MINIREVIEW

Inherited Disorders of Glycoprotein Synthesis: Cell Biological Insights (44121)

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Abstract. **Disorders of glycoprotein synthesis have been described only recently, and few have been studied extensively at both the clinical and biochemical level. The identification and characterization of these rare diseases are important, not only for the patients and their families, but because they offer enormous insight into biological processes. For example, the targeting of acid hydrolases to lysosomes by mannose-6-phosphate was discovered as a direct result of the elucidation of the defect in I-cell disease. The notion of carbohydrates as targeting agents continues to have ramifications today, with the success of macrophage-targeted enzyme replacement therapy for Gaucher disease. Likewise, confirmation of the *in vivo* role of fucose-containing glycans and selectins in neutrophil function came from studies using specimens from patients with leucocyte adhesion deficiency type II due to reduced availability of GDP-fucose. Identification of the *in vivo* ligands of selectins also has implications for anti-inflammatory therapies. Macular corneal dystrophy and spondyloepiphyseal dysplasia tarda offer an opportunity to investigate the number of different sulfotransferases in cells, their substrates, and their tissue expression. The Ehlers-Danlos progeroid variant offers insight into the function and regulation of the proteoglycan decorin, and suggests that several of the enzymes involved in proteoglycan synthesis may function as a multienzyme complex. The common occurrence of hypergonadotropic hypogonadism in patients with galactosemia or carbohydrate-deficient glycoprotein syndrome, due to defective N-linked glycosylation, suggests that ovarian function is particularly dependent on proper glycan synthesis. A host of other concepts await discovery as a fuller contingent of human disorders of glycan synthesis achieves recognition.** [P.S.E.B.M. 1997, Vol 215]

Carbohydrates, when covalently attached to proteins and lipids, play a myriad of critical roles in human metabolism (1). They facilitate cell adhesion and migration, and thereby help mediate the process of development. Carbohydrates aid protein function by ensuring correct protein folding, providing solubility and protease resistance, and targeting molecules both within cells and to specific cell types. Their roles in host defense include cell recognition and antigenicity. In all these pursuits, their service proceeds unnoticed until interrupted by some critical dysfunction which reveals the essential nature of a biochemical reaction.

Many more disorders of glycan catabolism have been described than disorders of synthesis. Hence, the biomedical community has become familiar with storage diseases such as the mucopolysaccharidoses (e.g., Hunter, Hurler, and Sanfilippo syndromes, due to enzymatic defects in glycosaminoglycan degradation), the glycoproteinoses (e.g., mannosidosis, sialidosis, and fucosidosis, due to impaired enzymatic breakdown of the oligosaccharides of N-linked glycoproteins), and the glycolipidoses (e.g., Gaucher, Fabry,

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and Tay-Sachs diseases, due to defects in the catabolism of glycolipids). These disorders, as well as defects of glycogen metabolism, have been reviewed extensively elsewhere and will not be discussed here (2–4).

Why have so few inherited disorders of glycan synthesis come to our attention? Given the roles of carbohydrates in development, many such disorders are undoubtedly prenatally lethal. Other disorders of glycan synthesis probably go unrecognized because it is difficult to detect the absence of important synthetic metabolites compared with, for example, the storage of undegraded carbohydrates. Nevertheless, several human defects of glycan synthesis have been described recently, and more are likely to be found as awareness of these disorders increases and analytical techniques in glycobiology improve. This identification and characterization of rare diseases not only assist affected patients and their families but also offer enormous insight into normal biological processes. Glycosylation is a complex process. A single glycan may be glycosylated differently depending on the cell type in which it is expressed, the nutritional state of the cell, and the developmental stage of the cell. Glycans also interact with many different molecules. The *in vivo* effects of these different modifications and interactions cannot be fully appreciated *in vitro.*

This review addresses the inherited disorders of humans, both known and hypothesized to result from defects in the synthesis of two classes of glycans: glycoproteins, which contain relatively small oligosaccharide moieties; and proteoglycans, which are comprised of long chains of repeating disaccharide units attached to a protein core. Current understanding of the basic defect in each disorder will be presented, along with a discussion of the questions and conclusions these disorders elicit concerning the mechanisms of glycan synthesis.

Defects in Glycoprotein Synthesis

Many proteins synthesized by mammalian cells are modified by carbohydrates in the form of either monosaccharides or, more often, oligosaccharide polymers. Oligosaccharides are constructed by the addition of monosaccharides, as high-energy nucleotide diphosphate or monophosphate sugars, to growing carbohydrate chains by the action of glycosyltransferases. Unlike proteins and DNA, oligosaccharides are not made from templates. Rather, the oligosaccharide structure is determined by the specificity of more than 100 different glycosyltransferases for their monosaccharide donors and their oligosaccharide acceptors. Also influencing the structure are the specific contingent of glycosyltransferases expressed in a given cell type, and the competition among glycosyltransferases for different acceptors.

Most glycoprotein glycosylation proceeds from common oligosaccharide cores, which are linked to proteins through GlcNAc-Asn, in the case of N-linked glycosylation, or GalNAc-Ser or -Thr, in the case of O-linked glycosylation. N-linked glycosylation takes place through a lipid intermediate (5) (Fig. 1). The precursor structure $Glc_3Man_9GlcNAc_2$-pyrophosphoryldolichol is synthesized in the rough endoplasmic reticulum (RER) and transferred *en bloc* within the RER lumen to accessible asparagines occurring in the sequence Asn-X-Ser, where X is any amino acid except proline. Once attached to a peptide, the oligosaccharide chain undergoes further processing as the protein continues through the RER and Golgi. The terminal glucose units are removed in the RER by the sequential actions of α-glycosidases I and II. If the oligosaccharide is destined to be of the "complex" or "hybrid" type, GlcNAc transferase I in the medial Golgi adds GlcNAc to the Manα1–3 arm of the N-glycan core. Otherwise, the oligosaccharide remains of the "high mannose" type. Complex oligosaccharides are further processed in the Golgi by glycosyltransferase-mediated addition of GlcNAc, galactose, fucose, and sialic acid. Between two and four such branches commonly occur on N-glycans. The details of complex N-linked glycoprotein synthesis have been reviewed elsewhere (6).

O-linked glycosylation is generally associated with mucins, such as blood group antigens, and with GlcNAc addition to several nucleoplasmic proteins (7, 8). Unlike N-linked oligosaccharides, O-linked oligosaccharides are not attached to proteins *via* a lipid intermediate. Rather, a sugar,

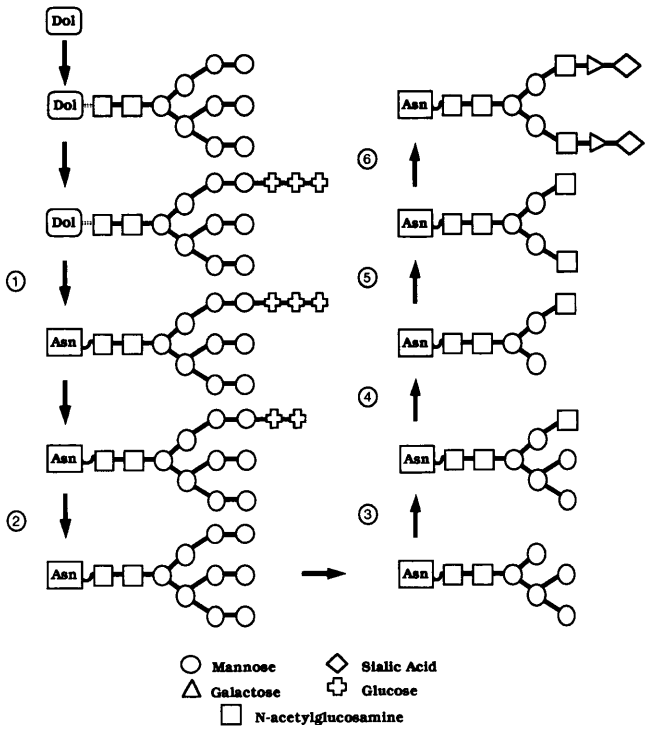

Figure 1. Synthesis, transfer, and processing of Asn-linked oligosaccharides. 1, dolichylpyrophosphoryloligosaccharide:polypeptide oligosaccharyltransferase transfers the precursor oligosaccharide *en bloc* to nascent protein; 2, α-glucosidases I and II trim the oligosaccharide, leaving a "high-mannose" type structure; 3, GlcNAc transferase I adds a GlcNAc; 4, α-mannosidase II removes mannose residues; 5, GlcNAc transferase II adds a GlcNAc; 6, glycosyltransferases sequentially produce "complex" type oligosaccharides.

usually GalNAc, is attached directly to the hydroxyl group of either Ser or Thr and extended by the stepwise action of glycosyltransferases (7). No consensus sequence has been identified for the amino acid residues surrounding the Ser/Thr sites of O-linked addition.

Numerous examples exist in which a mutation in a single amino acid disrupts a protein's glycosylation and renders it dysfunctional (9, 10). These mutations provide insight into the effect of glycosylation on the function of a particular protein. In this review, however, we will discuss human defects that disrupt the general pathway of oligosaccharide biosynthesis, thereby enhancing our understanding of the fundamental processes of glycosylation and its effects on many different molecules.

I-Cell Disease. In 1967, Leroy and DeMars (11) described two patients with clinical features reminiscent of a disorder of glycosaminoglycan catabolism. Their fibroblasts had unusual, dark cytoplasmic inclusions associated with lysosomes. This inclusion cell, or "I-cell," disease, is a particularly severe form of lysosomal storage disease. Typical patients present with congenital dislocation of the hip, coarse facial features, inguinal hernias, and gingival hyperplasia, and eventually develop corneal opacities, hepatosplenomegaly, and short-trunk dwarfism (12). Mental retardation is usually severe, and patients generally die before their seventh year. This rare disorder is inherited in an autosomal recessive manner.

