam

65 De Jong G., van Noort W. L., Feelders R. A., de Jeu-Jaspars C. M. H. and van Eijk H. G. (1992) Adaptation of transferrin protein and glycan synthesis. Clin. Chim. Acta. **212:** 27–45

66 Yamashita K., Ideo H., Ohkura T., Fukushima K., Yuasa I., Ohno K. et al. (1993) Sugar chains of serum transferrin from patients with carbohydrate deficient glycoprotein syndrome: evidence of asparagine-N-linked oligosaccharide transfer deficiency. J. Biol. Chem. **268:** 5783–5789

67 Yamashita K., Ohkura T., Ideo H., Ohno K. and Kanai M. (1993) Electrospray ionization-mass spectrometric analysis of serum transferrin isoforms in patients with carbohydrate-deficient glycoprotein syndrome. J. Biochem. Tokyo **114:** 766–769

68 Wada Y., Nishikawa A., Okamoto N., Inui K., Tsukamoto H., Okada S. et al. (1992) Structure of serum transferrin in carbohydrate-deficient glycoprotein syndrome. Biochem. Biophys. Res. Commun. **189:** 832–836

69 Wada Y., Gu J. G., Okamoto N. and Inui K. (1994) Diagnosis of carbohydrate-deficient glycoprotein syndrome by matrix-assisted laser desorption time-of-flight mass spectrometry. Biol. Mass Spectrom. **23:** 108–109

70 Clayton P., Winchester B., Di Tomaso E., Young E., Keir G. and Rodeck C. (1993) Carbohydrate-deficient glycoprotein syndrome: normal glycosylation in the fetus. Lancet **341:** 956

71 Stibler H. (1991) Carbohydrate-deficient transferrin in serum: a new marker of potentially harmful alcohol consumption reviewed. Clin. Chem. **37:** 2029–2037

72 Stibler H., von Dobeln U., Kristiansson B. and Guthenberg C. (1997) Carbohydrate-deficient transferrin in galactosaemia. Acta Paediatr. **86:** 1377–1378

73 Ornstein K. S., McGuire E. J., Berry G. T., Roth S. and Segal S. (1992) Abnormal galactosylation of complex carbohydrates in cultured fibroblasts from patients with galactose-1-phosphate uridyltransferase deficiency. Pediatr. Res. **31:** 508–511

74 Charlwood J., Clayton P., Keir G., Mian N. and Winchester B. (1998) Defective galactosylation of serum transferrin in galactosemia. Glycobiology **8:** 351–357

75 Adamowicz M. and Pronicka E. (1996) Carbohydrate deficient glycoprotein syndrome-like transferrin isoelectric focusing pattern in untreated fructosaemia. Eur. J. Pediatr. **155:** 347–348

76 Jaeken J., Pirard M., Adamowicz M., Pronicka E. and Van Schaftingen E. (1996) Inhibition of phosphomannose isomerase by fructose 1-phosphate: an explanation for defective N-glycosylation in hereditary fructose intolerance. Pediatr. Res. **40:** 764–766

77 Pohl S., Hoffmann A., Rudiger A., Nimtz M., Jaeken J. and Conradt H. S. (1997) Hypoglycosylation of a brain glycoprotein (beta-trace protein) in CDG syndromes due to phosphomannomutase deficiency and N-acetylglucosaminyltransferase II deficiency. Glycobiology **7:** 1077–1084

78 Stibler H., Holzbach U. and Kristiansson B. (1998) 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. **58:** 55–61

79 Van Geet C. and Jaeken J. (1993) A unique pattern of coagulation abnormalities in carbohydrate-deficient glycoprotein syndrome. Pediatr. Res. **33:** 540–541

80 Stibler H., Holzbach U., Tengborn L. and Kristiansson B. (1996) Complex functional and structural coagulation abnormalities in the carbohydrate-deficient glycoprotein syndrome type I. Blood Coagulat. Fibrinol. **7:** 118–126

81 Okamoto N., Wada Y., Kobayashi M., Otani K., Tagawa T., Futagi Y. et al. (1993) Decreased blood coagulation activities in carbohydrate-deficient glycoprotein syndrome. J. Inherit. Metab. Dis. **16:** 435–440

82 Yuasa I., Ohno K., Hashimoto K., Iijima K., Yamashita K. and Takeshita K. (1995) Carbohydrate-deficient glycoprotein syndrome: electrophoretic study of multiple serum glycoproteins. Brain Dev. **17:** 13–19

83 Fiumara A., Barone R., Buttitta P., Musso R., Pavone L., Nigro F. et al. (1996) Haemostatic studies in carbohydrate-deficient glycoprotein syndrome type I. Thromb. Haemost. **76:** 502–504

84 De Zegher F. and Jaeken J. (1995) Endocrinology of the carbohydrate-deficient glycoprotein syndrome type 1 from birth through adolescence. Pediatr. Res. **37:** 395–401

85 Kristiansson B., Stibler H. and Wide L. (1995) Gonadal function and glycoprotein hormones in the carbohydrate-deficient glycoprotein (CDG) syndrome. Acta Paediatr. **84:** 655–659

86 Burda P., Borsig L., de Rijk van Andel J. F., Wevers R. A., Jaeken J., Carchon H. et al. (1998) A novel carbohydrate-deficient glycoprotein syndrome characterized by a deficiency in glucosylation of the dolichol-linked oligosaccharide. J. Clin. Invest. **102:** 647–652

87 Krasnewich D. M., Holt G. D., Brantly M., Skovby F., Redwine J. and Gahl W. A. (1995) Abnormal synthesis of dolichol-linked oligosaccharides in carbohydrate-deficient glycoprotein syndrome. Glycobiology **5:** 503–510

88 Imtiaz F., Worthington V., Champion M., Beesley C., Charlwood J., Clayton P. et al. (2000) Genotypes and phenotypes of patients in the UK with carbohydrate-deficient glycoprotein syndrome type 1. J. Inherit. Metab. Dis. **23:** 162–74

