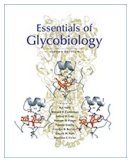

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Chapter 42 Genetic Disorders of Glycosylation

Hudson H Freeze and Harry Schachter.

This chapter discusses inherited human diseases that affect glycan biosynthesis and metabolism. Mutuations affecting each of the major glycan families are described. Disorders affecting the degradation of glycans are described in Chapter 41.

INHERITED PATHOLOGICAL MUTATIONS OCCUR IN ALL MAJOR GLYCAN FAMILIES

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Because 1–2% of the genome encodes enzymes involved in glycan formation, it is surprising that inherited disorders in glycan biosynthesis were not discovered until relatively recently. These rare human diseases are biochemically and clinically heterogeneous and usually affect multiple organ systems. Several of the diseases directly or indirectly impact one or more classes of glycans. Defects have been found in the activation, presentation, and transport of sugar precursors, in the glycosidases and glycosyltransferases involved in glycan synthesis and processing, and in proteins that control the traffic of components of the glycosylation machinery within the cell. In rare instances, patients have been successfully treated by oral administration of simple sugars. Increasing awareness of this group of disorders in the clinical community should foster new insights into their pathogenesis and treatment.

Table 42.1 lists the disorders in humans. Most of the human diseases have been discovered recently, although some are well-known diseases whose links to glycosylation are only now appreciated. The disorders are grouped together according to the major pathway affected. The specific nomenclature of each group is still evolving.

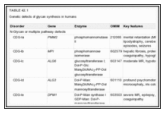

TABLE 42.1

Genetic defects of glycan synthesis in humans

DEFECTS IN N-GLYCAN BIOSYNTHESIS

Go to:

Clinical and Laboratory Features and Diagnosis

The congenital disorders of glycosylation (CDG) were originally called carbohydrate-deficient glycoprotein syndromes (CDGS) and are a subset of genetic defects affecting primarily N-glycan assembly. The broad clinical features involve many organ systems but especially the development of certain regions of the brain and functions of the gastrointestinal, hepatic, visual, and immune systems, indicating the importance of normal glycosylation in their functions. The variability of clinical features makes it difficult for physicians to recognize CDG patients. The first were identified in the early 1980s based on clinical symptoms and deficiencies in multiple plasma glycoproteins. The patients had psychomotor retardation, low muscle tone, incomplete brain development, visual problems, coagulation defects, and endocrine abnormalities. Many of these symptoms are seen in patients with other inherited multisystemic metabolic disorders, such as mitochondrial-based diseases. However, many CDG patients can be identified because they display abnormal glycosylation. Nearly all patients show undersialylation of serum glycoproteins as detected by examination of serum transferrin. This protein has two N-glycosylation sites with a typical disialylated biantennary glycan on each site (“tetrasialo” glycoform). About 10–15% of the protein molecules contain one or two trisialylated triantennary glycans (“pentasialo” or “hexasialo” forms), but only a very small proportion of glycans have just a single sialic acid residue (“disialo” or “monosialo” forms). These glycoforms are identified using isoelectric focusing (IEF), using ion-exchange chromatography, and, more recently, by mass spectrometric analysis. In CDG, many other serum glycoproteins have altered glycosylation, but transferrin is the most reliable, most sensitive, and simplest indicator. Transferrin provides a simple litmus test that has enabled physicians to spot CDG patients without knowing the molecular basis of the diseases.

For convenience, CDG defects are divided into types, I and II. The type I patients lack sialic acid residues on transferrin because they have unoccupied Asn-X-Ser/Thr glycosylation sequons; that is, entire sugar chains are absent. However, the N-glycan chains that remain in CDG type I patients are normal or only slightly altered. In the type II patients, both glycosylation sequons of transferrin are occupied, but the structure of the protein-bound glycan is altered. All defects in the biosynthesis of the lipid-linked oligosaccharide (LLO) and its transfer to proteins in the endoplasmic reticulum (ER) fall into type I, whereas defects in all subsequent processing steps are in type II. Individual type I CDGs (caused by defects in different genes) are given lowercase letters (e.g., CDG-Ia). The CDG nomenclature is in transition and will be replaced soon by the gene name, for example, CDG-PMM2. A list of these disorders is given in Table 42.1, and a few highlights are discussed below. The synthetic

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pathways and locations of the defects are shown in Figures 42.1 and 42.2.

FIGURE 42.1

Location of defects in type I congenital disorders of glycosylation (CDG-I). The figure shows the biosynthesis of the lipid-linked oligosaccharide (LLO) precursor and the defective steps leading to type I CDG. The monosaccharides must first be activated: (more...)