Fibroblasts from I-cell disease patients are deficient in most lysosomal enzymes, while the same enzyme activities are elevated in sera (11). This biochemical finding suggests that I-cell disease patients do not properly target hydrolases ordinarily destined for lysosomes (13). The characterization of the basic defect in this disease led directly to the finding that lysosomal enzymes normally contain a mannose-6-phosphate recognition marker which binds to specific receptors (14). These receptors, located in the Golgi, effect transport to a prelysosomal compartment, while those residing in the plasma membrane participate in receptor-mediated endocytosis.

Most lysosomal enzymes are now recognized to be N-linked glycoproteins whose oligosaccharides contain a mannose-6-phosphate moiety. Two enzymes combine to add the mannose-6-phosphate marker to lysosomal enzymes (15) (Fig. 2). First, GlcNAc-1-P is added to the 6 position of a mannose on a high-mannose type oligosaccharide by UDP-N-acetylglucosamine:lysosomal enzyme N-acetylglucosaminyl-1-phosphotransferase. Then N-acetylglucosamine-1-phosphodiester-N-acetylglucosaminidase removes the GlcNAc residue. I-cell disease and its milder variant, pseudo-Hurler polydystrophy, result from defects in the phosphotransferase (16). The gene encoding the phosphotransferase has not been cloned, but genetic linkage studies have mapped it to human chromosome 4q21–q23 (17). No defects have been reported in the phosphodiesterase or in the mannose-6-phosphate receptor.

I-cell disease provides an example of a synthetic glycoprotein defect that abrogates proper enzyme targeting and, somewhat paradoxically, results in failed lysosomal degradation of macromolecules, mimicking a catabolic enzyme defect. Interestingly, at least two lysosomal hydrolases, β-glucocerebrosidase and acid phosphatase, have normal activities in patients' cells, indicating that these enzymes are targeted to lysosomes by a mechanism not requiring a mannose-6-phosphate marker (18).

The discovery that carbohydrates target molecules to certain cell receptors has important practical applications. Gaucher disease patients, with a deficiency of the lysosomal enzyme β-glucocerebrosidase, now receive enzyme replacement therapy based on just this concept (19). Commercially purified β-glucocerebrosidase is modified enzy-

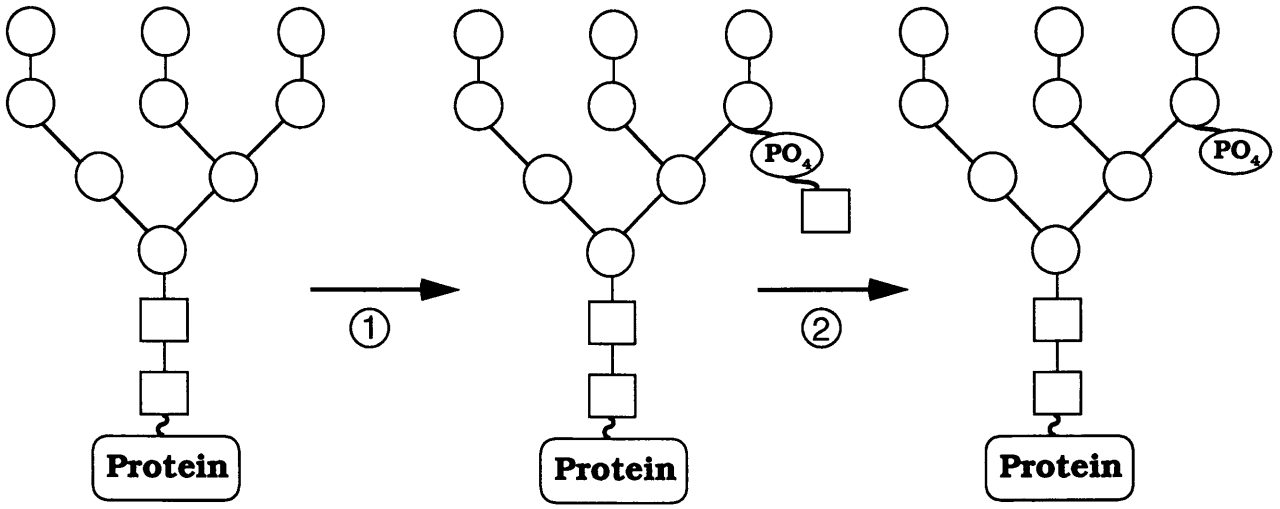

Figure 2. Synthesis of the mannose-6-phosphate marker. 1, UDP-GlcNAc:lysosomal enzyme N-acetylglucosaminyl-1-phosphotransferase adds GlcNAc-1-phosphate to the 6 position of mannose; 2, GlcNAc 1-phosphodiester-N-acetylglucosaminidase removes GlcNAc. Symbols are as in Figure 1.

matically to expose mannose residues on its N-glycans. The terminal mannose residues, in turn, target the enzyme to mannose (not mannose-6-phosphate) receptors on macrophages, where stored glucocerebroside can then be degraded. This therapy has been hugely successful and will no doubt serve as a model for future carbohydrate-targeted drugs.

Carbohydrate-deficient Glycoprotein Syndromes. The carbohydrate-deficient glycoprotein syndromes (CDGS) are a group of inherited disorders first described in 1980. The original patients were monozygotic twin girls with psychomotor retardation, increased cerebrospinal fluid protein, delayed nerve conduction velocity, thyroxin-binding globulin deficiency, and increased lysosomal arylsulfatase A activity. Later reports demonstrated that these patients exhibited a deficiency of sialic acid on their serum and CSF transferrin, which has two sites of N-linked glycosylation (20). Since the initial description of CDGS, the disorder has been divided into four groups based upon clinical presentation and biochemical findings. The basic defect has been identified in CDGS types I and II.

CDGS type I. The vast majority of CDGS patients identified have type I. Over 100 patients with this autosomal recessive disorder have been reported worldwide (21). Affected individuals have a dynamic clinical course (22). As they age, the dysmorphic features of infancy are lost, while new signs typical of adult patients emerge. All infants with CDGS have neurologic abnormalities, failure to thrive, developmental delay, and dysmorphic features, including a high nasal bridge, prominent jaw, and inverted nipples. Some affected infants also have hepatic dysfunction, a coagulopathy, and subclinical pericardial effusions. Perhaps the most striking physical feature of infants with CDGS is an abnormal distribution of subcutaneous fat over the suprapubic region and in the upper outer area of the buttocks. These unusual fat pads disappear over time. The neurologic findings include hypotonia, alternating esotropia, cerebellar hypoplasia, and brainstem atrophy. The predominant features of CDGS type I in childhood are ataxia, dysequilibrium, and severe developmental delay without regression. Progressive retinitis pigmentosa occurs in a small percentage of children. Adults suffer from variable degrees of progressive muscular atrophy and develop severe kyphoscoliosis.

CDGS is diagnosed by demonstrating the presence of abnormally glycosylated serum glycoproteins, usually transferrin, detected by isoelectric focusing (IEF). Type I patients have considerable amounts of serum asialo- and disialotransferrins, in contrast to normal serum in which these are absent or represent minor components (23). Tetrasialotransferrin, present in normal serum, is reduced in type I patients.

Metabolic labeling of CDGS fibroblasts with [^{3}H]mannose revealed lower than normal levels of intracellular total mannose, free mannose, and phosphorylated mannose (24). Mannose incorporation into both protein-linked and dolichol-linked oligosaccharides was reduced, indicating a defect in the earliest steps of N-glycan biosynthesis (24, 25). Panneerselvam and Freeze have demonstrated that normal incorporation of mannose into both protein-linked and dolichol-linked oligosaccharides can be restored to CDGS type I fibroblasts in culture by the addition of exogenous mannose (26). This finding suggests a potential strategy for therapy.

In 1995, Van Schaftingen and Jaeken reported that fibroblasts and leucocytes from patients with CDGS type I had virtually absent phosphomannomutase activity, and the parents of two patients had 50% normal activity in leucocytes (27). Phosphomannomutase is one of the enzymes responsible for converting glucose to GDP-mannose (Fig. 3). GDP-mannose donates its sugar for incorporation into a variety of oligosaccharides, including the dolichol-oligosaccharide precursor necessary for N-glycosylation. Linkage analysis has mapped the CDGS type I gene to human chromosome 16p13.3–p13.12 (28). The gene for phosphomannomutase has not been mapped or cloned.

The spectrum of biochemical and clinical abnormalities in patients with CDGS type I has been studied in great detail, perhaps more so than for any of the other disorders discussed in this review. From these studies, the roles of glycosylation in different biological processes begin to emerge. For example, CDGS patients have multiple endocrine disturbances (29), but some are age specific, such as FSH and LH, which are increased only in adolescents. Others are gender specific. Female patients with CDGS type I have hypergonadotropic hypogonadism and do not attain secondary sexual characteristics, while affected males undergo normal pubertal development.

In addition, expression of the CDGS defect seems dependent on developmental stage. Before phosphomannomutase deficiency was recognized as the basic defect in CDGS type I, prenatal diagnosis was attempted using IEF to detect abnormal glycosylation of transferrin and α_1-fetoprotein from chorionic villi, fetal blood, and amniotic fluid samples (30, 31). Prior to 36 weeks' gestation, the isoforms of these glycoproteins appeared normal in two unrelated affected pregnancies. This tells us that proper glycosylation might be achieved *in utero* by the existence of a fetal isozyme of phosphomannomutase or by an alternative source of GDP-mannose. At the same time, the clinical finding of physical stigmata at birth in CDGS indicates that abnormal glycosylation of proteins must occur in some tissues *in utero.* Once the mechanism of normal glycosylation in CDGS fetuses is ascertained, we can approach an understanding of the regulation of GDP-mannose synthesis, and prenatal treatment of this disorder may become possible. For now, the presence of phosphomannomutase activity in amniocytes should permit definitive prenatal diagnosis in affected type I CDGS fetuses.