89 Verbert A. (1995) From $Glc_3Man_9GlcNAc_2$-protein to $Man_5GlcNAc_2$-protein: transfer 'en bloc' and processing. In: New Comprehensive Biochemistry, vol. 29 a, Glycoproteins, pp. 145–152, Montreuil J. Vliegenthart J. F. G. and Schachter H. (eds), Elsevier, Amsterdam

90 Knauer R. and Lehle L. (1994) The N-oligosaccharyltransferase complex from yeast. FEBS Lett. **344:** 83–86

91 Knauer R., Lehle L., Hanefeld F. and von Figura K. (1994) Normal N-oligosaccharyltransferase activity in fibroblasts from patients with carbohydrate-deficient glycoprotein syndrome. J. Inher. Metab. Dis. **17**: 541–544

92 Hemming F. W. (1995) The coenzymic role of phosphodolichols. In: Glycoproteins, New Comprehensive Biochemistry, vol. 29a, pp 127–143, Montreuil J., Vliegenthart J. F. G. and Schachter H., (eds), Elsevier, Amsterdam

93 Powell L. D., Panreerselvam K., Vij R., Diaz S., Manzi A., Buist N. et al. (1994) Carbohydrate-deficient glycoprotein syndrome: not an N-linked oligosaccharide processing defect, but an abnormality in lipid-linked oligosaccharide biosynthesis? J. Clin. Invest. **94:** 1901–1909

94 Panneerselvam K. and Freeze H. H. (1996) Mannose corrects altered N-glycosylation in carbohydrate-deficient glycoprotein syndrome fibroblasts. J. Clin. Invest. **97:** 1478–1487

95 Körner C., Lehle L. and von Figura K. (1998) Abnormal synthesis of mannose 1-phosphate derived carbohydrates in carbohydrate-deficient glycoprotein syndrome type I fibroblasts with phosphomannomutase deficiency. Glycobiology **8:** 165–171

96 Ohkura T., Fukushima K., Kurisaki A., Sagami H., Ogura K., Ohno K. et al. (1997) A partial deficiency of dehydrodolichol reduction is a cause of carbohydrate-deficient glycoprotein syndrome type I. J. Biol. Chem. **272:** 6868–6875

97 Van Schaftingen E. and Jaeken J. (1995) Phosphomannomutase deficiency is a cause of carbohydrate-deficient glycoprotein syndrome type I. FEBS Lett. **377:** 318–320

98 Charlwood J., Clayton P., Johnson A., Keir G., Mian N. and Winchester B. (1997) A case of the carbohydrate-deficient glycoprotein syndrome type 1 (CDGS type 1) with normal phosphomannomutase activity. J. Inher. Metab. Dis. **20:** 817–827

99 Jaeken J., Artigas J., Barone R., Fiumara A., de Koning T. J., Poll-The B. T. et al. (1997) Phosphomannomutase deficiency is the main cause of carbohydrate-deficient glycoprotein syndrome with type I isoelectrofocusing pattern of serum sialotransferrins. J. Inher. Metab. Dis. **20:** 447–449

100 Struck D. K. and Lennarz W. J. (1980) The function of saccharide-lipids in synthesis of glycoproteins. In: The Biochemistry of Glycoproteins and Proteoglycans, pp. 35–83, Lennarz W. J. (ed.), Plenum, New York

101 Kornfeld R. and Kornfeld S. (1985) Assembly of asparagine-linked oligosaccharides. Annu. Rev. Biochem. **54:** 631–664

102 Rush J. S., Panneerselvam K., Waechter C. J. and Freeze H. H. (2000) Mannose supplementation corrects GDP-mannose deficiency in cultured fibroblasts from some patients with congenital disorders of glycosylation (CDG). Glycobiology **10:** 829–835

103 Panneerselvam K., Etchison J. R., Skovby F. and Freeze H. H. (1997) Abnormal metabolism of mannose in families with carbohydrate-deficient glycoprotein syndrome type 1. Biochem. Mol. Med. **61:** 161–167

104 Körner C., Lehle L. and von Figura K. (1998) Carbohydrate-deficient glycoprotein syndrome type 1: correction of the glycosylation defect by deprivation of glucose or supplementation of mannose. Glycoconj. J. **15:** 499–505

105 Panneerselvam K. and Freeze H. H. (1996) Mannose enters mammalian cells using a specific transporter that is insensitive to glucose. J. Biol. Chem. **271:** 9417–9421

106 Panneerselvam K., Etchison J. R. and Freeze H. H. (1997) Human fibroblasts prefer mannose over glucose as a source of mannose for N-glycosylation – evidence for the functional importance of transported mannose. J. Biol. Chem. **272:** 23123–23129

107 Panneerselvam K., Etchison J. R. and Freeze H. H. (1997) Human fibroblasts prefer mannose over glucose as a source of mannose for N-glycosylation: evidence for the functional importance of transported mannose (Vol 272, pg 23123, 1997). J. Biol. Chem. **272:** 33444

108 Etchison J. R. and Freeze H. H. (1997) Enzymatic assay of D-mannose in serum. Clin. Chem. **43:** 533–538

109 Alton G., Kjaergaard S., Etchison J. R., Skovby F. and Freeze H. H. (1997) Oral ingestion of mannose elevates blood mannose levels: a first step toward a potential therapy for carbohydrate-deficient glycoprotein syndrome type I. Biochem. Mol. Med. **60:** 127–133

110 Jaeken J. and Casaer P. (1997) Carbohydrate-deficient glycoconjugate (CDG) syndromes: a new chapter of neuropaedriatrics. Eur. J. Paediatr. Neurol. **2/3:** 61–66