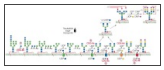

FIGURE 42.2

Location of defects in type II congenital disorders of glycosylation (CDG-II). After the glycan is added to the protein, the trimming/processing reactions generate multiantennary complex-type glycans. Three glucose and four mannose residues are removed (more...)

Type I Congenital Disorders of Glycosylation

A complete absence of N-glycans is lethal. Therefore, the type I CDG mutations create hypomorphic alleles, not complete knockouts. A deficiency in any of the steps required for the assembly of LLO in the ER (e.g., nucleotide sugar synthesis or sugar addition catalyzed by a glycosyltransferase) produces a structurally incomplete LLO. Because the oligosaccharyltransferase prefers full-sized LLO glycans, this results in hypoglycosylation of multiple glycoproteins. Importantly, many deficiencies in LLO synthesis produce incomplete intermediates. Most of the LLO assembly steps are not easy to assay by standard biochemical methods, but functional assays for LLO assembly have been developed. Because LLO assembly is conserved from yeast to humans, the intermediates that accumulate in CDG patients often correspond to the intermediates that accumulate in mutant *Saccharomyces cerevisiae* strains with known defects in LLO assembly. Some mutant mammalian cells (e.g., Chinese hamster ovary cells) have been shown to have similar defects (see Chapter 46). The close homology between yeast and human genes enabled the normal human ortholog to rescue defective glycosylation in mutant yeast strains, whereas the cDNA from patients with mutations do not. This provided substantial clues to the likely human defect, and sequencing of the patients' genes can reveal mutations. This approach was invaluable for tracking the human defects that cause CDG-Ic, -Id, -Ig, -Ih, -Ii, -Ik, and -IL. In a few types of CDG where yeast and mammalian pathways diverged, mammalian cell lines with known glycosylation defects are available for complementation by a similar strategy. This is especially important for identifying the defects in CDG-Ie and -If. Established enzyme assays help identify the defects in CDG-Ia, -Ib, -Ie, and -Ij. One critical clue for solving CDG-Ia, the most common type, is that patient cells synthesize a series of truncated LLO species when incubated in medium containing reduced glucose, whereas normal cells do not. This points to a general limitation in early precursors that originally led to the identification of phosphomannomutase as the defective enzyme in CDG-Ia, resulting in deficiency of GDP-mannose.

CDG-Ia is the most common type of CDG, with more than 600 cases identified worldwide. The patients have moderate to severe psychomotor retardation, hypotonia, dysmorphic features, failure to thrive, liver dysfunction, coagulopathy, abnormal endocrine functions, and a pronounced susceptibility to infection. Scores of mutations have been found in *phosphomannomutase 2* (*PMM2*), the defective gene in CDG-Ia. *PMM2* encodes an enzyme that catalyzes the conversion of Man-6-P to Man-1-P, which is a precursor required for the synthesis of GDP-mannose (GDP-Man) and dolichol-P-mannose (Dol-P-Man). Both donors are substrates for the mannosyltransferases involved in the synthesis of $Glc_3Man_9GlcNAc_2$-PP-Dol and their levels are decreased in CDG-Ia patients. Patients have hypomorphic alleles and complete loss of activity is lethal. In fact, mouse embryos lacking *Pmm2* die 2–4 days after fertilization, whereas those with homozygous hypomorphic alleles survive. There are currently no therapeutic options for CDG-Ia patients. In vitro studies suggested that supplements of mannose might improve glycosylation, but mannose therapy for CDG-Ia patients is ineffective.

CDG-Ib is caused by a deficiency in phosphomannose isomerase activity encoded by the gene *MPI*. The enzyme interconverts fructose-6-phosphate and Man-6-P, and deficiency leads to the synthesis of glycoproteins with unoccupied glycosylation sequons. In contrast to CDG-Ia, patients with CDG-Ib do not have psychomotor or developmental abnormalities. Instead, they show hypoglycemia, coagulopathy, severe vomiting and diarrhea, protein-losing enteropathy, and hepatic fibrosis. Several patients died of severe bleeding before the basis of this CDG had been described.

