Published in final edited form as:

Annu Rev Neurosci. 2015 July 8; 38: 105–125. doi:10.1146/annurev-neuro-071714-034019.

NEUROLOGICAL ASPECTS OF HUMAN GLYCOSYLATION DISORDERS

Hudson H. Freeze¹, Erik A. Eklund², Bobby G. Ng¹, and Marc C. Patterson³

Hudson H. Freeze: hudson@sanfordburnham.org; Erik A. Eklund: Erik.Eklund@med.lu.se; Bobby G. Ng: bobbyng@sanfordburnham.org; Marc C. Patterson: patterson.marc@mayo.edu

1

2

3

Abstract

This review will present principles of glycosylation, describe the relevant glycosylation pathways and their related disorders, and highlight some of the neurological aspects and issues that continue to challenge researchers. Over 100 rare human genetic disorders that result from deficiencies in the different glycosylation pathways are known today. Most of these disorders impact the central and/or peripheral nervous systems. Patients typically have developmental delay/intellectual disability, hypotonia, seizures, neuropathy, and metabolic abnormalities in multiple organ systems. Between these disorders there is great clinical diversity because all cell types differentially glycosylate proteins and lipids. The patients have hundreds of mis-glycosylated products afflicting a myriad of processes including cell signaling, cell-cell interaction and cell migration. This vast complexity in glycan composition and function, along with limited analytic tools has impeded the identification of key glycosylated molecules that cause pathologies, and to date few critical target proteins have been pinpointed.

Keywords

glycans; congenital disorders; epilepsy; seizures; glycoprotein; CDG

INTRODUCTION

Whether you study the central or the peripheral nervous systems, gross architecture or molecular details, patients or model systems, sooner or later you will encounter glycosylation. Adding the correct sugar chains (glycans) to proteins or lipids employs at least 2% of the translated genome to generate 1000's of molecular structures. Every cell

Corresponding Author: Hudson H. Freeze, Ph.D., Professor and Director, Human Genetics Program, Sanford Children's Health Research Center, Sanford-Burnham Medical Research Institute, 10901 North Torrey Pines Road, La Jolla, CA 92037, Phone: 858-646-3142, Fax: 858-795-5381, hudson@sanfordburnham.org.

CONFLICTS OF INTEREST

Marc C. Patterson: Consulting: Actelion, Agios, Amicus, Cydan, Stem Cells, Shire HGT. Editorial: Journal of Child Neurology, Child Neurology Open (Editor-in-Chief); Journal of Inherited Metabolic Disease (Editor); Up-To-Date (Section Editor); Hudson H. Freeze: Consulting: Agios.

makes glycans and all the multiple theories about their function are correct but cannot be applied to all cases (Varki 1993). If so, how do we determine which pathways and genes are most important? We believe that one way to answer this question is to identify neurologically impaired individuals who carry mutations in glycosylation-related genes. Over 100 distinct Congenital Disorders of Glycosylation (CDG) are known and >80% have neurological abnormalities. Here, we describe several of them together with emerging insights that may inform both clinical and basic science perspectives.

First, a review of relevant glycosylation pathways and basic principles gives the background to discuss classes of genes that cause defects. Then we select examples from the clinic that highlight neurological perspectives. Unfortunately, most mechanisms and specific target proteins are unknown. As the BRAIN initiative (NIH) in the United States moves forward in the coming decades, studying glycosylation will provide new opportunities for unexplored areas. Table S1 in the Supplemental material lists the known mutated genes that cause a neurological phenotype, their function, and typical patient abnormalities. These examples may enthuse or bewilder, but deeper consideration will benefit basic scientists, clinicians, and patients.

FUNDAMENTALS and COMMON FEATURES

Most sugar metabolism focuses on glucose (Glc), which also causes diabetic complications from non-enzymatic glycation (Sharma et al. 2014). Glycosylation and glycation are sometimes confused. Glycosylation is a template-independent, enzymatic process that adds one or more sugars to proteins and lipids. In mammals, nine sugars (monosaccharides) are unequally distributed between 10 major pathways (Spiro 2002), each one being defined by the linkage between the first sugar and the protein or lipid. Genetic defects are known in most of them. The potential complexity of the glycome— all the sugar chains in a cell or organism—is orders of magnitude larger than the genome or proteome. Much of this complexity arises from the variable length, modifications and arrangements of repeating disaccharides in glycosaminoglycans. In addition, the family of sialic acid molecules has over 50 members and linking 6 different, unmodified sugars to each other generates a trillion unique combinations (Varki and Sharon, 2009). All of this complexity provides mechanisms for ultrafine tuning of some processes, superimposed on a background of seemingly inconsequential effects (Freeze 2013, Varki 1993). The same sugar chain expressed on different proteins can have functional consequences ranging from indifference to destruction. Outcome is context dependent and case-by-case analysis is the rule.