111 Kjaergaard S., Kristiansson B., Stibler H., Freeze H. H., Schwartz M., Martinsson T. et al. (1998) Failure of short-term mannose therapy of patients with carbohydrate-deficient glycoprotein syndrome type 1A. Acta Paediatr. **87:** 884–888

112 Mayatepek E. and Kohlmuller D. (1998) Mannose supplementation in carbohydrate-deficient glycoprotein syndrome type I and phosphomannomutase deficiency. Eur. J. Pediatr. **157:** 605–606

113 Mayatepek E., Schroder M., Kohlmuller D., Bieger W. P. and Nutzenadel W. (1997) Continuous mannose infusion in carbohydrate-deficient glycoprotein syndrome type I. Acta Paediatr. **86:** 1138–1140

114 Martinsson T., Bjursell C., Stibler H., Kristiansson B., Skovby F., Jaeken J. et al. (1994) Linkage of a locus for carbohydrate-deficient glycoprotein syndrome type I (CDG1) to chromosome 16p, and linkage disequilibrium to microsatellite marker D16S406. Hum. Mol. Genet. **3:** 2037–2042

115 Matthijs G., Legius E., Schollen E., Vandenberk P., Jaeken J., Barone R. et al. (1996) Evidence for genetic heterogeneity in the carbohydrate-deficient glycoprotein syndrome type I (CDG1). Genomics **35:** 597–599

116 Bjursell C., Stibler H., Wahlstrom J., Kristiansson B., Skovby F., Stromme P. et al. (1997) Fine mapping of the gene for carbohydrate-deficient glycoprotein syndrome, type I (CDG1): linkage disequilibrium and founder effect in Scandinavian families. Genomics **39:** 247–253

117 Matthijs G., Schollen E., Pirard M., Budarf M. L., van Schaftingen E. and Cassiman J. J. (1997) PMM (PMM1), the human homologue of SEC53 or yeast phosphomannomutase, is localized on chromosome 22q13. Genomics **40:** 41–47

118 Wada Y. and Sakamoto M. (1997) Isolation of the human phosphomannomutase gene (PMM1) and assignment to chromosome 22q13. Genomics **39:** 416–417

119 Hansen S. H., Frank S. R. and Casanova J. E. (1997) Cloning and characterization of human phosphomannomutase, a mammalian homologue of yeast SEC53. Glycobiology **7:** 829–834

120 Matthijs G., Schollen E., Pardon E., Veiga-Da-Cunha M., Jaeken J., Cassiman J. J. et al. (1997) Mutations in PMM2, a phosphomannomutase gene on chromosome 16p13, in carbohydrate-deficient glycoprotein type I syndrome (Jaeken syndrome). Nat. Genet. **16:** 88–92

121 Matthijs G., Schollen E., Pardon E., Veiga-Da-Cunha M., Jaeken J., Cassiman J. J. et al. (1997) Mutations in PMM2, a phosphomannomutase gene on chromosome 16p13, in carbohydrate-deficient glycoprotein type I syndrome (Jaeken syndrome) (Vol 16, pg 88, 1997). Nat. Genet. **16:** 316

122 Matthijs G., Schollen E., Van Schaftingen E., Cassiman J. J. and Jaeken J. (1998) Lack of homozygotes for the most frequent disease allele in carbohydrate-deficient glycoprotein syndrome type 1A. Am. J. Hum. Genet. **62:** 542–550

123 Schollen E., Pardon E., Heykants L., Renard J., Doggett N. A., Callen D. F. et al. (1998) Comparative analysis of the phosphomannomutase genes PMM1, PMM2 and PMM2 psi: the sequence variation in the processed pseudogene is a reflection of the mutations found in the functional gene. Hum. Mol. Genet. **7:** 157–164

124 Kjaergaard S., Skovby F. and Schwartz M. (1998) Absence of homozygosity for predominant mutations in PMM2 in Danish patients with carbohydrate-deficient glycoprotein syndrome type 1. Eur. J. Hum. Genet. **6:** 331–336

125 Bjursell C., Wahlstrom J., Berg K., Stibler H., Kristiansson B., Matthijs G. et. al. (1998) Detailed mapping of the phosphomannomutase 2 (PMM2) gene and mutation detection enable improved analysis for Scandinavian CDG type I families. Eur. J. Hum. Genet. **6:** 603–611

126 Matthijs G., Schollen E., Cassiman J. J., CormierDaire V., Jaeken J. and van Schaftingen E. (1998) Prenatal diagnosis in CDG1 families: beware of heterogeneity. Eur. J. Hum. Genet. **6:** 99–104

127 Charlwood J., Clayton P., Keir G., Mian N., Young E. and Winchester B. (1998) 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. **18:** 693–699

128 Matthijs G., Schollen E., Bjursell C., Erlandson A., Freeze H., Imtiaz F. et al. (2000) Mutations in PMM2 that cause congenital disorders of glycosylation, type Ia (CDG-Ia). Hum. Mutat. **16:** 386–94

129 Schollen E., Kjaergaard S., Legius E., Schwartz M. and Matthijs G. (2000) Lack of Hardy-Weinberg equilibrium for the most prevalent PMM2 mutation in CDG-Ia (congenital disorders of glycosylation type Ia). Eur. J. Hum. Genet. **8:** 367–71

130 Pirard M., Matthijs G., Heykants L., Schollen E., Grünewald S., Jaeken J. et al. (1999) Effect of mutations found in carbohydrate-deficient glycoprotein syndrome type IA on the activity of phosphomannomutase 2. FEBS Lett. **452:** 319–322

131 Pirard M., Achouri Y., Collet J. F., Schollen E., Matthijs G. and Van Schaftingen E. (1999) Kinetic properties and tissular distribution of mammalian phosphomannomutase isozymes. Biochem. J. **339:** 201–207

132 Freeze H. H. and Aebi M. (1999) Molecular basis of carbohydrate-deficient glycoprotein syndromes type I with normal phosphomannomutase activity. Biochim. Biophys. Acta. **1455:** 167–178