As mentioned in Chapter 4, Man-6-P can be generated directly by hexokinase-catalyzed phosphorylation of mannose. This pathway is intact in CDG-Ib patients. In humans, the plasma mannose level is about 50 μM, resulting from glycan degradation or processing. Mannose can be taken up into cells by a hexose transporter. Oral mannose therapy for phosphomannose-isomerase-deficient CDG-Ib patients was quite successful and corrected coagulopathy, hypoglycemia, protein-losing enteropathy, and intermittent gastrointestinal problems, as well as normalizing the glycosylation of plasma transferrin and other serum glycoproteins. Because orally administered mannose is well tolerated, this approach is clearly a satisfyingly effective, if not curative, therapy for this life-threatening condition. Complete loss of the single *Mpi* gene in mice is lethal at about E11.5. N-Glycosylation is normal, but death results from accumulation of intracellular Man-6-P, which depletes ATP, activates hexokinase, and inhibits several glycolytic enzymes. Providing dams with extra mannose during pregnancy only hastens the embryo's demise via the "honeybee effect." This unusual condition happens when bees are given only mannose instead of glucose. They continue flying for a short time and then literally drop dead. The reason is that they have low phosphomannose isomerase activity compared to hexokinase and therefore accumulate Man-6-P, which they degrade, only to phosphorylate it again using an ever-decreasing amount of ATP. Entry of Man-6-P into glycolysis is very slow and thus the bees become energy starved and die within a few minutes. CDG-Ib patients have sufficient residual phosphomannose isomerase activity that they do not accumulate intracellular

Man-6-P when given mannose, but it is sufficient to correct impaired glycosylation.

Other types of CDG-I have a broad range of clinical phenotypes. Some have characteristic phenotypes: for example, low LDL, low IgG, kidney failure, genital hypoplasia, and cerebellar hypoplasia. The reasons for these effects are unknown, but accumulated LLO intermediates may be toxic or there may be specific requirements for polymannosyl glycans that a truncated LLO cannot provide. It is likely that patients will be found with mutations in all the remaining steps of LLO biogenesis. See Table 42.1 and Figure 42.1 for known other types of CDG-I. Defects were recently found in genes encoding dolichol kinase, a putative LLO flippase, and two oligosaccharyltransferase subunits.

Type II Congenital Disorders of Glycosylation

Type II CDG disorders (Figure 42.2) are more diverse than type I disorders because mutations occur in glycosyltransferases, nucleotide sugar transporters, and cytoplasmic proteins that traffic the glycosylation machinery into and within the Golgi.

Structural analysis of serum protein glycans helped to pinpoint the defect in CDG-IIa. It is caused by mutations in *MGAT2*, which encodes N-acetylglucosaminyltransferase-II (GlcNAcT-II), the enzyme that adds the second *N*-acetylglucosamine of biantennary complex-type chains, the most common glycan in serum glycoproteins. In CDG-IIa patients, these glycans are replaced with monosialylated hybrid chains. The patients have dysmorphic features and psychomotor retardation, but do not have peripheral neuropathy or abnormal cerebellar development. Very rare (1%) survivors of a mouse line ablated in *Mgat2* provide a near phenocopy of the patients' pathology, including some of the dysmorphic features (see Chapter 8).

In CDG-IId, serum protein glycan structural analysis showed the loss of both galactose and sialic acid from transferrin as a result of the loss of nearly all β1-4 galactosyltransferase activity. Determining the cause of CDG-IIc, also called leukocyte adhesion deficiency type II (LAD-II), was more complex. Here transferrin sialylation was normal, so this defect was not detected by the usual test, but the N-glycans of IgM and O-linked glycans on leukocyte surface proteins are deficient in fucose. This group of proteins includes the selectin ligand carrying the glycan, sialyl Lewis^x that mediates leukocyte rolling prior to extravasation of leukocytes from the capillary lumen into the tissues (see Chapter 31). This defect greatly elevates circulating leukocytes and leads to frequent infections. The defect is due to mutations in the GDP-fucose transporter, which explains why it affects fucosylation of both N- and O-linked glycans. The success of mannose therapy for CDG-Ib prompted the use of orally administered fucose as a therapy for CDG-IIc. Several patients responded well to daily supplements of fucose in their diet. Sialyl Lewis^x reappeared on the leucocytes, and elevated circulating neutrophils promptly returned to normal levels. As mentioned in Chapter 4, fucose is converted into Fuc-1-P by fucose kinase and Fuc-1-P is converted to GDP-Fuc by GDP-Fuc pyrophosphorylase. A mouse model of fucose deficiency has been described that lacks de novo biosynthesis of GDP-Fuc from GDP-Man (see Chapter 4) because of the loss of the FX protein (GDP-4-keto-6-deoxy-mannose 3,5-epimerase-4-reductase). The mice die without fucose supplements, but providing fucose in the drinking water rapidly and reversibly normalizes their elevated neutrophils. The treatment also corrects abnormal hematopoeisis resulting from disrupted Notch signaling, emphasizing the crucial importance of its O-fucose glycans for signaling. CDG-IIf is caused by a defect in the CMP-sialic acid transporter (see Chapter 4). The patient completely lacks sialyl Lewis^x on leukocytes because of a deficiency of sialic acid, leading to severe neutropenia, formation of giant platelets, and abnormal platelet glycoproteins.