CDGS type II. In 1991, a 3-year-old female was reported with coarse facial features, low-set ears, widely spaced nipples, and a ventricular septal defect (32). The

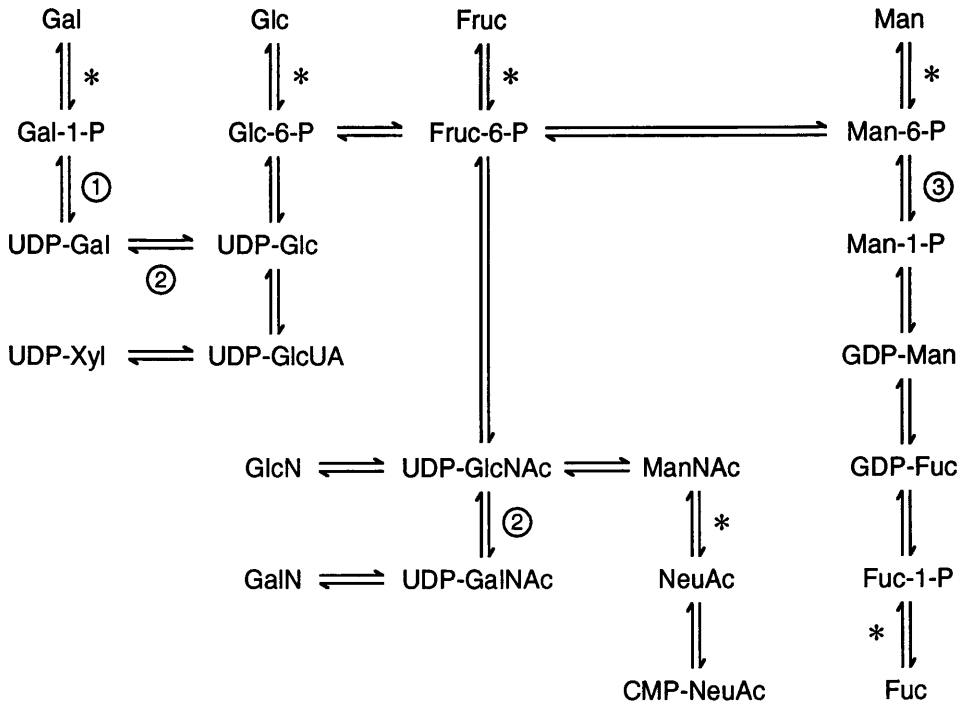

Figure 3. Synthesis of sugar nucleotides. 1, galactose-1-phosphate uridyltransferase; 2, UDP galactose-4-epimerase; 3, phosphomannomutase. Not all intermediate steps are shown. *Reactions that require ATP.

patient had severe developmental delay, generalized hypotonia, and limb weakness, with preservation of deep tendon reflexes. MRI revealed delayed myelination and global cortical atrophy. Levels of serum thyroxin-binding globulin, α_1-antitrypsin (α1AT), and haptoglobin were low, while IgM and cerebrospinal fluid protein were elevated. Unlike type I CDGS patients, this child did not have a peripheral neuropathy, and the cerebellum was normal on MRI. However, she was considered to have a form of CDGS because her serum transferrin had an abnormal IEF pattern. The pattern differed from that of type I patients in displaying a considerable elevation of disialotransferrin, dramatically reduced levels of pentasialo- and tetrasialo-transferrins, and an unidentified band between monosialo- and asialotransferrin. The autosomal recessive nature of this disorder was supported by the presence of consanguinity in the parents and of a similarly affected sibling who died in infancy. A second, unrelated patient has been described with this same disorder (33).

The defect in CDGS type II is a deficiency of N-acetylglucosamine transferase II (GnTII), the enzyme that adds GlcNAc to the Manα1–6 arm of the N-glycan core (Fig. 1). Complex type N-linked oligosaccharides cannot be produced without the action of GnTII, although further processing occurs on the single antenna resulting from the action of GnTI. Fibroblasts from the two patients with CDGS type II had GnTII activities that were 1.4% and 0.9% of normal. Their parents had normal serum transferrin IEF patterns, and mononuclear cell extracts from the parents of one patient had approximately 50% normal activity of GnTII. The gene encoding GnTII (MGAT2) has been cloned and mapped to human chromosome 14q21 (34).

A deficiency of GnTII as a cause of CDGS was unexpected, since GnTII deficiency was already associated with another very different inherited disorder (35), hereditary erythroblastic multinuclearity with positive acidified-serum test (HEMPAS) (see below). The dramatic differences between these two disorders suggests that there are complex aspects to the expression and function of GnTII that have yet to be explored.

CDGS type III. Two unrelated patients have been reported with CDGS type III (36). Both patients were hypotonic and had infantile spasms, optic atrophy, and depigmented areas of their skin. In addition, the second reported patient had hepatomegaly, a Dandy-Walker malformation of the brain, and hypoplasia of the corpus callosum. Clinically, these individuals differ from type I patients in that they do not have peripheral neuropathy, cerebellar hypoplasia, or retinitis pigmentosa. Type III patients have serum transferrin with an abnormal IEF pattern in which the tri-, di-, mono-, and asialo- forms are equally elevated. The basic defect in CDGS type III remains unknown.

CDGS type IV. Type IV is the most recent category of CDGS to be described (37). Two unrelated patients reported in 1995 presented with microcephaly, severe epilepsy, absent psychomotor development, and minor dysmorphic features, including adducted thumbs, high-arched palate, and dysplastic ears. Serum transferrin from the type IV patients exhibited elevations of the disialo form upon IEF.

One might have predicted that different defects having

the common result of abnormal N-glycosylation would be clinically similar. The multiple forms of CDGS show that this is not the case. Different abnormal carbohydrate structures produce different clinical phenotypes. A comparison of the biochemical and clinical findings in all forms of CDGS will reveal important structure/function relationships for many N-glycans.

Hereditary Erythroblastic Multinuclearity with Positive Acidified-Serum Test. Hereditary erythroblastic multinuclearity with positive acidified-serum (HEMPAS), or congenital dyserythropoietic anemia type II, is a rare autosomal recessive disorder in which patients suffer from a mild anemia, hepatomegaly, cirrhosis, and hemosiderosis of the liver (38). Patients often have jaundice, diabetes, and gallstones as well. The disorder is characterized hematologically by erythroid hyperplasia and the finding of mature erythroblasts which are bi- or multinucleated, indicating a defect in cell division. Band 3 glycoprotein, a major erythrocyte membrane protein, was found to be abnormally glycosylated in HEMPAS patients (39). Their band 3 glycoprotein lacks the polylactosaminoglycan that is normally added to one branch of its N-glycans. Instead, the patients' band 3 glycoprotein contains truncated oligosaccharides corresponding to hybrid type N-glycans and N-glycans with three mannose residues and only one normally processed branch.

This defect appears to affect glycoproteins specifically, since lipid polylactosamines are actually increased in HEMPAS erythrocyte membranes. GnTII activities in lymphocytes from two HEMPAS patients were 11% and 30% of normal (35). However, another patient has been identified with normal GnTII activity (40). This patient had virtually no activity and considerably reduced mRNA expression of the Golgi enzyme α-mannosidase II (α-MII). Like GnTII, α-MII is necessary to convert high-mannose type N-glycans to complex type N-glycans. Additional evidence that α-MII deficiency is a primary cause of HEMPAS comes from *in vitro* studies in which normal erythrocytes produced a HEMPAS phenotype when cultured in the presence of the swainsonine, an inhibitor of α-MII (41). The gene for α-MII (MANA2) has been cloned and mapped to human chromosome 5q21–q22 (42). Another α-MII isozyme which produces two different transcripts by alternate splicing has also been cloned and mapped to human chromosome 15q25. Mutation analysis has not been reported in HEMPAS patients. It remains unknown whether HEMPAS represents a genetically heterogeneous disorder, or a single defect that interferes with several processing enzymes.

The reason why the HEMPAS phenotype is limited to certain tissues has yet to be explained. Fukuda speculated that other cell types, such as granulocytes, which are rich in polylactosaminoglycans, may be unaffected because the polylactosamines are extended on tri- and tetra-antennary cores, whereas erythrocyte band 3 glycoprotein has only a bi-antennary core (41). Although the clinical defect is present only in erythrocytes, the biochemical defect is present in other tissues, such as liver. Serum transferrin, which is produced by the liver, contains no normal complex-type N-glycans in HEMPAS patients (43). Why then do HEMPAS patients not appear more like CDGS type II patients, and why do CDGS patients not have the HEMPAS phenotype? CDGS type II patients probably are spared because the polylactosamine content of erythrocyte band 3 and band 4.5 glycoproteins is reduced by only 50%, while these glycoproteins in HEMPAS patients lack polylactosamine completely (44). Different isozymes of the α-MII gene have been identified, and the possibility exists that there are fetal forms of α-MII and GnTII that do not express the defect and therefore spare HEMPAS patients from developmental abnormalities.

Other severe metabolic defects are known which have variant forms that are expressed only in red blood cells and are either clinically benign or present with mild anemia. Gal-4-epimerase deficiency (see below) and glutathione synthetase deficiency are two examples (45). In one case of glutathione synthetase deficiency, a hemolytic variant results from a temperature-sensitive mutation with considerable residual activity. Presumably, the lack of new protein synthesis in red cells allows this unstable enzyme to have a more protein production. That GnTII-deficient HEMPAS patients have higher enzyme activity than CDGS type II patients is consistent with this hypothesis. Clearly there is much to be learned about the function and forms of N-glycan processing enzymes through delineation of the defects in HEMPAS and CDGS type II.