133 Niehues R., Hasilik M., Alton G., Körner C., Schiebe-Sukumar M., Koch H. G. et al. (1998) Carbohydrate-deficient glycoprotein syndrome type Ib – phosphomannose isomerase deficiency and mannose therapy. J. Clin. Invest. **101:** 1414–1420

134 Jaeken J., Matthijs G., Saudubray J. M., Dionisi-Vici C., Bertini E., de Lonlay P. et al. (1998) Phosphomannose isomerase deficiency: a carbohydrate-deficient glycoprotein syndrome with hepatic-intestinal presentation. Am. J. Hum. Genet. **62:** 1535–1539

135 de Koning T. J., Dorland L., van Diggelen O. P, Boonman A. M. C., de Jong G. J., van Noort W. L. et al. (1998) A novel disorder of N-glycosylation due to phosphomannose isomerase deficiency. Biochem. Biophys. Res. Commun. **245:** 38–42

136 Schollen E., Dorland L., de Koning T. J., van Diggelen O. P., Huijmans J. G., Marquardt T. et al. (2000) Genomic organization of the human phosphomannose isomerase (MPI) gene and mutation analysis in patients with congenital disorders of glycosylation type Ib (CDG-Ib). Hum. Mutat. **16:** 247–252

137 de Koning T. J., Nikkels P. G., Dorland L., Bekhof J., De Schrijver J. E., van Hattum J. et al. (2000) Congenital hepatic fibrosis in 3 siblings with phosphomannose isomerase deficiency. Virch. Arch. **437:** 101–105

138 Freeze H. H. (1999) New diagnosis and treatment of congenital hepatic fibrosis. J. Pediatr. Gastroenterol. Nutr. **29:** 104–106

139 de Lonlay P., Cuer M., Vuillaumier-Barrot S., Beaune G., Castelnau P., Kretz M. et al. (1999) Hyperinsulinemic hypoglycemia as a presenting sign in phosphomannose isomerase deficiency: a new manifestation of carbohydrate-deficient glycoprotein syndrome treatable with mannose. J. Pediatr. **135:** 379–383

140 Babovic-Vuksanovic D., Patterson M. C., Schwenk W. F., O'Brien J. F., Vockley J., Freeze H. H. et al. (1999) Severe hypoglycemia as a presenting symptom of carbohydrate-deficient glycoprotein syndrome. J. Pediatr. **135:** 775–781

141 Cole R. N. and Hart G. W. (1997) Glycosyl-phosphatidylinositol anchors: structure, biosynthesis and function. In: New Comprehensive Biochemistry, vol. 29B, Glycoproteins II, pp. 69–88, Montreuil J., Vliegenthart J. F. G. and Schachter H. (eds), Elsevier, Amsterdam

142 Chiba A., Matsumura K., Yamada H., Inazu T., Shimizu T., Kusunoki S. et al. (1997) Structures of sialylated O-linked oligosaccharides of bovine peripheral nerve alpha-dystroglycan: the role of a novel O-mannosyl-type oligosaccharide in the binding of alpha-dystroglycan with laminin. J. Biol. Chem. **272:** 2156–2162

143 Finne J. (1997) Carbohydrate units of nervous tissue glycoproteins. In: New Comprehensive Biochemistry vol. 29B, Glycoproteins II, pp. 55–67, Montreuil J., Vliegenthart J. F. G. and Schachter H. (eds), Elsevier, Amsterdam

144 Doucey M. A., Hess D., Cacan R. and Hofsteenge J. (1998) Protein C-mannosylation is enzyme-catalyzed and uses dolichyl-phosphate-mannose as a precursor. Mol. Biol. Cell **9:** 291–300

145 Körner C., Knauer R., Holzbach U., Hanefeld F., Lehle L. and von Figura K. (1998) Carbohydrate-deficient glycoprotein syndrome type V: deficiency of dolichyl-P-Glc: Man(9) GlcNAc(2)-PP-dolichyl glucosyltransferase. Proc. Natl. Acad. Sci. USA. **95:** 13200–13205

146 Hanefeld F., Korner C., Holzbach-Eberle U. and von Figura K. (2000) Congenital disorder of glycosylation-Ic: case report and genetic defect. Neuropediatrics **31:** 60–62

147 Imbach T., Burda P., Kuhnert P., Wevers R. A., Aebi M., Berger E. G. et al. (1999) A mutation in the human ortholog of the *Saccharomyces cerevisiae* ALG6 gene causes carbohydrate-deficient glycoprotein syndrome type-Ic. Proc. Natl. Acad. Sci. USA. **96:** 6982–6987

148 Reiss G., Heesen S. T., Zimmerman J., Robbins P. W. and Aebi M. (1996) Isolation of the ALG6 locus of *Saccharomyces cerevisiae* required for glucosylation in the N-linked glycosylation pathway. Glycobiology **6:** 493–498

149 Imbach T., Grunewald S., Schenk B., Burda P., Schollen E., Wevers R. A. et al. (2000) Multi-allelic origin of congenital disorder of glycosylation (CDG)-Ic. Hum. Genet. **106:** 538–545