CDG-IIe is a new type of CDG-II defect caused by mutations in the *COG7* gene that disrupt multiple glycosylation pathways by impeding the normal trafficking of multiple glycosyltransferases and nucleotide sugar transporters. This mutation affects the synthesis of both N- and O-linked glycans including glycosaminoglycan (GAG) chains, so the phenotypic effects are far reaching. Cog7 is part of the eight-subunit COG (conserved oligomeric Golgi) complex, which is thought to have multiple roles in trafficking within the Golgi. Mammalian cells deficient in Cog1, Cog2, Cog3, and Cog5 also show various degrees of altered glycosylation. Many unclassified CDG-II patients who show defects in multiple pathways may have alterations in trafficking proteins that will likely impact synthesis of GAG chains and the normal assembly of the extracellular matrix. Several patients have defects in Cog8, and one has a defect in Cog1, but the phenotypes are surprisingly mild compared to the Cog7 deficiency. It is likely that mutations will be found in other Cog subunits as well as in other proteins that control protein trafficking or lumenal pH homeostasis in the Golgi. A series of mutations in a vacuolar H^+-ATPase subunit causes a CDG-II that affects multiple glycosylation pathways.

Other genetic defects in N-glycan synthesis are known. I-cell disease, which results from the lack of Man-6-P on lysosomal enzyme N-glycans, is covered in Chapter 30, but for historical reasons it was not classified as a CDG.

Is it possible to have diseases caused by "excessive" glycosylation? Not all potential N-glycosylation sites are occupied, and site occupancy for some proteins varies by tissue. The location of N-glycan sites tends to be conserved in most proteins, and point mutations that randomly create novel glycosylation sites could cause protein misfolding and rapid degradation. Perhaps the survival of these abnormally glycosylated proteins would have even worse consequences because new glycans might impair an important function or the ability to form multimeric complexes. For example, Marfan syndrome is caused by known mutations in fibrillin1 (FBN1). One of these creates an N-glycosylation site that disrupts multimeric assembly.

A good example of hyperglycosylation is seen in patients with heightened susceptibility to mycobacterial infections because they have an N-glycan site mutation in interferon γ receptor 2 (IFNγR2). Protein folding and surface localization are normal, but the function of the hyperglycosylated protein is dramatically decreased. Enzymatic removal of the additional glycan at the cell surface restores IFNγR2 activity. Thus, inserting the N-glycan at that site destroyed the receptor's function. This may not be an isolated case. A survey of 577 missense mutations of proteins traveling the ER-Golgi pathway showed that 13% of them potentially create inappropriate glycosylation sites. This is far greater than predicted for the effects of random mutations and may mean that hyperglycosylation has

a greater impact on function than loss of glycosylation. It is therefore not surprising that random insertion of bulky glycans into a finely tuned receptor has negative effects on formation of signaling complexes.

Congenital Dyserythropoieic Anemia Type II

More than 200 cases of autosomal recessive congenital dyserythropoieic anemia type II (CDA-II) are known. This disorder is also called HEMPAS (hereditary erythroblastic multinuclearity with a positive acidified serum lysis test). Patients generally have a normal life span, although complications may develop with age, including an enlarged liver, jaundice, gallstones, or diabetes. Ineffective erythropoiesis causes anemia and morphological abnormalities of the majority of erythroblasts in the bone marrow. In erythroid cells, the presence of poly-*N*-acetyllactosamines on glycosphingolipids is greatly increased along with a loss of protein-bound complex N-glycans and an increase in hybrid forms. This suggested a deficiency of N-glycan processing enzymes α-mannosidase II or GlcNAcT-II (encoded by the *MGAT2* gene). Reduced expression of Golgi α-mannosidase II and/or GlcNAcT-II activities has been observed in some, but not all, cases of HEMPAS. The α-mannosidase II knockout mouse also develops dyserythropoietic anemia. However, no CDA-II patients have proven mutations in these genes and *MGAT2* mutations cause CDG-IIa. Linkage analysis located the defective locus in CDA type II to 20q11.2, which eliminates both of these candidates. Sequencing candidate genes in this region in a large CDA-II population also failed to find mutations in other known genes. The cause(s) of CDA-II therefore remains unknown; however, alteration of a transcription factor or other regulatory molecule that affects several enzymes has been suggested. Alternatively, appreciation of the function of glycosylation-promoting chaperone proteins in the ER (e.g., COSMC) and cytoplasm (e.g., COG complex) offers new possibilities to consider.

GALACTOSEMIA

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Galactosemia refers to a group of diseases caused by inherited defects in the genes encoding three enzymes involved in galactose metabolism. One of these disorders, termed “classical galactosemia”, is caused by a deficiency of galactose-1-phosphate uridyltransferase (GALT; Figure 42.3). This disease increases intracellular galactose-1-phosphate and decreases synthesis and availability of UDP-galactose. Defects in UDP-galactose-4′-epimerase (GALE; Figure 42.3) or galactokinase (GALK; Figure 42.3) also cause the disease, but they are rare.