Defects of Sugar Nucleotide Pools

As observed in the case of phosphomannomutase deficiency in CDGS type I, aberrant glycosylation can result from an insufficient pool of a specific sugar nucleotide donor. The sequence of an oligosaccharide depends on a dynamic interplay among the specificity of glycosyltransferases, the contingent of glycosyltransferases actually expressed by a given cell, and the availability of monosaccharide donors and oligosaccharide acceptors. Therefore, disturbances in sugar nucleotide pools can result in improper synthesis of N-linked and O-linked oligosaccharides, as well as other glycans.

The monosaccharide donors for the glycosylation reactions are in the form of different but somewhat interchangeable high-energy nucleotide sugars (Fig. 3), whose availability is tightly regulated by a variety of mechanisms. First, each sugar nucleotide must be brought to the site of the glycosyltransferase. Specific transporters in the Golgi membrane take up sugar nucleotides in exchange for the free nucleotides generated by the transferase reaction (e.g., GDP-mannose is exchanged for GMP [46]). Second, the concentration of each sugar nucleotide is extensively regulated. For example, UDP-GlcNAc inhibits glutamine-fructose-6-P transaminase (47), the first enzyme in the synthetic pathway leading to UDP-GlcNAc, and CMP-NeuAc inhibits UDP-GlcNAc-2-epimerase, the first enzyme in

CMP-NeuAc synthesis. Furthermore, several steps in the biosynthesis of sugar nucleotides require ATP (Fig. 3). Therefore, the metabolic state of the cell affects the concentration of the sugar nucleotide product. The tight regulation of sugar nucleotide pools, their compartmentalization, and their limiting concentrations mean that alterations in a single sugar nucleotide can significantly impair glycosylation. Examples of this phenomenon are provided by two forms of galactosemia, Gal-1-P uridyltransferase deficiency and Gal-4-epimerase deficiency.

Gal-1-P Uridyltransferase Deficiency. Galactose is a major source of nutrition for infants who consume considerable amounts of the galactose-glucose disaccharide, lactose. Galactose also serves as a component of N-linked and O-linked oligosaccharides, proteoglycans, and the glycosylated hydroxylysines of collagen. The main pathway of galactose metabolism requires the phosphorylation of galactose to form Gal-1-P, followed by the conversion of Gal-1-P and UDPGlc to Glc-1-P and UDPGal by the enzyme Gal-1-P uridyltransferase (Fig. 3). Glc-1-P then enters the Emden-Meyerhoff glycolytic pathway and UDPGal can be utilized by the different galactosyltransferases. UDPGal is reversibly converted to UDPGlc by UDPGal-4-epimerase, which also catalyzes the epimerization of UDPGalNAc and UDPGlcNAc.

A deficiency of Gal-1-P uridyltransferase activity causes "classical," autosomal recessive galactosemia with an incidence of 1 in 62,000 live births (48). The gene responsible for this disorder has been cloned and localized to human chromosome 9p13 (49). Shortly after receiving milk, affected infants present with vomiting, jaundice, lethargy, and hepatomegaly. They are particularly prone to *Escherichia coli* sepsis in the neonatal period. Galactosuria is present and red cell Gal-1-P levels are greatly elevated. Removing virtually all galactose from the diet results in resolution of acute symptoms. Red cell Gal-1-P levels fall after several weeks of strict dietary therapy, but usually remain above the normal range. Since there is no evidence that older patients acquire an ability to metabolize galactose, a galactose-free diet should be maintained throughout life. Despite presymptomatic identification of patients and galactose restriction, long-term outcome remains poor. Patients are often developmentally delayed, with characteristic speech abnormalities and, occasionally, ataxia. Ovarian failure occurs in females and appears to correlate inversely with residual transferase activity but not directly with red cell Gal-1-P levels (50). This hypergonadotropic hypogonadism resembles that seen in CDGS type I.

Identifying the causes of these complications is of great importance for treatment. Some or all may result from abnormalities of galactosylation. Although patients cannot make UDPGal from Gal-1-P and UDPGlc, the epimerase enzyme should be able to provide UDPGal directly from UDPGlc. However, galactosylation may still be disturbed if the epimerase reaction cannot provide enough UDPGal, especially if some tissues have higher UDPGal requirements than others. In addition, some reactions which provide UDPGal may be inhibited by accumulated metabolites such as Gal-1-P.

Attempts to demonstrate low pools of UDPgal in different tissues of patients with Gal-1-P uridyltransferase-deficiency have been controversial because measurements of UDPGal and UDPGlc vary considerably according to the method employed. Overall results indicate that, on average, red cell UDPGal levels are lower in transferase-deficient patients than controls, but patient values overlap considerably with the normal range (51). The UDPGlc-to-UDPGal ratio is consistently elevated, indicating some disturbance in the steady-state relationship of these sugar nucleotides.

Several indirect lines of evidence suggest that glycoconjugates are indeed galactosylated abnormally. Compositional analysis of the total carbohydrate content of fibroblasts from six transferase-deficient patients revealed only slightly reduced levels of galactose but a significantly reduced ratio of galactose to mannose (52). In the presence of exogenous galactosyltransferase, fibroblast lysates from another group of patients proved better acceptors of radiolabeled UDPGal than controls (53). This experiment strongly suggests that cells from the transferase-deficient patients have proteins that are under-galactosylated.

Direct evidence of abnormal glycosylation in an untreated transferase-deficient newborn was reported by Jaeken and colleagues, who showed that the IEF patterns of serum β-hexosaminidase and α-fucosidase had abnormal, cathodally migrating bands compared with age-matched controls (54). β-Hexosaminidase activity was elevated in serum, but α-fucosidase activity was normal. The IEF patterns and enzyme activities were normal after the patient was treated with a galactose-free diet for only 1 week. No parameters of dietary control such as red cell Gal-1-P concentrations were reported.

While untreated patients appear to undergo abnormal galactosylation, it is not clear whether those on galactose-restricted diets have defects as well. The presence of hypergonadotropic hypogonadism in both galactosemia and CDGS type I suggests that they do. The importance of proper glycosylation to the function of gonadotropins has been previously documented (55). Recently, a point mutation in the gene encoding the follicle-stimulating hormone (FSH) receptor has been described in families with an autosomal recessive form of hypergonadotropic ovarian failure (56). This mutation lies in an evolutionarily conserved region adjacent to a glycosylation site necessary for FSH binding. Further investigations into galactosemia, CDGS type I, and hypergonadotropic ovarian failure will broaden our understanding of glycosylation, ovarian function, and the mechanism behind inherited forms of ovarian failure.

Galactose-4-Epimerase Deficiency. Another cause of galactosemia in humans, gal-4-epimerase deficiency, results in a nucleotide sugar pool deficiency which may cause abnormalities in glycoconjugate synthesis. The most convincing data derive from investigations into cul-

tured animal cells. A Chinese hamster ovary cell line has been identified which has a complete deficiency of UDP-Gal-4-epimerase (57). Despite normal Gal-1-P uridyltransferase activity, which should provide an alternate source of UDPGal, these cells displayed significant defects in the synthesis of several glycoproteins and glycolipids when grown in media containing glucose as the only sugar source. The cells lack functional LDL receptors, which have many sites of both N-linked and O-linked glycosylation. LDL receptor activity was restored when exogenous galactose and GalNAc were present.

Human Gal-4-epimerase deficiency, a rare autosomal recessive condition, causes no clinical symptoms since it is confined to peripheral blood cells (48). Stimulation of leucocytes from such patients with phytohemagglutinin, as well as lymphocyte transformation with Epstein-Barr virus, restores epimerase activity. This could be another example of an unstable enzyme with considerable residual activity manifesting only in blood cells because no new enzyme is being synthesized. The gene for human Gal-4-epimerase has been cloned and mapped to chromosome 1p36-p35 (58). Mutations in epimerase patients have not been reported.

Two patients have been reported with a deficiency of UDPGal-4-epimerase in all tissues examined (59, 60). Both patients showed only residual activity of UDPGal-4-epimerase in erythrocytes and fibroblasts. UDPGalNAc-4-epimerase deficiency was documented as well in fibroblasts from one patient. Activities in fibroblasts from the parents were reduced compared with controls. These severely affected individuals excreted excess amounts of Gal-1-P and galactose. Like transferase-deficient galactosemic patients, these individuals presented as neonates with jaundice, failure to thrive, hepatosplenomegaly, and abnormal liver function tests. A galactose-free diet led to the resolution of all acute symptoms and normalization of red cell Gal-1-P levels. However, because epimerase-deficient patients are presumably unable to synthesize UDPGal in the absence of dietary galactose, their diets were supplemented with a small quantity of galactose. Despite treatment, the patients were severely developmentally delayed, with poor growth. Fibroblasts from one patient, grown in media with glucose as the only sugar source, synthesized LDL receptors of normal size and function, unlike Chinese hamster ovary cells with the same defect (61). Most likely, the residual epimerase activity demonstrated in the human cells provided adequate UDPGal and UDPGalNAc concentrations without an exogenous source of galactose. A complete deficiency of the epimerase would probably be lethal. Further studies determining whether Gal-4-epimerase–deficient patients exhibit subtle or tissue-specific defects in galactosylation have not been reported.

Leucocyte Adhesion Deficiency Type II (LADII). Although the primary defect in leucocyte adhesion deficiency type II (LADII) patients has not been identified, this disorder of neutrophil function most likely also falls into the category of a sugar nucleotide deficiency.