150 Grünewald S., Imbach T., Huijben K., Rubio-Gozalbo M. E., Verrips A., de Klerk J. B. et al. (2000) Clinical and biochemical characteristics of congenital disorder of glycosylation type Ic, the first recognized endoplasmic reticulum defect in N-glycan synthesis. Ann. Neurol. **47:** 776–781
151 Stibler H., Stephani U. and Kutsch U. (1995) Carbohydrate-deficient glycoprotein syndrome – a fourth subtype. Neuropediatrics **26:** 235–237
152 Körner C., Knauer R., Stephani U., Marquardt T., Lehle L. and von Figura K. (1999) Carbohydrate deficient glycoprotein syndrome type IV: deficiency of dolichyl-P-Man: Man(5)GlcNAc(2)-PP-dolichyl mannosyltransferase. EMBO J. **18:** 6816–6822
153 Kim S., Westphal V., Srikrishna G., Mehta D. P., Peterson S., Filiano J. et al. (2000) Dolichol phosphate mannose synthase (DPM1) mutations define congenital disorder of glycosylation Ie (CDG-Ie). J. Clin. Invest. **105:** 191–198
154 Tomita S., Inoue N., Maeda Y., Ohishi K., Takeda J. and Kinoshita T. (1998) A homologue of *Saccharomyces cerevisiae* Dpm1p is not sufficient for synthesis of dolichol-phosphate-mannose in mammalian cells. J. Biol.Chem. **273:** 9249–9254
155 Colussi P. A., Taron C. H., Mack J. C. and Orlean P. (1997) Human and *Saccharomyces cerevisiae* dolichol phosphate mannose synthases represent two classes of the enzyme, but both function in *Schizosaccharomyces pombe*. Proc. Natl. Acad. Sci. USA **94:** 7873–7878
156 Imbach T., Schenk B., Schollen E., Burda P., Stutz A., Grünewald S. et al. (2000) Deficiency of dolichol-phosphate-mannose synthase-1 causes congenital disorder of glycosylation type-Ie. J. Clin. Invest. **105:** 233–239
157 Anand M., Ray S. and Lehrman M. A. (2000) The Lec35 gene is essential for glucose-P-dolichol utilization in Chinese hamster ovary cells. Glycobiology **10:** 1099
158 Lehrman M. A. (2000) The general role of the Lec35 gene in monosaccharide-P-dolichol dependent glycosyltransferase reactions in hamster cells. Glycobiology **10:** 1079
159 Camp L. A., Chauhan P., Farrar J. D. and Lehrman M. A. (1993) Defective mannosylation of glycosylphosphatidylinositol in Lec35 Chinese hamster ovary cells. J. Biol. Chem. **268:** 6721–6728
160 Ware F. E. and Lehrman M. A. (1996) Expression cloning of a novel suppressor of the Lec15 and Lec35 glycosylation mutations of Chinese hamster ovary cells. J. Biol. Chem. **271:** 13935–13938
161 Mao M., Fu G., Wu J. S., Zhang Q. H., Zhou J., Kan L. X. et al. (1998) Identification of genes expressed in human CD34(+) hematopoietic stem/progenitor cells by expressed sequence tags and efficient full-length cDNA cloning. Proc. Natl. Acad. Sci. USA **95:** 8175–8180
162 Schenk B., Aebi M., Imbach T., Berger E. G., Hennet T., Matthijs G. et al. (2000) N-linked protein glycosylation in the ER of *Saccharomyces cerevisiae*: a tool to define the molecular cause of different types of CDG. In: Congenital Disorders of Glycosylation, 2000, p. 7, Freeze H. (ed.), The Burnham Institute, La Jolla Calif.
163 Ramaekers V. T., Stibler H., Kint J. and Jaeken J. (1991) A new variant of the carbohydrate deficient glycoproteins syndrome. J. Inher. Metab. Dis. **14:** 385–388
164 Jaeken J., Decock P., Stibler H., Vangeet C., Kint J., Ramaekers V. et al. (1993) Carbohydrate-deficient glycoprotein syndrome type II. J Inher. Metab. Dis. **16:** 1041
165 Jaeken J., Schachter H., Carchon H., De Cock P., Coddeville B. and Spik G. (1994) Carbohydrate deficient glycoprotein syndrome type II: a deficiency in Golgi localised N-acetylglucosaminyltransferase II. Arch. Dis. Child **71:** 123–127
166 Charuk J. H. M., Tan J., Bernardini M., Haddad S., Reithmeier R. A. F., Jaeken J. et al. (1995) Carbohydrate-deficient glycoprotein syndrome type II – an autosomal recessive N-acetylglucosaminyltransferase II deficiency different from typical hereditary erythroblastic multinuclearity, with a positive acidified-serum lysis test (HEMPAS). Eur. J. Biochem. **230:** 797–805