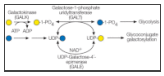

FIGURE 42.3

UDP-galactose synthesis and galactosemia. The most common form of galactosemia is due to a deficiency of galactose-1-phosphate uridyltransferase (GALT). This enzyme normally utilizes galactose-1-phosphate derived from dietary galactose. In the absence (more...)

As infants, GALT-deficient patients fail to thrive and have enlarged liver, jaundice, and cataracts. Lactose-free diet ameliorates most of the acute symptoms because it reduces the amount of galactose entering the metabolic pathway and diminishes the accumulation of galactose and galactose-1-phosphate that is thought to contribute to the symptoms of the disease. Reducing galactose accumulation also helps to inhibit the formation of galactitol and galactonate, which are produced via reductive or oxidative metabolism of galactose, respectively. Galactitol is not metabolized further and has osmotic properties that contribute to cataract formation. Unfortunately, a galactose-free diet apparently does not prevent the appearance of cognitive disability, ataxia, growth retardation, and ovarian dysfunction that are characteristic of this disease. The long-term complications in treated GALT-deficient individuals may be due to small amounts of toxic metabolites that continue to accumulate in these patients. Another possibility is that the complications are long-term outcomes of dysfunctions that originated during fetal life. GALT deficiency may decrease UDP-galactose and galactosylated glycans. Hypogalactosylation of glycoproteins and glycolipids has been observed in some GALT-deficient individuals. Some patients who mistakenly receive galactose develop a hypoglycosylated transferrin that is missing both entire N-glycans and individual monosaccharides on the remaining chains. The basis of this combined loss is not understood, but the pattern returns to normal when the patients are placed on a galactose-free diet. It is possible that accumulation of hypogalactosylated glycans is secondary to a general metabolic abnormality in these patients, but how abnormal structures lead to the complications in these patients remains unsolved.

MUSCULAR DYSTROPHIES

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Congenital Muscular Dystrophies

Mutations altering glycans on α-dystroglycan (αDG) cause at least five types of congenital muscular dystrophies (CMDs) (Table 42.1 and Figure 42.4) and other CMDs with unknown etiologies will likely involve glycosylation. The O-mannosylation pathway is presented in Chapter 12. The initial step occurs in the ER lumen using a Dol-P-Man donor and a heterodimeric enzyme (protein O-mannosyltransferase; POMT1/POMT2) to add mannose in α1-O linkage to serine/threonine. In the Golgi, a pathway-specific protein O-mannoside β1-2GlcNAc-transferase (POMGNT1) creates a disaccharide and then galactose and sialic acid are added by unspecified transferases. αDG is the only identified carrier of these glycans. It is a vital component of the dystrophin glycoprotein complex (DGC) on the sarcolemma of skeletal muscle cells. Dystroglycan is a major component of the DGC that connects the ECM to the cytoskeleton in many tissues. Both components (αDG and βDG) are derived from a single gene, *DAG1*. In muscle, actin in the cytoskeleton is linked to βDG, which spans the cell membrane. The extracellular domain of βDG binds to αDG, which in turn binds laminin-2 in the ECM via its glycan-containing extracellular domain. Traditional naming of CMDs based on clinical symptoms is being replaced by designation of the specific defective genes.

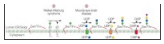

FIGURE 42.4

Location of glycosylation defects in the O-mannosyl glycosylation pathway leading to congenital muscular dystrophies. The biosynthesis of O-mannosyl glycans primarily on α-dystroglycan first involves the addition of mannose to serine or threonine (more...)

The degree and type of αDG glycosylation vary in different tissues. The presence of sialic acid may be required for laminin-2 binding. Monoclonal antibodies against the glycans show very low or undetectable binding to αDG in some patients and provide the key tool to identify glycosylation-related defects, similar to the use of transferrin for N-glycosylation defects in the CDGs. Normal αDG runs as a diffuse band of 130–190 kD as detected by an antibody that recognizes the protein core, but in CMD patients anti-αDG recognizes a less diffuse band of about 90 kD, regardless of which step is defective. The size and amount of βDG are unaffected. Although no pathological mutations have been found in αDG itself, the name α-dystroglycanopathy has been suggested to describe these glycosylation disorders.