The recruitment of neutrophils to sites of inflammation as part of the cell's defense against bacterial infection requires three distinct steps (62). First, the circulating neutrophils slow and roll against the enormous shear force within blood vessels. Once slowed, the neutrophils adhere to endothelial cells and, finally, extravasate into the inflamed tissue. The initial rolling step is mediated by a family of molecules called selectins, which contain amino-terminal, lectin-like domains that bind fucosylated oligosaccharides such as sialyl Lewis X (NeuAcα2,3,Galβ1,4,(Fucα1,3)GlcNAc) found on many glycoproteins. E- and P-selectins are expressed on endothelial cells upon induction by inflammatory stimuli, and L-selectin is constitutively expressed on leucocytes. The modulation of selectins for anti-inflammatory therapies is of considerable interest, and a number of oligosaccharides have been shown to act as selectin ligands *in vitro*. The identification of selectins' *in vivo* carbohydrate ligands, and the precise interactions between ligand, selectins, and the other adhesion molecules are still being delineated.

In 1992, two unrelated boys were reported with recurrent bacterial infections and defective neutrophil motility (63). They differed from patients with the well-described disorder, leucocyte adhesion deficiency type I (64), in having severe mental retardation, short stature, and dysmorphic features. These included a low hairline, hypertelorism, a broad nasal bridge, protruding tongue, prominent mandible, and a narrow or high-arched palate. Their condition was designated LADII. Both patients had the rare Bombay blood group phenotype, which occurs in the absence of the red cell H antigen, produced by fucosyltransferase I. In addition, the patients were both nonsecretors and were negative for the Lewis blood group, indicating that they also lacked the products of fucosyltransferase II and fucosyltransferase III, respectively. Indeed, antibodies against the fucosylated tetrasaccharide sialyl Lewis X did not react with the patients' neutrophils. Since sialyl Lewis X or a structurally similar glycan is considered the *in vivo* ligand for selectins, its absence provides the likely basis for the immune deficiency in LADII patients. The presence of mental retardation and dysmorphic features suggests that the defect of fucosylation extends beyond blood group antigens and selectins. Clearly, reduced availability of GDP-fucose mitigates against its use in the different fucosyltransferase reactions. This could be due to a defect in the transport of fucose to the Golgi lumen, or in one of the enzymes in the synthetic pathway of GDP-fucose.

Cell lines from LADII patients have been critical to studies confirming the role of fucosylated carbohydrates in selectin-mediated neutrophil adhesion *in vivo* (65). Neutrophils from normal individuals will adhere to endothelial cells when E-selectin is induced by interleukin-1β. In studies that observed the behavior of neutrophils in mesenteric venules of rabbits by intravital microscopy, LADII neutrophils rolled poorly and failed to adhere upon interleukin-1β stimulation. Normal adhesion and extravasation did occur if

blood flow was stopped for several minutes, showing that this initial slowing of neutrophils, rather than later steps, was defective in LADII patients. These experiments confirm the role of fucosylated glycans and selectins in this process.

Defects in Proteoglycan Synthesis

Proteoglycans are a class of glycans found in all connective tissues and extracellular matrices, as well as on the surface of many cell types (66). They consist of a core protein modified by the addition of specific repeating disaccharide polymers called glycosaminoglycans (Fig. 4). Proteoglycans may have a single glycosaminoglycan chain or more than 100 chains; glycosaminoglycans can account for up to 90% of the molecular weight of a proteoglycan. The repeating disaccharide units are usually a uronic acid (i.e., glucuronic or iduronic acid) linked to either GalNAc or GlcNAc. These disaccharides are further modified by the addition of sulfates, making glycosaminoglycans highly negatively charged molecules of diverse composition. This structure allows them to bind large quantities of water molecules. As a result, proteoglycans have a viscous quality, which promotes cell migration and provides a cushioned environment for connective tissue.

Glycosaminoglycans usually are linked to their core proteins by a unique serine-xylose linkage at the amino acid sequence Ser-Gly-X-Gly, where X represents any amino acid (Fig. 4). After the addition of xylose, a linking region is formed by the stepwise addition of sugars *via* the actions of galactosyltransferase I, galactosyltransferase II, and glucuronyltransferase I. The repeating disaccharide units are added by the similar, sequential action of glycosyltransferases. Sulfation of glycosaminoglycans takes place by a two-step process in which sulfate is first "activated" by reacting with ATP to form adenosine-3′-phosphate-5′-phosphosulfate (PAPS) (67). PAPS then serves as the donor for any of several sulfotransferases.

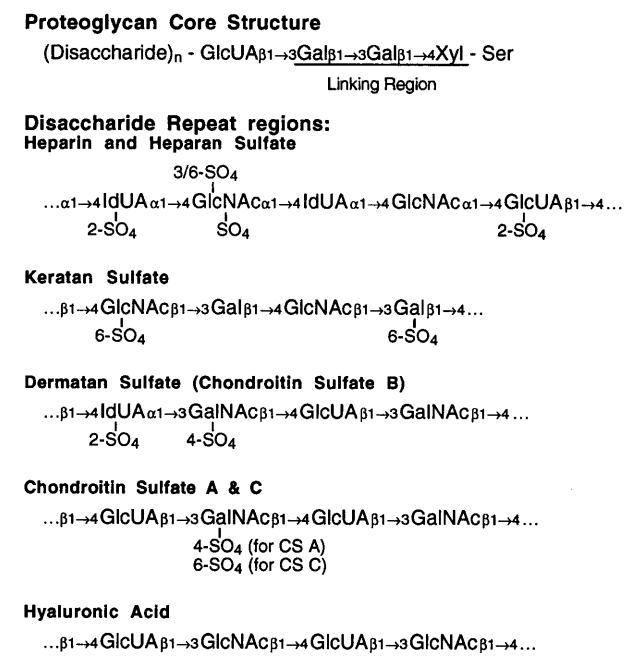

Figure 4. Proteoglycan core and disaccharide repeat structures.

Keratan sulfate and hyaluronic acid represent two exceptions to the general rules of proteoglycan structure. One type of keratan sulfate, found in the cornea, is attached to its core protein by an Asn-type linkage (68). The keratan sulfates also differ from most proteoglycans in that GlcNAc alternates with galactose rather than one of the uronic acids. Hyaluronic acid is unique in that the large GlcUA-GlcNAc polymer is not covalently attached to a protein, nor is it modified by sulfate addition (69).

Ehlers-Danlos Syndrome, Progeroid Type. In 1987, Kresse and co-workers described a 4-year-old Danish male, born to normal unrelated parents, with an Ehlers-Danlos type of connective tissue disorder (70). The boy had a small triangular face, flat forehead, broad nasal bridge, prominent eyes, hypoplastic earlobes, and a small mouth. His skin was light in complexion and loose, and his palms and soles were thickened. Wound healing was delayed with the formation of thin, atrophic scars. The patient's scalp hair and eyebrows were very thin and his teeth were brown and crumbling. During the first year of life he failed to thrive, and by 2 years of age his height was below the third percentile. He was proportionate with several skeletal abnormalities, including bony protruberances of the clavicle, radius, and ulna, a distal radioulnar dislocation, widening of the anterior ribs, short, broad clavicles, a valgus deformity of the hip, flat feet, and progressive demineralization of the bones. Initially, he exhibited low muscle tone with hypermobile joints, delayed motor development, and delayed speech. However, after 3 years of age he began to grow at a normal rate and attended kindergarten at a normal age. Other reports of Ehlers-Danlos–like syndromes with progeroid features have appeared, but none have been shown to have the same disorder described by Kresse.

The patient's fibroblasts were partially deficient in synthesizing the small dermatan sulfate proteoglycan, decorin, although some amounts of both mature, abnormally elongated decorin and glycosaminoglycan-free core protein were secreted by the cells (71). Decorin is an extracellular matrix protein that binds the surface of collagen fibrils, and which contains a single dermatan sulfate chain. The patient's decorin had normal N-linked glycosylation, and the amounts of both cell-associated and secreted proteoheparan sulfate from fibroblasts were normal.

The activity of galactosyltransferase I in homogenized patient fibroblasts, assayed using xylosylserine as a substrate, was 5% of that in normal fibroblasts. The apparent K_m with respect to xylosylserine was 2-fold normal, and the patient's enzyme was abnormally thermolabile. Cells from each parent had 50% normal activity, although no defect in decorin or in dermatan sulfate synthesis could be detected. In addition, the activity of galactosyltransferase II towards galactosylxylosylserine was 20% of normal in the patient's

fibroblasts and reduced in both parents' cells as well. Xylosyltransferase activity, measured using bovine nasal cartilage as an acceptor protein, was increased in the patient's fibroblasts.

Because this defect affects the linking unit common to other glycosaminoglycans, it is surprising that no other abnormal proteoglycans were noted. The altered K_m of galactosyltransferase I in this patient may change its substrate specificity such that only selected proteins are affected. The patient's abnormalities in two other enzyme activities related to proteoglycan synthesis must also be explained. These enzymes could form a complex *in vivo* which is disrupted by the mutation in galactosyltransferase I. Mutation analysis may resolve many questions about the structure of the galactosyltransferases and their substrate specificities.

Abnormalities in decorin synthesis appear to play a role in the pathophysiology of other inherited connective tissue disorders with progeroid features (72). Patients with neonatal Marfan, Wiedemann-Rautenstrauch, and Hutchison-Gilford syndromes all have reduced decorin transcription secondary to their inherited defects. Cell lines from all of these patients will be useful tools with which to study the complex interactions and regulation of extracellular matrix proteins.