167 Tan J., Dunn J., Jaeken J. and Schachter H. (1996) Mutations in the MGAT2 gene controlling complex N-glycan synthesis cause carbohydrate-deficient glycoprotein syndrome type II, an autosomal recessive disease with defective brain development. Am. J. Hum. Genet. **59:** 810–817
168 Schachter H. (2000) Diseases with deficiencies in asparagine-linked glycosylation. A. Carbohydrate-deficient glycoprotein syndromes. In: Frontiers in Molecular Biology, vol. 30, Molecular and Cellular Glycobiology, pp. 133–157, Fukuda M. and Hindsgaul O. (eds), Oxford University Press, Oxford
169 Schachter H. and Jaeken J. (1999) Carbohydrate-deficient glycoprotein syndrome type II. Biochim. Biophys. Acta **1455:** 179–192
170 Tulsiani D. R., Opheim D. J. and Touster O. (1977) Purification and characterization of alpha-D-mannosidase from rat liver Golgi membranes. J. Biol. Chem. **252:** 3227–3233
171 Harpaz N. and Schachter H. (1980) Control of glycoprotein synthesis. V. Processing of asparagine-linked oligosaccharides by one or more rat liver Golgi α-D-mannosidases dependent on the prior action of UDP-N-acetylglucosamine: α-D-mannoside β-2-N-acetylglucosaminyltransferase I. J. Biol. Chem. **255:** 4894–4902
172 Schachter H. (1995) Glycosyltransferases involved in the synthesis of N-glycan antennae. In: New Comprehensive Biochemistry, vol. 29 a, Glycoproteins, pp. 153–199, Montreuil J., Vliegenthart J. F. G. and Schachter H. (eds), Elsevier, Amsterdam
173 Schachter H. (1991) The 'yellow brick road' to branched complex N-glycans. Glycobiology **1:** 453–461
174 D'Agostaro G. A. F., Zingoni A., Moritz R. L., Simpson R. J., Schachter H. and Bendiak B. (1995) Molecular cloning and expression of cDNA encoding the rat UDP-N-acetylglucosamine: alpha-6-D-mannoside beta-1,2-N-acetylglucosaminyltransferase II. J. Biol. Chem. **270:** 15211–15221
175 Tan J., D'Agostaro G. A. F., Bendiak B., Reck F., Sarkar M., Squire J. A. et al. (1995) The human UDP-N-acetylglucosamine: alpha-6-D-mannoside-beta-1,2-N-acetylglucosaminyltransferase II gene (MGAT2)–cloning of genomic DNA, localization to chromosome 14q21, expression in insect cells and purification of the recombinant protein. Eur. J. Biochem. **231:** 317–328
176 Schachter H. (1994) Molecular cloning of glycosyltransferase genes. In: Frontiers in molecular biology, vol. 4, Molecular Glycobiology, pp. 88–162, Fukuda M. and Hindsgaul O. (eds), Oxford University Press, Oxford
177 Cormier-Daire V., Amiel J., Vuillaumier-Barrot S., Tan J., Durand G., Munnich A. et al. (2000) Congenital disorders of glycosylation IIa cause growth retardation, mental retardation, and facial dysmorphism. J. Med. Genet. **37:** 875–877
178 De Praeter C. M., Gerwig G. J., Bause E., Nuytinck L. K., Vliegenthart J. F., Breuer W. et al. (2000) A novel disorder caused by defective biosynthesis of N-linked oligosaccharides due to glucosidase I deficiency. Am. J. Hum. Genet. **66:** 1744–1756
179 Moore S. and Spiro R. G. (1990) Demonstration that Golgi endo-alpha-D-mannosidase provides a glucosidase-independent pathway for the formation of complex N-linked oligosaccharides of glycoproteins. J. Biol. Chem. **265:** 13104– 13112
180 Spiro M. J. and Spiro R. G. (2000) Use of recombinant endomannosidase for evaluation of the processing of N-linked oligosaccharides of glycoproteins and their oligosaccharide-lipid precursors. Glycobiology **10:** 521–529
181 Crookston J. H., Godwin T. F., Wightman K. J. R., Dacie J. V., Davis J. A., Lewis S. M. et al. (1966) Congenital dyserythropoietic anaemia. Int. Soc. Haematol. **11:** 18
182 Crookston J. H., Crookston M. C., Burnie K. L., Francombe W. H., Dacie J. V., Davis J. A. et al. (1969) Hereditary erythro-