Walker–Warburg syndrome (WWS) is the most severe form of the CMD caused by defective αDG glycosylation. Patients have a short life span (less than 1 year on average), multiple brain abnormalities, and severe muscular dystrophy. About 20% of patients have mutations in *POMT1* and a few have mutations in *POMT2*. As with *DAG1*-null mice, *POMT1* ablation is an early embryonic lethal; both mutant strains of mice fail to synthesize Reichert's membrane, a basement membrane that surrounds the blastocyst, supporting a functional connection between the two genes. A few clinically defined WWS patients have defects in the *fukutin* and *FKRP* (fukutin-related protein) genes, which are thought to function in this glycosylation pathway. Mutations in *fukutin* and *FKRP* also cause other, milder forms of CMD (see below). Linkage studies of consanguineous WWS families suggest that mutations in at least two other genes can cause disease.

The *POMGnT1* gene is mutated in muscle–eye–brain disease (MEB), which is characterized by symptoms similar to, but milder than, WWS. The most severely affected patients die during the first years of life, but the majority of mild cases survive to adulthood.

Fukuyama muscular dystrophy (FCMD) is caused by a single 3-kb 3′-retrotransposon insertional event into the *fukutin* gene, which occurred 2000–2500 years ago. This partially reduces the stability of the mRNA, making it a relatively mild mutation. FCMD is one of the most common types of CMD in Japan with a carrier frequency of 1/188. The protein has a putative glycosyltransferase domain (DXD) and localizes to the *cis*-Golgi, but no transferase activity has yet been demonstrated. *Fukutin*-null mice die by E9.5 in embryogenesis and appear to have basement membrane defects. Chimeric mice with less than 50% contribution of cells that are heterozygous for loss of *fukutin* show typical muscular dystrophy, compromised survival, and disorganized laminar structures in the brain, along with ocular and retinal abnormalities.

Studies of two other CMDs provide further examples of novel, as yet uncharacterized, proteins (putative enzymes) that play a role in αDG glycosylation. MDCIC is a relatively mild disorder that is caused by mutations in *FKRP*. The protein has a glycosyltransferase signature (DXD) but no known transferase activity. It is Golgi localized, but a portion may also reside in the ER. Patients with MDC1D, a limb-girdle muscular dystrophy, contain mutations in *LARGE*, originally described in myodystrophic mice (*myd,* now called *Large* ^{myd}). The protein has two glycosyltransferase signatures (DXD motifs) in different domains, suggesting the possibility of a bifunctional enzyme. No enzymatic activity has been demonstrated, but mutations of the DXD motifs prevent LARGE from rescuing defective glycosylation. The protein resides in the Golgi and appears to recognize an aminoterminal region of αDG defining an enzyme-substrate recognition motif necessary to initiate functional glycosylation. Molecular recognition of αDG by the product of *LARGE* is required for the biosynthesis of a functional dystroglycan and to prevent muscle degeneration.

Overexpression of *LARGE* results in hyperglycosylation of αDG by an as yet unknown compensatory mechanism and phenotypically rescues the *Large* ^{myd} mouse. The glycan-enriched αDG shows a high affinity for extracellular ligands. LARGE circumvents the αDG glycosylation defect in cells from individuals with genetically distinct types of CMD without increasing the expression of the other dystroglycan complex proteins. Transfection of the *LARGE* gene into cultured cells from CMD patients restores αDG receptor function and rescues defective FCMD myoblasts, MEB fibroblasts, and WWS myoblasts. These results are significant because they may mean that *LARGE* could be a broad-based gene therapy for CMD. LARGE is not the only protein with this effect. Overexpression of a specific cytotoxic T cell α-N-acetylgalactosaminyltransferase can also create a novel glycan in αDG and prevent the onset of pathology in the *mdx* mouse; this mouse is deficient in dystrophin, the cause of Duchenne's muscular dystrophy, showing that formation of an effective extracellular complex can override and substitute for the loss of the intracellular link to actin. The mechanism appears to involve increased binding to utrophin, a dystrophin homolog, which is not normally present at the muscle membrane in sufficient levels.

Inclusion Body Myopathy 2

Autosomal recessive, adult-onset inclusion body myopathy type 2 (IBM2) occurs worldwide, but is especially common among Persian Jews (1:1500). It is allelic with distal myopathy with rimmed vacuoles (Nonaka myopathy) and patients accumulate vacuoles containing β-amyloid, tau, and presenilin. They are caused by mutations in the *GNE* gene, which encodes a bifunctional, two-domain enzyme UDP-GlcNAc 2-epimerase/N-acetylmannosamine kinase that catalyzes successive steps in the de novo pathway for sialic acid biosynthesis (see Chapter 12). Dominant mutations in *GNE* also cause sialuria, resulting in continuous secretion of sialic acid, but IBM2 patients do not show this phenotype. Sialuria patients have mutations that inactivate an allosteric binding site for CMP-sialic acid. In IBM2, mutations occur in various combinations in both domains, with the most common (M712T) occurring in the kinase domain. In vitro assays show only moderately reduced enzyme