Osteochondrodysplasias. Three well-described osteochondrodysplasias, diastrophic dystrophy (DTD), atelosteogenesis type II (AOII), and achondrogenesis type IB (ACG-IB) result from defective sulfation of cartilage proteoglycans (73). AOII, also known as neonatal osseous dysplasia type I, and ACG-IB are both perinatally lethal disorders with limb shortening and a small chest. Death results from respiratory insufficiency or pulmonary hypoplasia. Patients with AOII also have cleft palate, club feet, adducted thumbs and great toes, and a variety of other skeletal abnormalities. DTD is a milder disorder, which shares many findings with AOII and ACG-IB. Clinical manifestations are restricted to cartilage and bone, and include bilateral club feet, kyphoscoliosis, premature calcification of the costal cartilages, and a deformity of the first metacarpal commonly referred to as "hitchhiker's thumb." Patients can have cleft palate. Mortality is increased in infancy, but DTD patients whose disease is mild enough to survive this period have only a moderate decrease in life expectancy. Morbidity includes progressive joint arthritis and muscle contractures, causing considerable functional impairment. All three disorders are inherited in an autosomal recessive manner. Over 160 DTD patients have been reported in Finland.

Biochemical investigations revealed that proteoglycans from the cartilage matrix of ACG-IB patients were not modified by sulfate, despite normal fibroblast sulfotransferase activity. When incubated with [^{35}S]sodium sulfate, their fibroblasts produced little adenosine phosphosulfate and PAPS (74). Sulfate uptake was found deficient, though not entirely absent, in fibroblasts from a Finnish DTD patient; sulfate uptake in an obligate DTD carrier was normal (75).

The DTD gene, DTDST, maps to human chromosome 5q31–33.1 and encodes a sulfate transporter (75). AOII and ACG-IB are also caused by defects in the DTDST gene, which appears to be expressed in all cell types. This expression pattern was unexpected, since the phenotypes of these disorders are limited to cartilage and bone.

Mutation analysis has revealed that patients with DTD, AOII, and ACG-IB all represent compound heterozygotes, with one allele bearing a single null mutation (75–77). As might be predicted, when this null allele is present with another null allele or with a potentially severe mutation such as an amino acid substitution in one of the transmembrane domains, the result is the lethal disorder ACG-IB. When the null allele combines with a mutation causing a single amino acid substitution in a conserved region of the gene, the phenotype is that of AOII. While the mutation responsible for DTD in the Finnish population has not been identified, it will presumably be a milder mutation conferring residual transport activity. Phenotype-genotype correlations will help to identify the different functional regions of the DTDST protein. The limited consequences of this generalized defect also remain to be explained.

Macular Corneal Dystrophy. Macular corneal dystrophy is a progressive autosomal recessive disorder in which grey opacities form in the corneal stroma, reducing corneal sensitivity and leading to painful attacks of photophobia and corneal erosions (78). Onset usually occurs in the second half of the first decade. Patients are classified biochemically into two groups. In MCD I, both serum and cornea lack material cross-reacting with an antibody that recognizes sulfated keratan sulfate proteoglycans. In MCD II, the reaction with sulfated keratan sulfate antibodies is normal in both serum and corneal cells. Genetic mapping by linkage analysis has placed the gene responsible for MCD I on human chromosome 16q22 (79). MCD II may be an allelic form of MCD I.

Corneas of MCD patients produce an abnormal keratan sulfate proteoglycan which is reduced in size. This proteoglycan does not contain sulfate groups (80). In contrast, macular chondroitin sulfate and dermatan sulfate proteoglycans from an MCD patient were larger, had a greater [^{14}C]glucosamine-to-[^{3}H]mannose ratio, and contained a higher proportion of sulfated disaccharides compared with the control. This may have been a secondary effect related to the absence of sulfated keratan sulfate proteoglycan in the macula. The basic defect in MCD has not been identified, but a likely explanation for these findings would be the absence of a keratan sulfate sulfotransferase.

A single enzyme with 6-sulfotransferase activity for both GalNAc and galactose residues of chondroitin and keratan sulfate has been purified from chicken serum (81). The human homologue of this sulfotransferase could be a candidate for the gene responsible for either MCD or spondyloepiphyseal dysplasia tarda (see below).

Spondyloepiphyseal Dysplasia Tarda. At least six patients from three unrelated families have been described with an inherited form of spondyloepiphyseal dys-

plasia tarda in which there is a qualitative defect in urinary chondroitin-6-sulfate (82). These patients have moderately short stature with a reduced upper to lower body segment ratio and abnormalities of their spine and pelvis. Spine radiographs showed flattened vertebral bodies, reduced space between the vertebrae, and anterior curvature of the lower back. The skin was not affected and mental development was normal. Five of the six patients had peripheral punctate corneal opacities, visible only upon slit lamp examination.

When proteoglycans from the urine of four related patients were separated by agarose gel electrophoresis and subjected to hydrolysis by chondroitinase 4/6, a higher proportion of unsulfated disaccharides was present compared with normal controls (83). The quantity of glycosaminoglycans in patient urine was normal. To test the hypothesis that these patients had defective chondroitin sulfate-sulfotransferase, sera from the four patients were incubated with desulfated chondroitin 4- and 6-sulfate, and [^{35}S]PAPS. Incorporation of labeled sulfate was significantly reduced compared with normal. Both parents, expected to have intermediate levels of sulfate-sulfotransferase activity, in fact had normal levels of incorporation. This could have occurred because the assay measured more than one sulfotransferase activity, or because the patients' defect was only partial; the parents' activities would then be only slightly reduced. Normal activity in the parents could also indicate that reduced sulfotransferase activity is a secondary effect in the patients.

In contrast to the serum results, the sulfation of chondroitin sulfates synthesized by skin fibroblasts from one patient was normal. The different tissue distributions for separate sulfotransferases finds precedent in brachymorphic mice, which synthesize undersulfated proteoglycans in their cartilage due to a defect in the synthesis of PAPS, but show normal PAPS synthesis and proteoglycan synthesis in their skin. As with macular corneal dystrophy, spondyloepiphyseal dysplasia offers an opportunity to investigate the number of sulfotransferases, their substrate specificities, and their tissue distribution.

Conclusion

The handful of known disorders of glycan synthesis have provided a window revealing the functional roles normally played by glycosylation. There remains a great deal yet to see and to learn, however, from these and other rare inherited diseases. Although these illustrative disorders are rare, insights gained from the study of inherited disorders can be used to understand processes involved in more common diseases. Improper glycosylation has been reported in conditions such as cancer (84), rheumatoid arthritis (85), and alcoholism (86). In fact, alcoholics have serum transferrin IEF patterns similar to those seen in CDGS type I patients. Novel therapies may also arise. Already, compounds which inhibit normal glycan synthesis are being considered as possible treatments for acquired as well as inherited disorders. N-butyldeoxynojirimycin (NBDNJ), an inhibitor of the Golgi processing enzyme α-glucosidase I, has been suggested as an antiviral therapy against hepatitis B (87) and HIV (88). Because it inhibits glycolipid synthesis, it could also act to attenuate certain lysosomal storage disorders such as Gaucher disease (89). Basic and clinical investigations can benefit enormously and synergistically from shared information obtained at the bench and bedside.

Decades of experience have demonstrated that glycans interact with other molecules within an organism in ways that cannot be duplicated in the laboratory. These interactions may be appreciated through the natural history of disorders of glycan synthesis. Enhanced study of these interactions in humans is vital to continued progress in basic and applied carbohydrate research. The few disorders discussed here represent only a fraction of the illustrative human diseases that must exist, that should be discovered, and that are certainly poised to illuminate further the field of glycan synthesis.

1. Varki A. Biological roles of oligosaccharides: All of the theories are correct. Glycobiology **3:**97–130, 1993.
2. Neufeld EF, Muenzer J. The mucopolysaccharidoses. In: Scriver CR, Beaudet AL, Sly WS, Valle D, Eds. The Metabolic and Molecular Basis of Inherited Disease (7th ed.) New York: McGraw-Hill, Vol **2:**pp2465–2494, 1995.
3. Thomas GH, Beaudet AL. Disorders of glycoprotein degradation and structure: α-Mannosidosis, β-mannosidosis, fucosidosis, sialidosis, aspartylglucosaminuria and carbohydrate-deficient glycoprotein syndrome. In: Scriver CR, Beaudet AL, Sly WS, Valle D, Eds. The Metabolic and Molecular Basis of Inherited Disease (7th ed). New York: McGraw-Hill, Vol **2:**pp2529–2561, 1995.
4. Chen Y-T, Burchell A. Glycogen storage diseases In: Scriver CR, Beaudet AL, Sly WS, Valle D, Eds. The Metabolic and Molecular Basis of Inherited Disease (7th ed). New York: McGraw-Hill, Vol **1:**pp935–965, 1995.
5. Struck DK, Lennarz EJ. The function of saccharide-lipids in synthesis of glycoproteins. In: Lennarz WJ, Ed. The Biochemistry of Glycoproteins and Proteoglycans. New York: Plenum, pp35–83, 1980.
6. Kornfeld R, Kornfeld S. Assembly of asparagine-linked oligosaccharides. Annu Rev Biochem **54:**631–664, 1985.
7. Schachter H, Roseman S. Mammalian glycosyltransferases: Their role in the synthesis and function of complex carbohydrates and glycolipids. In: Lennarz WJ, Ed. The Biochemistry of Glycoproteins and Proteoglycans. New York: Plenum, pp85–160, 1980.
8. Holt GD, Haltiwanger RS, Torres C-R, Hart GW. Nuclear pore complex glycoproteins contain cytoplasmically disposed O-linked N-acetylglucosamine. J Biol Chem **262:**14847–14850, 1987.
9. Cohn DH, Byers PH, Steinmann B, Gelinas RE. Lethal osteogenesis imperfecta resulting from a single nucleotide change in one human proα1(1) collagen allele. Proc Natl Acad Sci U S A **83:**6045–6047, 1986.
10. Kretz KA, Carson GS, Morimoto S, Kishimoto Y, Fluharty AL, O'Brien JS. Characterization of a mutation in a family with saposin B deficiency: A glycosylation site defect. Proc Natl Acad Sci U S A **87:**2541–2544, 1990.
11. Leroy JG, Demars RI. Mutant enzymatic and cytological phenotypes in cultured human fibroblasts. Science **157:**804–806, 1967.
12. Kornfeld S, Sly WS. I-Cell disease and pseudo-Hurler polydystrophy: Disorders of lysosomal enzyme phosphorylation and localization. In: Scriver CR, Beaudet AL, Sly WS, Valle D, Eds. The Metabolic and Molecular Basis of Inherited Disease (7th ed). New York: McGraw-Hill, Vol **2:**pp2495–2508, 1995.
13. Hickman S, Neufeld EF. A hypothesis for I-cell disease: Defective