blastic multinuclearity associated with a positive acidified-serum test: a typical congenital dyserythropoietic anaemia. Br. J. Haematol. **17:** 11–26
183 Heimpel H. and Wendt F. (1968) Congenital dyserythropoietic anemia with karyorrhexis and multinuclearity of erythroblasts. Hel. Med. Acta. **34:** 103–115
184 Nathan D. G. and Oski F. A. (1987) Hematology of Infancy and Childhood, Saunders Philadelphia
185 Iolascon A., D'Agostaro G., Perrotta S., Izzo P., Tavano R. and delGiudice E. M. (1996) Congenital dyserythropoietic anemia type II: molecular basis and clinical aspects. Haematologica **81:** 543–559
186 Fukuda M. N. (1999) HEMPAS: hereditary erythroblastic multinuclearity with positive acidified serum lysis test. Biochim. Biophys. Acta. **1455:** 231–239
187 Crookston J. H., Crookston M. C. and Rosse W. F. (1972) Red-cell abnormalities in HEMPAS (hereditary erythroblastic multinuclearity with a positive acidified-serum test). Br. J. Haematol. **23 (suppl.):** 83–91
188 Verwilghen R. L., Lewis S. M., Dacie J. V., Crookston J. H. and Crookston M. C. (1973) HEMPAS: congenital dyserythropoietic anaemia (type II). Q. J. Med. N. S. **42:** 257–278
189 Fukuda M. N., Gaetani G. F., Izzo P., Scartezzini P. and Dell A. (1992) Incompletely processed N-glycans of serum glycoproteins in congenital dyserythropoietic anaemia type II (HEMPAS). Br. J. Haematol. **82:** 745–752
190 Mawby W. J., Tanner M. J. A., Anstee D. J. and Clamp J. R. (1983) Incomplete glycosylation of erythrocyte membrane proteins in congenital dyserythropoietic anaemia type II (CDA II). Br. J. Haematol. **55:** 375–368
191 Zdebska E., Anselsetter V., Pacuszka T., Krauze R., Chelstowska A., Heimpel H. et al. (1987) Glycolipids and glycopeptides of red cell membranes in congenital dyserythropoietic anemia type II (CDAII). Br. J. Haematol. **66:** 385–391
192 Scartezzini P., Forni G. L., Baldi M., Izzo C. and Sansone G. (1982) Decreased glycosylation of band 3 and band 4.5 glycoproteins of erythrocyte membrane in congenital dyserythropoietic anaemia type II. Br. J. Haematol. **51:** 569–576
193 Baines A. J., Banga J. P. S., Gratzer W. B., Linch D. C. and Huehns E. R. (1982) Red cell membrane anomalies in congenital dyserythropoietic anaemia, type II (HEMPAS). Br. J. Haematol. **50:** 563–574
194 Fukuda M. N., Papayannopoulou T., Gordon-Smith E. C., Rochant H. and Testa U. (1984) Defect in glycosylation of erythrocyte membrane proteins in congenital dyserythropoietic anaemia type II (HEMPAS). Br. J. Haematol. **56:** 55–68
195 Fukuda M. N., Klier G., Yu J. and Scartezzini P. (1986) Anomalous clustering of underglycosylated band 3 in erythrocytes and their precursor cells in congenital dyserythropoietic anemia type II. Blood **68:** 521–529
196 Fukuda M. N., Bothner B., Scartezzini P. and Dell A. (1986) Isolation and characterization of poly-N-acetyllactosaminylceramides accumulated in the erythrocytes of congenital dyserythropoietic anemia type II patients. Chem. Phys. Lipids. **42:** 185–197
197 Fukuda M. N., Dell A. and Scartezzini P. (1987) Primary defect of congenital dyserythropoietic anemia type II: failure in glycosylation of erythrocyte lactosaminoglycan-proteins caused by lowered N-acetylglucosaminyltransferase II. J. Biol. Chem. **262:** 7195–7206
198 Fukuda M. N., Masri K. A., Dell A., Luzzatto L. and Moremen K. W. (1990) Incomplete synthesis of N-glycans in congenital dyserythropoietic anemia type II caused by a defect in the gene encoding alpha-mannosidase II. Proc. Natl. Acad. Sci. USA **87:** 7443–7447
199 Misago M., Liao Y. F., Kudo S., Eto S., Mattei M. G., Moremen K. W. et al. (1995) Molecular cloning and expression of cDNAs encoding human alpha-mannosidase II and a previously unrecognized alpha-mannosidase IIx isozyme. Proc. Natl. Acad. Sci. USA **92:** 11766–11770
200 Fukuda M., Dell A., Oates J. E. and Fukuda M. N. (1984) Structure of branched glycosaminoglycan, the carbohydrate moiety of band 3 isolated from adult human erythrocytes. J. Biol. Chem. **259:** 8260–8273
201 Fukuda M. N. (1990) HEMPAS disease: genetic defect of glycosylation. Glycobiology **1:** 9–15
202 Fukuda M. N. (1993) Congenital dyserythropoietic anaemia type II (HEMPAS) and its molecular basis. Baillieres Clin. Haematol. **6:** 493–511
203 Fukuda M. N. (2000) Diseases with deficiencies in asparagine-linked glycosylation. B. Congenital dyserythropoietic anemia type II (HEMPAS). In: Frontiers in Molecular Biology, vol. 30, Molecular and Cellular Glycobiology, pp. 158–168, Fukuda M. and Hindsgaul O. (eds), Oxford University Press, Oxford
204 van den Eijnden D. H., Koenderman A. H. L. and Schiphorst W. E. C. M. (1988) Biosynthesis of blood group i-active polylactosaminoglycans: partial purification and properties of an UDP-GlcNAc: N-acetyllactosaminide β1-→3-N-acetylglucosaminyltransferase from Novikoff tumor cell ascites fluid. J. Biol. Chem. **263:** 12461–12471
205 Gasparini P., del Giudice E. M., Delaunay J., Totaro A., Granatiero M., Melchionda S. et al. (1997) Localization of the congenital dyserythropoietic anemia II locus to chromosome 20q11.2 by genomewide search. Am. J. Hum. Genet. **61:** 1112–1116
206 Iolascon A., delGiudice E. M., Perrotta S., Granatiero M., Zelante L. and Gasparini P. (1997) Exclusion of three candidate genes as determinants of congenital dyserythropoietic anemia type II (CDA-II). Blood **90:** 4197–4200
207 Iolascon A., De Mattia D., Perrotta S., Carella M., Gasparini P. and Deliliers G. L. (1998) Genetic heterogeneity of congenital dyserythropoietic anemia type II. Blood **92:** 2593–2594
208 Boogaerts M. A. and Verwilghen R. L. (1982) Variants of congenital dyserythropoietic anemia: an update. Haematologia **15:** 211–219
209 Vermylen C., Scheiff J. M., Rodhain J., Ninane J. and Cornu G. (1986) A variant of the congenital dyserythropoietic anemia type II with structural abnormalities in the granulocyte series. Eur. J. Pediatr. **145:** 232–235
210 Fukuda M. N., Masri K. A., Dell A., Thonar E. J.-M., Klier G. and Lowenthal R. M. (1989) Defective glycosylation of erythrocyte membrane glycoconjugates in a variant of congenital dyserythropoietic anemia type II: association of low level of membrane-bound form of galactosyltransferase. Blood **73:** 1331–1339
211 Kornfeld S., Gregory W. and Chapman A. (1979) Class E Thy-1 negative mouse lymphoma cells utilize an alternate pathway of oligosaccharide processing to synthesize complex-type oligosaccharides. J. Biol. Chem. **254:** 11649–11654
212 Rearick J., Chapman A. and Kornfeld S. (1981) Glucose starvation alters lipid-linked oligosaccharide biosynthesis on Chinese hamster ovary cells. J. Biol. Chem. **256:** 6255–6261
213 Moremen K. W., Trimble R. B. and Herscovics A. (1994) Glycosidases of the asparagine-linked oligosaccharide processing pathway. Glycobiology **4:** 113–125
214 Tulsiani D. R. and Touster O. (1985) Characterization of a novel α-D-mannosidase from rat brain microsomes. J. Biol. Chem. **260:** 13081–13087
215 Bonay P., Roth J. and Hughes R. C. (1992) Subcellular distribution in rat liver of a novel broad-specificity (alpha1→2, alpha1→3 and alpha1→6) mannosidase active on oligomannose glycans. Eur. J. Biochem. **205:** 399–407
216 Bonay P. and Hughes R. C. (1991) Purification and characterization of a novel broad-specificity (alpha1→2, alpha1→3 and alpha1→6) mannosidase from rat liver. Eur. J. Biochem. **197:** 229–238