activity (20–60%) in both domains and it is unproven whether such modest decreases in measured activity cause defective sialylation, especially on αDG. It is unknown whether GNE has additional roles besides sialic acid biosynthesis. Since sialic acid is efficiently salvaged from degraded glycoproteins, some cells may be less reliant on de novo synthesis, but there is little information on the cell-type preference or age-dependent contributions of the de novo versus salvage pathways. *GNE*-null mice have an embryonic lethal outcome. Most of the mice homozygous for the M712T mutation die a few days after birth; however, they do not develop a myopathy. Instead, they have severe hematuria and proteinuria as a result of abnormalities in the glomerular basement membrane. The major sialoprotein in foot podocytes (podocalexin) is undersialylated. Providing *N*-acetylmannosamine to the pups rescues a portion of the pups and increases sialylation of podocalexin. This may provide a therapy for IBM2 patients, although it is not yet proven that the muscle defect is due to reduced sialyation. Another mouse model, carrying a *GNE* mutation common in the Japanese population, develops a pathological muscle phenotype involving β-amyloid deposition that precedes the accumulation of inclusion bodies. No therapeutic studies have been done on this model.

DEFECTS IN O-GalNAc GLYCANS

Go to:

A defect in O-linked glycosylation causes familial tumoral calcinosis. This severe autosomal recessive metabolic disorder shows phosphatemia and massive calcium deposits in the skin and subcutaneous tissues. The disorder is due to mutations in *GALNT3*, one of the O-GalNAc transferases in mucin O-glycosylation. Mutations in the O-glycosylated fibroblast growth factor 23 (FGF23) also cause phosphatemia, suggesting that *GALNT3* modifies FGF23. The rare autoimmune disease, Tn syndrome, is caused by somatic mutations in the X-linked gene *COSMC*, which encodes a highly specific chaperone required for the proper folding and normal activity of β1-3galactosyltransferase needed for synthesis of core 1 O-glycans (see Chapter 43 for additional details).

DEFECTS IN PROTEOGLYCAN SYNTHESIS

Go to:

Proteoglycans and their GAG chains are critical components in extracellular matrices. For a discussion of their biosynthesis, core proteins, and function, see Chapter 16.

Ehlers–Danlos Syndrome (Progeroid Type)

Ehlers–Danlos syndrome (progeroid type) is a connective tissue disorder characterized by failure to thrive, loose skin, skeletal abnormalities, hypotonia, and hypermobile joints, together with delayed motor development and delayed speech. The molecular basis of the disorder is in the synthesis of the core region of xylose-based GAG chains. Decorin, a dermatan sulfate proteoglycan that binds to collagen fibrils, is partially deficient, and some molecules are made without an extended GAG chain. Galactosyltransferase I, the enzyme that adds galactose to xylosyl-serine has only 5% of normal activity. The activity of galactosyltransferase II, the enzyme responsible for adding the second galactose residue to the GAG chain core, has only 20% of normal activity. One possible explanation for the dual effect is that the primary mutation affects the formation or stability of a biosynthetic complex involving several GAG-chain biosynthetic enzymes. Because all proteoglycans contain this common linkage region, it is unclear why the defect has a selective effect on decorin.

Congenital Exostosis

Defects in the formation of heparan sulfate (HS) cause hereditary multiple exostosis (HME), an autosomal dominant disease with a prevalence of about 1:50,000. It is caused by mutations in two genes *EXT1* and *EXT2*, which are involved in HS synthesis. HME patients have bony outgrowths, usually at the growth plates of the long bones. Normally, the growth plate contains chondrocytes in various stages of development, which are enmeshed in an ordered matrix composed of collagen-chondroitin sulfate. In HME, however, the outgrowths are often capped by disorganized cartilagenous masses with chrondrocytes in different stages of development. About 1–2% of patients also develop osteosarcoma.

HME mutations occur in *EXT1* (60–70%) and *EXT2* (30–40%). The proteins encoded by these genes are thought to exist as a complex in the Golgi and both are required for polymerizing GlcNAcα1–4 and GlcAβ1–3 into HS. However, the partial loss of one allele of either gene appears sufficient to cause HME. This means that haploinsufficiency decreases the amount of HS and that EXT activity is rate limiting for HS biosynthesis. This is unusual because most glycan biosynthetic enzymes are in substantial excess.