hydrolases that do not enter lysosomes. Biochem Biophys Res Commun **49:**992–999, 1972.
14. Natowicz MR, Chi MM-Y, Lowry OH, Sly WS. Enzymatic identification of mannose-6-phosphate on the recognition marker for receptor mediated pinocytosis of β-glucuronidase by human fibroblasts. Proc Natl Acad Sci U S A **74:**4322–4326, 1979.
15. Waheed A, Hasilik A, von Figura K. Processing of the phosphorylated recognition marker in lysosomal enzymes. J Biol Chem **256:**5717–5721, 1981.
16. Reitman ML, Varki A, Kornfeld S. Fibroblasts from patients with I-cell disease and pseudo-Hurler polydystrophy are deficient in uridine 5′-diphosphate-N-acetylglucosamine: Glycoprotein N-acetylglucosaminylphosphotransferase activity. J Clin Invest **67:**1574–1579, 1981.
17. Mueller OT, Wasmuth JJ, Murray JC, Lozzio CB, Lovrien EW, Shows TB. Chromosomal assignment of N-acetylglucosaminylphosphotransferase, the lysosomal hydrolase targeting enzyme deficient in mucolipidosis II and III. Cytogenet Cell Genet **46:**664, 1987.
18. Barriocanal JG, Bonifacino JS, Yuan L, Sandoval IV. Biosynthesis, glycosylation, movement through the Golgi system, and transport to lysosomes by an N-linked carbohydrate-independent mechanism of three lysosomal integral membrane proteins. J Biol Chem **261:**16755–16763, 1986.
19. Barton NW, Brady RO, Dambrosia JM, Di Bisceglie AM, Doppelt SH, Hill SC, Mankin HJ, Murray GJ, Parker RI, Argoff CE, Grewal RP, Yu K-T. Replacement therapy for inherited enzyme deficiency—macrophage targeted glucocerebrosidase for Gaucher's disease. N Engl J Med **324:**1464–1470, 1991.
20. Jaeken J, van Eijk HG, van der Heul C, Corbeel L, Eeckels R, Eggermont E. Sialic acid-deficient serum and cerebrospinal fluid transferrin in a newly recognized genetic syndrome. Clin Chim Acta **144:**245–247, 1984.
21. Eeg-Olofsson KE, Wahlstrom J. Genetic and epidemiological aspects of the carbohydrate-deficient glycoprotein syndrome. Acta Paediatr Scand Suppl **375:**63–65, 1991.
22. Krasnewich DM, Gahl WA. Carbohydrate-deficient Glycoprotein Syndrome. Adv Pediatr, 1996, in press.
23. Stibler H, Jaeken J, Kristiansson B. Biochemical characteristics and diagnosis of the carbohydrate-deficient glycoprotein syndrome. Acta Paediatr Scand Suppl **375:**21–31, 1991.
24. Krasnewich DM, Holt GD, Brantly M, Skovby F, Redwine J, Gahl WA. Abnormal synthesis of dolichol-linked oligosaccharides in carbohydrate-deficient glycoprotein syndrome. Glycobiology **5:**503–510, 1995.
25. Powell LD, Paneerselvam K, Vij R, Diaz S, Manzi A, Buist N, Freeze H, Varki A. 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, 1994.
26. Panneerselvam K, Freeze HH. Mannose corrects altered N-glycosylation in carbohydrate-deficient glycoprotein syndrome fibroblasts. J Clin Invest **97:**1478–1487, 1996.
27. Van Schaftingen E, Jaeken J. Phosphomannomutase deficiency is a cause of carbohydrate-deficient glycoprotein syndrome type I. FEBS Letters **377:**318–320, 1995.
28. Martinsson T, Bjursell C, Stibler H, Kristiansson B, Skovby F, Jaecken J, Blennow G, Stromme P, Hanefeld F, Wahlstrom J. Linkage of a locus for carbohydrate-deficient glycoprotein syndrome type I (CDGI) to chromosome 16p, and linkage disequilibrium to microsatellite marker D16S406. Hum Mol Genet **3:**2037–2042, 1994.
29. De Zegher F, Jaeken J. Endocrinology of the carbohydrate-deficient glycoprotein syndrome type I from birth through adolescence. Pediatr Res **37:**395–401, 1995.
30. Clayton P, Winchester B, Di Tomaso E, Young E, Keir G, Rodeck C. Carbohydrate-deficient glycoprotein syndrome: Normal glycosylation in the fetus. Lancet **341:**956, 1993.
31. Stibler H, Skovby F. Failure to diagnose carbohydrate-deficient glycoprotein syndrome prenatally. Pediatr Neurol **11:**71, 1994.
32. Ramaekers VT, Stibler H, Kint J, Jaeken J. A new variant of the carbohydrate-deficient glycoprotein syndrome. J Inherit Metab Dis **14:**385–388, 1991.
33. Jaeken J, Schachter H, Carchon H, De Cock P, Coddeville B, Spik G. Carbohydrate-deficient glycoprotein syndrome type II: A deficiency in Golgi localized N-acetyl-glucosaminyltransferase II. Arch Dis Child **71:**123–127, 1994.
34. Tan J, D'Agostaro GAF, Bendiak BK, Squire J, Schachter H. Molecular cloning and localization to chromosome 14 of the human UDP-N-acetylglucosamine: α-6-D-mannoside β-1,2-N-acetylglucosaminyltransferase II gene (MGAT2). Glycoconj J **10:**232–233, 1993.
35. Fukuda MN, Dell A, Scartezzini P. 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, 1987.
36. Stibler H, Westerberg B, Hanefeld F, Hagberg B. Carbohydrate-deficient glycoprotein (CDG) syndrome—A new variant, type III. Neuropediatrics **24:**51–52, 1993.
37. Stibler H, Stephani U, Kutsch U. Carbohydrate-deficient glycoprotein syndrome—A fourth subtype. Neuropediatrics **26:**235–237, 1995.
38. Verwilghen RL, Lewis SM, Dacie JV, Crookston MC. HEMPAS: congenital dyserythropoietic anaemia (type II). Q J Med **42:**257–278, 1973.
39. Fukuda MN, Papayannopoulou T, Gordon-Smith EC, Rochant H, Testa U. Defect in glycosylation of erythrocyte membrane proteins in congenital dyserythropoietic anaemia type II (HEMPAS). Br J Haemat **56:**55–68, 1984.
40. Fukuda MN, Masri KA, Dell A, Luzzatto L, Moremen KW. Incomplete synthesis of N-glycans in congenital dyserythropoietic anemia type II caused by a defect in the gene encoding α-mannosidase II. Proc Natl Acad Sci U S A **87:**7443–7447, 1990.
41. Fukuda MN. Congenital dyserythropoietic anaemia type II (HEMPAS) and its molecular basis. Bailliere's Clin Haemat **6:**493–511, 1993.
42. Misago M, Liao Y-F, Kudo S, Eto S, Mattei M-G, Moremen KW, Fukuda MN. Molecular cloning and expression of cDNAs encoding human α-mannosidase II and a previously unrecognized α-mannosidase II^x isozyme. Proc Natl Acad Sci U S A **92:**11766–11770, 1995.
43. Fukuda MN, Gaetani GF, Izzo P, Scartezzini P, Dell A. Incompletely processed N-glycans of serum glycoproteins in congenital dyserythropoietic anaemia type II (HEMPAS). Br J Haemat **82:**745–752, 1992.
44. Charuk JH, Tan J, Bernardini M, Haddad S, Reithmeier RA, Jaeken J, Schachter H. 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, 1995.
45. Spielberg SP, Garrick MD, Corash LM, Butler JD, Tietze F, Gorgers LV, Schulman JD. Biochemical heterogeneity in glutathione synthetase deficiency. J Clin Invest **61:**1417–1420, 1978.
46. Capasso JM, Hirschberg CB. Mechanisms of glycosylation and sulfation in the Golgi apparatus: evidence for nucleotide sugar/nucleoside monophosphate and nucleotide sulfate/nucleoside monophosphate antiports in the Golgi apparatus membrane. Proc Natl Acad Sci U S A **81:**7051–7055, 1984.
47. Kornfeld S, Kornfeld R, Neufeld EF, O'Brien PJ. The feedback control of sugar nucleotide biosynthesis in liver. Proc Natl Acad Sci U S A **52:**371–379, 1964.
48. Segal S, Berry GT. Disorders of galactose metabolism. In: Scriver CR, Beaudet AL, Sly WS, Valle D, Eds. The Metabolic and Molecular Basis of Inherited Disease (7th ed). New York: McGraw-Hill, Vol **1:**pp967–1000, 1995.
49. Reichardt JKV, Berg P. Cloning and characterization of a cDNA encoding human galactose-1-phosphate uridyltransferase. Mol Biol Med **7:**107–122, 1988.
50. Kaufman FR, Xu YK, Ng WG, Donnell GN. Correlation of ovarian function with galactose-1-phosphate uridyl transferase levels in galactosemia. J Pediatr **112:**754–756, 1988.
51. Holton JB, de la Cruz F, Levy HL. Galactosemia: The uridine diphosphate galactose deficiency—Uridine treatment controversy. J Pediatr **123:**1009–1014, 1993.