217 Becker D. J. and Lowe J. B. (1999) Leukocyte adhesion deficiency type II. Biochim. Biophys. Acta. **1455:** 193–204
218 Etzioni A., Frydman M., Pollack S., Avidor I., Phillips M. L., Paulson J. C. et al. (1992) Recurrent severe infections caused by a novel leukocyte adhesion deficiency. N. Engl. J. Med. **327:** 1789–1792
219 Frydman M., Etzioni A., Eidlitz-Markus T., Avidor I., Varsano I., Shechter Y. et al. (1992) Rambam-Hasharon syndrome of psychomotor retardation, short stature, defective neutrophil motility, and Bombay phenotype. Am. J. Med. Genet. **44:** 297–302
220 Larsen R. D., Ernst L. K., Nair R. P. and Lowe J. B. (1990) Molecular cloning, sequence, and expression of a human GDP-L-fucose : beta-D-galactoside 2-alpha-L-fucosyltransferase cDNA that can form the H blood group antigen. Proc. Natl. Acad. Sci. USA **87:** 6674–6678
221 Kelly R. J., Rouquier S., Giorgi D., Lennon G. G. and Lowe J. B. (1995) Sequence and expression of a candidate for the human secretor blood group alpha(1,2)fucosyltransferase gene (FUT2) – Homozygosity for an enzyme-inactivating nonsense mutation commonly correlates with the non-secretor phenotype. J. Biol. Chem. **270:** 4640–4649
222 Kukowska-Latallo J. F., Larsen R. D., Rajan V. P. and Lowe J. B. (1990) Structure and function of a cloned human cDNA encoding a α(1,3/1,4)fucosyltransferase (α(1,3/1,4)FT) analogous to the Lewis blood group α(1,3/1,4)FT. FASEB J. **4:** A1930
223 Von Andrian U. H., Berger E. M., Ramezani L., Chambers J. D., Ochs H. D., Harlan J. M. et al. (1993) In vivo behavior of neutrophils from two patients with distinct inherited leukocyte adhesion deficiency syndromes. J. Clin. Invest. **91:** 2893–2897
224 Etzioni A., Phillips L. M., Paulson J. C. and Harlan J. M. (1995) Leukocyte adhesion deficiency (LAD) II. In: Ciba Foundation Symposia, vol. 189, Cell Adhesion and Human Disease, pp. 51–62, Marsh J. and Goode J. A., (eds.), Wiley, Chichester
225 Phillips M. L., Schwartz B. R., Etzioni A., Bayer R., Ochs H. D., Paulson J. C. et al. (1995) Neutrophil adhesion in leukocyte adhesion deficiency syndrome type 2. J. Clin. Invest. **96:** 2898–2906
226 Karsan A., Cornejo C. J., Winn R. K., Schwartz B. R., Way W., Lannir N. et al. (1998) Leukocyte adhesion deficiency type II is a generalized defect of de novo GDP-fucose biosynthesis – endothelial cell fucosylation is not required for neutrophil rolling on human nonlymphoid endothelium. J. Clin. Invest. **101:** 2438–2445
227 Sturla L., Etzioni A., Bisso A., Zanardi D., DeFlora G., Silengo L. et al. (1998) Defective intracellular activity of GDP-D-mannose-4,6-dehydratase in leukocyte adhesion deficiency type II syndrome. FEBS Lett. **429:** 274–278
228 Lübke T., Marquardt T., von Figura K. and Körner C. (1999) A new type of carbohydrate-deficient glycoprotein syndrome due to a decreased import of GDP-fucose into the golgi. J. Biol. Chem. **274:** 25986–25989
229 Körner C., Linnebank M., Koch H. G., Harms E., von Figura K. and Marquardt T. (1999) Decreased availability of GDP-L-fucose in a patient with LAD II with normal GDP-D-mannose dehydratase and FX protein activities. J. Leukoc. Biol. **66:** 95–98
230 Puglielli L. and Hirschberg C. B. (1999) Reconstitution, identification, and purification of the rat liver Golgi membrane GDP-fucose transporter. J. Biol. Chem. **274:** 35596–35600
231 Marquardt T., Brune T., Lühn K., Zimmer K. P., Körner C., Fabritz L. et al. (1999) Leukocyte adhesion deficiency II syndrome, a generalized defect in fucose metabolism. J. Pediatr. **134:** 681–688
232 Marquardt T., Luhn K., Srikrishna G., Freeze H. H., Harms E. and Vestweber D. (1999) Correction of leukocyte adhesion deficiency type II with oral fucose. Blood. **94:** 3976–3985
233 Freeze H. H. (1999) Human glycosylation disorders and sugar supplement therapy. Biochem. Biophys. Res. Commun. **255:** 189–193
234 Etzioni A. and Tonetti M. (2000) Leukocyte adhesion deficiency II – from A to almost Z. Immunol. Rev. **178:** 138–147
235 Foo Y. and Rosalki S. B. (1998) Carbohydrate deficient transferrin measurement. Ann. Clin. Biochem. **35:** 345–350
236 Iourin O., Mattu T. S., Mian N., Keir G., Winchester B., Dwek R. A. et al. (1996) The identification of abnormal glycoforms of serum transferrin in carbohydrate deficient glycoprotein syndrome type I by capillary zone electrophoresis. Glycoconj. J. **13:** 1031–1042
237 Oda R. P., Prasad R., Stout R. L., Coffin D., Patton W. P., Kraft D. L. et al. (1997) Capillary electrophoresis-based separation of transferrin sialoforms in patients with carbohydrate-deficient glycoprotein syndrome. Electrophoresis **18:** 1819–1826
238 Henry H., Tissot J. D., Messerli B., Markert M., Muntau A., Skladal D. et al. (1997) Microheterogeneity of serum glycoproteins and their liver precursors in patients with carbohydrate-deficient glycoprotein syndrome type I: apparent deficiencies in clusterin and serum amyloid P. J. Lab. Clin. Med. **129:** 412–421
239 Harrison H. H., Miller K. L., Harbison M. D. and Slonim A. E. (1992) Multiple serum protein abnormalities in carbohydrate-deficient glycoprotein syndrome: pathognomonic finding of two-dimensional electrophoresis? Clin. Chem. **38:** 1390–1392