The mechanism of HME pathology is likely rooted in a disruption of the normal distribution of HS-binding growth factors, which include FGF and morphogens such as hedgehog, Wnt, and members of the TGF-β family. The loss of HS disrupts these pathways in *Drosophila*. Mice that are null for either *Ext* gene are embryonic lethal and fail to gastrulate; however, *Ext* heterozygous animals are viable and about one third develop a visible exostoses on the ribs. No exostoses develop on the long bones of these animals (in contrast to patients with HME), but subtle chondrocyte growth abnormalities were seen in the growth plates of these bones. Further studies are needed to understand how truncation of the HS chains leads to ectopic growth plate formation and the phenotype abnormalities.

Achondrogenesis, Diastrophic Dystrophy, and Atelosteogenesis

Three autosomal recessive disorders, diastrophic dystrophy (DTD), atelosteogenesis type II (AOII), and achondrogenesis type IB (ACG-IB), all result from defective cartilage proteoglycan sulfation. These forms of osteochondrodysplasia have various outcomes. AOII and ACG-IB are perinatally lethal because of respiratory insufficiency, whereas DTD patients develop symptoms only in cartilage and bone, including cleft palate, club feet, and other skeletal abnormalities. Those DTD patients surviving infancy often live a nearly normal life span. All of these disorders result from different mutations in the DTD gene that encodes a plasma membrane sulfate transporter. Unlike monosaccharides, sulfate released from degraded macromolecules in the lysosome is not salvaged well. The heavy demand for sulfate in bone and cartilage proteoglycan synthesis probably explains why the symptoms are most

evident in these locations. Defects in the UDP-GlcA/UDP-GalNAc Golgi transporter cause Schneckenbecken dysplasia. Patients have bone abnormalities similar to those seen in other chrondrodysplasias, and a mouse model of the disease shows similar features.

Macular Corneal Dystrophy

Keratan sulfate (KS) in the cornea is an N-linked oligosaccharide with poly-*N*-acetyllactosamine repeats (Galβ1-4GlcNAcβ1-3) variably sulfated at the 6-positions. Macular corneal dystrophy (MCD), an autosomal recessive disease, causes the cornea to become opaque and corneal lesions to develop. Two types of MCD have been described. MCD I appears to be due to a deficiency in sulfating the repeating units. Both galactose and *N*-acetylglucosamine are sulfated in KS; sulfation of galactose and *N*-acetylgalactosamine in chondroitin sulfate are also affected in MCD patients.

DEFECTS IN GLYCOSPHINGOLIPID SYNTHESIS

Go to:

Positional cloning identified *SIAT9* as the cause of autosomal recessive Amish infantile epilepsy syndrome. This gene encodes a sialyltransferase required for the synthesis of the ganglioside GM3 (Siaα2-3Galβ1-4Glc-ceramide) from lactosylceramide (Galβ1-4Glc-ceramide). GM3 is also a precursor for some more complex gangliosides. The nonsense mutation in these families truncates the protein and abolishes GM3 synthase activity. Analysis of plasma glycosphingolipids showed the accumulation of multiple nonsialylated glycolipids. In contrast to the human form of the disease, mice that lack GM3 do not have seizures or a shortened life span. However, mouse strains that are null for the sialyltransferase and an N-acetylgalactosaminyltransferase that is required for making other complex gangliosides do develop seizures, suggesting that it is the absence of these more complex gangliosides that may be the underlying problem (see Chapter 10).

PHENOTYPES, MULTIPLE ALLELES, AND GENETIC BACKGROUND

Go to:

One puzzling feature of the genetic disorders of glycosylation is that the phenotypic expression of the same mutation can have widely variable impact, even among affected siblings. Explanations based on the level of residual enzymatic activity for these “simple Mendelian disorders” are neither simple nor generally satisfying. Genotype–phenotype correlations are often difficult to establish. The most likely explanation is differences in the “genetic background,” a term meaning all the individual’s other genes. For instance, a very frequent single-nucleotide polymorphism (SNP) in *ALG6*, the cause of CDG-Ic, has a barely discernible effect on glycosylation of a model protein in yeast and yet when examined in CDG-Ia patients (PMM2 deficiency), the SNP is twice as frequent in severe cases relative to mild cases. In yeast, *Alg6* deletion decreases glycosylation, but it is not lethal; however, when it is combined with another nonlethal mutation in the oligosac-charyltransferase gene (*Wbp1*), lethality occurs. The effects of mutations are context dependent. As mentioned above, a knockout mutation may be lethal in one highly inbred mouse strain, but not in another because compensatory pathways may exist. Dietary and environmental impacts are substantial as seen in CDG-Ib patients with and without oral mannose therapy. As discussed in Chapter 43, the genetic deficiency in these patients makes them more susceptible to infections and to the impact of pro-inflammatory cytokines on their intestinal protein leakage, probably as a result of loss of epithelial heparan sulfate. The synergism of multiple simultaneous or sequential environmental insults on genetic insufficiencies may create a cascade leading to overt disease.

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Go to:

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