The most important factors determining glycosylation products are the identity of the protein/lipid, rate limiting enzymes involved in sequential or competing biosynthetic steps, their subcellular localization, supply and localization of activated sugars (sugar phosphates and nucleotide sugars), and the presence of competing acceptors (Freeze et al. 2014). Relatively little attention has focused on epigenetic or micro RNA regulation of these activities (Agarwal et al. 2014, Kasper et al. 2014, Kizuka et al. 2014, Pedersen et al. 2013, Shi & Ruvkun 2012). Nearly all the precursors (nucleotide sugars) are made in the cytoplasm and carried into the ER or Golgi using a set of transporters. This increases their concentration where most reactions occur. Glycosylated proteins can carry multiple types of

glycans. Since multiple pathways share these precursors, limiting their amount or delivery can impact multiple glycosylation pathways. Modification of a particular glycan can exclude or enhance subsequent extensions of that glycan. Rate-limiting steps vary and the outcome is context-dependent.

Glycosyltransferases are transcriptionally regulated, but another important feature is their localization and efficiency of recycling through the dynamic ER and Golgi. Glycosylation requires a functional Golgi system and defects in Golgi homeostasis, trafficking and composition cause glycosylation disorders (Willett et al. 2013). Such trafficking defects can impact single or multiple glycosylation pathways because they mis-localize coalitions of multiple glycosyltransferases and nucleotide sugar transporters (Freeze & Ng 2011). Many of these defects occur in cytoplasmic proteins that transiently associate with the Golgi and help guide vesicles containing the glycosylation machinery to their location.

BIOSYNTHETIC PATHWAYS AND CONSEQUENCES OF THEIR DISRUPTION

Eight major glycan-generating pathways populate the ER-Golgi network. Neurological defects occur in patients carrying mutations in most of them (see Table 1 and supplementary Table S1). Cellular model systems are common but the availability of published animal models varies greatly (Table 1). Pathway-specific glycosyltransferases initiate and extend the glycan chains, but addition of the more distal sugars sometimes involves transferases that can serve several pathways.

N-LINKED GLYCOSYLATION

The term defines the first sugar, N-acetylglucosamine (GlcNAc), added to selected asparagines (Asn) on nascent proteins as they emerge into the ER lumen. Clients include nearly all secreted, cell surface, receptor and signalling membrane proteins, as well as ER, Golgi or lysosomal proteins, i.e., nearly any protein passing through the ER-Golgi network. N-glycans promote protein folding, stability, trafficking, localization, and oligomerization (Stanley et al. 2009). They act as proof-reading monitors and play vital roles in cell-cell interactions and intracellular signalling (Dennis et al. 2009).

All N-linked glycan synthesis begins with a nearly universal 14-sugar precursor containing 2 GlcNAc, 9 Mannose (Man), and 3 Glc units assembled stepwise into a specific structure on a lipid carrier (dolichol), to form the lipid-linked oligosaccharide (LLO) (Stanley et al. 2009). Five of the Man units are derived from GDP-Man and four from dolichol-P-Man (Dol-P-Man). That entire glycan is transferred to Asn using the multi-subunit oligosaccharyltransferase, (OST) complex (Li et al. 2008, Zielinska et al. 2010) with the aid of associated complexes, such as translocon complex (TRAP) (Dejgaard et al. 2010, Shibatani et al. 2005). Incomplete LLO glycans are transferred less efficiently, leaving unoccupied glycosylation sites. After transfer to protein, all Glc units and up to 6 Man units are removed by a series of specific ER- and Golgi-localized glycosidases, and then GlcNAc, Galactose (Gal), Sialic acid (Sia), and Fucose (Fuc) are added creating multiple branches of different lengths and compositions. A few sugars can be modified by the addition of

sulphate or phosphate esters. N-glycan remodelling has a prescribed order in the early portion of the pathway, but multiple competing reactions can alter the final outcome in this non template-driven symphony (Figure S1).