52. Ornstein KS, McGuire EJ, Berry GT, Roth S, Segal S. Abnormal galactosylation of complex carbohydrates in cultured fibroblasts from patients with galactose-1-phosphate uridyltransferase deficiency. Pediatr Res **31:**508–511, 1992.
53. Dobbie JA, Holton JB, Clamp JR. Defective galactosylation of proteins in cultured skin fibroblasts from galactosemic patients. Ann Clin Biochem **27:**274–275, 1990.
54. Jaeken J, Kint J, Spaapen L. Serum lysosomal enzyme abnormalities in galactosemia. Lancet **340:**1472–1473, 1992.
55. Thotakura NR, Blithe DR. Glycoprotein hormones: Glycobiology of gonadotropins, thyrotropin and free alpha subunit. Glycobiology **5:**3–10, 1995.
56. Aittomaki K, Lucena JLD, Pakarinen P, Sistonen P, Tapanainen J, Gromoll J, Kaskikari R, Sankila E-M, Lehvaslaiho H, Engel AR, Nieschlag E, Huhtaniemi I, de la Chapelle A. Mutation in the follicle-stimulating hormone receptor gene causes hereditary hypergonadotropic ovarian failure. Cell **82:**959–968, 1995.
57. Kingsley DM, Kozarsky KF, Hobbie L, Krieger M. Reversible defects in O-linked glycosylation and LDL receptor expression in a UDP-gal/UDP-galNAc 4-epimerase deficient mutant. Cell **44:**749–759, 1986.
58. Daude N, Gallaher TK, Zeschnigk M, Starzinski-Powitz A, Petry KG, Haworth IS, Reichardt JK. Molecular cloning, characterization, and mapping of a full-length cDNA encoding human UDP-galactose 4-epimerase. Biochem Mol Med **56:**1–7, 1995.
59. Holton JB, Gillett MG, MacFaul R, Young R. Galactosemia: A new severe variant due to uridine diphosphate galactose-4-epimerase deficiency. Arch Dis Child **56:**885–887, 1981.
60. Sardharwalla IB, Wraith JE, Bridge C, Fowler B, Roberts SA. A patient with severe type of epimerase deficiency galactosemia. J Inherit Metab Dis **11**(Suppl 2):249–251, 1988.
61. Kingsley DM, Krieger M, Holton JB. Structure and function of low-density-lipoprotein receptors in epimerase-deficient galactosemia. N Engl J Med **314:**1257–1258, 1993.
62. Bevilacqua MP, Nelson RM. Selectins. J Clin Invest **91:**379–387, 1993.
63. Frydman M, Etzioni A, Eidlitz-Markus T, Avidor I, Varsano I, Shechter Y, Orlin JB, Gershoni-Baruch R. Rambam-Hasharon syndrome of psychomotor retardation, short stature, defective neutrophil motility, and Bombay phenotype. Am J Med Genet **44:**297–302, 1992.
64. Anderson DC, Kishimoto TK, Smith CW. Leukocyte adhesion deficiency and other disorders of leukocyte adherence and motility. In: Scriver CR, Beaudet AL, Sly WS, Valle D, Eds. The Metabolic and Molecular Basis of Inherited Disease (7th ed). New York: McGraw-Hill, Vol **3:**pp3955–3994, 1995.
65. von Adrian UH, Berger EM, Ramezani L, Chambers JD, Ochs HD, Harlan JM, Paulson JC, Etzioni A, Arfors KE. In vivo behavior of neutrophils from two patients with distinct inherited leukocyte adhesion deficiency syndromes. J Clin Invest **91:**2893–2897, 1993.
66. Roden L. Structure and metabolism of connective tissue proteoglycans. In: Lennarz WJ, Ed. The Biochemistry of Glycoproteins and Proteoglycans. New York: Plenum, pp267–371, 1980.
67. Lipmann F. Biological sulfate activation and transfer. Science **128:**575–580, 1958.
68. Hart GW, Lennarz WJ. Effects of tunicamycin on the biosynthesis of glycosaminoglycans by embryonic chick cornea. J Biol Chem **253:**5795–5801, 1978.
69. Carson DD. Proteoglycans in development. In: Fukuda M, Ed. Cell Surface Carbohydrates any Cell Development. Boca Raton, FL: CRC Press, pp258–283, 1992.
70. Kresse H, Rosthoj S, Quentin E, Hollmann J, Glossl J, Okada S, Tonnesen T. Glycosaminoglycan-free small proteoglycan core protein is secreted by fibroblasts from a patient with a syndrome resembling progeroid. Am J Hum Genet **41:**436–453, 1987.
71. Quentin E, Gladen A, Roden L, Kresse H. A genetic defect in the biosynthesis of dermatan sulfate proteoglycan: galactosyltransferase I deficiency in fibroblasts from a patient with a progeroid syndrome. Proc Natl Acad Sci U S A **87:**1342–1346, 1990.
72. Beavan LA, Quentin-Hoffmann E, Schonherr E, Snigula F, Leroy JG, Kresse H. Deficient expression of decorin in infantile progeroid patients. J Biol Chem **268:**9856–9862, 1993.
73. Rimoin DL, Lachman RS. The chondrodysplasias. In: Emery AH, Rimoin DL, Eds. Principles and Practice of Medical Genetics (2nd ed). Edinburgh: Churchill Livingstone, pp895–932, 1990.
74. Superti-Furga A. A defect in the metabolic activation of sulfate in a patient with achondrogenesis type IB. Am J Hum Genet **55:**1137–1145, 1994.
75. Hastbacka J, de la Chapelle A, Mahtani MM, Clines G, Reeve-Daly MP, Daly M, Hamilton BA, Kusumi K, Trivedi B, Weaver A, Coloma A, Lovett M, Buckler A, Kaitila I, Lander ES. The diastrophic dysplasia gene encodes a novel sulfate transporter: Positional cloning by fine-structure linkage disequilibrium mapping. Cell **78:**1073–1087, 1994.
76. Superti-Furga A, Hastbacka J, Wilcox W, Cohn DH, van der Harten HJ, Rossi A, Blau N, Rimoin DL, Steinmann B, Lander ES, Gitzelmann R. Achondrogenesis type IB is caused by mutations in the diastrophic dysplasia sulphate transporter gene. Nat Genet **12:**100–102, 1996.
77. Hastbacka J, Superti-Furga A, Wilcox W, Rimoin DL, Cohn DH, Lander ES. Atelosteogenesis type II is caused by mutations in the diastrophic dysplasia sulfate-transporter gene (DTDST): Evidence for a phenotypic series involving three chondrodysplasias. Am J Hum Genet **58:**255–262, 1996.
78. Sugar J. Defects of the cornea. In: Emery AH, Rimoin DL, Eds. Principles and Practice of Medical Genetics (2nd ed). Edinburgh: Churchill Livingstone, pp655–667, 1990.
79. Vance JM, Jonasson F, Lennon F, Sarrica J, Damji KF, Stauffer J, Pericak-Vance MA, Klintworth GK. Linkage of a gene for macular corneal dystrophy to chromosome 16. Am J Hum Genet **58:**757–762, 1996.
80. Nakazawa K, Hassell JR, Hascall VC, Lohmander LS, Newsome DA, Krachmer J. Defective processing of keratan sulfate in macular corneal dystrophy. J Biol Chem **259:**13751–13757, 1984.
81. Sugumaran G, Katsman M, Drake RR. Purification, photoaffinity labeling, and characterization of a single enzyme for 6-sulfation of both chondroitin sulfate and keratan sulfate. J Biol Chem **270:**22483–22487, 1995.
82. Toledo SPA, Mourao PAS, Lamego C, Alves CAR, Dietrich CP, Assis LM, Mattar E. Recessively inherited, late onset spondylar dysplasia and peripheral corneal opacity with anomalies in urinary mucopolysaccharides: a possible error of chondroitin-6-sulfate synthesis. Am J Med Genet **2:**385–395, 1978.
83. Mourao PAS, Kato S, Donnelly PV. Spondyloepiphyseal dysplasia, chondroitin sulfate type: A possible defect of PAPS-chondroitin sulfate sulfotransferase in humans. Biochem Biophys Res Commun **98:**388–396, 1981.
84. Muramatsu T. Carbohydrate signals in metastasis and prognosis of human carcinomas. Glycobiology **3:**294–296, 1993.
85. Furukawa K, Matsuta K, Takeuchi F, Kosuge E, Miyamoto T, Kobata A. Kinetic study of a galactosyltransferase in the B cells of patients with rheumatoid arthritis. Int Immunol **2:**105–112, 1990.
86. Stibler H, Borg S, Joustra M. Micro anion exchange chromatography of carbohydrate-deficient transferrin in serum in relation to alcohol consumption (Swedish patent 8400587-5). Alcohol Clin Exp Res **10:**535–544, 1986.
87. Block TM, Lu X, Platt FM, Foster GR, Gerlich WH, Blumberg BS, Dwek RA. Secretion of human hepatitis B virus is inhibited by the imino sugar N-butyldeoxynojirimycin. Proc Natl Acad Sci U S A **91:**2235–2239, 1994.
88. Karlsson GB, Butters TD, Dwek RA, Platt FM. Effects of the imino sugar N-butyldeoxynojirimycin on the N-glycosylation of recombinant gp120. J Biol Chem **268:**570–576, 1993.
89. Platt FM, Neises GR, Dwek RA, Butters TD. N-Butyldeoxynojirimycin is a novel inhibitor of glycolipid biosynthesis. J Biol Chem **269:**8362–8365, 1994.