Dol-P-Man is also used as a substrate for 3 other pathways: Glycophosphatidylinositol (GPI)-anchors, O-mannose and C-mannose. The first two are described below.

PMM2-CDG—The most prevalent glycosylation disorder is caused by mutations in phosphomannomutase 2 (*PMM2*). The encoded enzyme (*PMM2*) converts mannose-6-phosphate to mannose-1-phosphate, which then generates GDP-Mannose and Dol-P-Man, the primary mannosylation donors. The *PMM2*-CDG defect reduces Man-1-P, GDP-Man, Dol-P-Man and LLO production used for N-glycosylation (Freeze 2013). This leaves many proteins with only partially occupied N-glycosylation sites, often decreasing their stability. Because Dol-P-Man is also used for GPI-anchor synthesis it is possible that this pathway could be affected, and one study offers suggestive evidence for this (de la Morena-Barrio et al. 2013).

Common neurological features of *PMM2*-CDG children include intellectual disabilities, seizures, hypotonia, microcephaly, cerebellar atrophy/hypoplasia, strabismus and stroke-like episodes (Table S1). Because of the large number of patients, it is the best characterized of all N-linked disorders, however, little is yet known about how *PMM2* deficiencies cause pathology in the nervous system because specific hypo-glycosylated proteins have not been identified. However, gated ion channels are often heavily glycosylated, contributing 5–50% of their molecular weight (Nowycky et al. 2014) and sialylation is especially significant (Ednie & Bennett 2012).

Mouse and zebrafish models mimic several of the neurological and developmental features seen in humans (Cline et al. 2012, Orr et al. 2013, Wang et al. 2002). It is becoming clear that genetic background dramatically influences patient phenotype. For example individuals with two of the more common *PMM2* mutations (F119L, R141H) can have a moderately severe phenotype while others die. One explanation is that some patients carry additional mutations in other genes in the N-glycosylation pathway, increasing mutation load for more severe cases. This has not been studied.

A common feature of *PMM2*-CDG children is cerebellar atrophy/hypoplasia (Barone et al. 2014). Autopsy studies show extensive loss of Purkinje and granule cells (CGC) (Aronica et al. 2005). To explain this loss, one study showed that mouse cerebellar granule cells are more sensitive than cortical neurons (CN) to inhibition of N-glycosylation either by LLO synthesis inhibitor, tunicamycin, or *PMM2* knockdown. Cultured CGC had a poorer ER-stress response, especially in GRP78/BiP, compared to CN. Over-expression of that chaperone rescues cell death arguing that ER stress may explain the cell-selective loss in the cerebellum (Sun et al. 2013).

TUSC3-CDG—*TUSC3*-CDG manifests as non-syndromic intellectual disability [ID] (Garshasbi et al. 2008, Molinari et al. 2008). *TUSC3* encodes a subunit of the oligosaccharyltransferase complex that plays a central role in N-glycosylation, but it also is

involved in plasma membrane magnesium transport. TUSC3 appears to enhance the efficiency of glycosylation of a subset of glycoproteins by slowing glycoprotein folding (Mohorko et al. 2014), raising the possibility of a structural substrate for ID when TUSC3 is deficient. Knockdown of TUSC3 decreases total and free intracellular magnesium in mammalian cell lines; developmental arrest in zebrafish can be rescued with excess magnesium (Zhou & Clapham 2009). Multiple pathways likely contribute to ID in TUSC3-CDG.

MYASTHENIC SYNDROME—Congenital myasthenic syndromes (CMS) impair signal transmission at the neuromuscular synapse (Engel et al. 1999). Most are due to post-synaptic defects (Muppidi et al. 2012) including mutations in one of the five acetylcholine receptor (AChR) subunits, *CHRNE* impairs assembly of the complex (Engel et al. 1999). A mutation that destroyed a glycosylation site and decreased protein levels first suggested that hypoglycosylation can cause CMS (Engel et al. 1999).

Thirteen families with limb-girdle CMS were reported with mutations in *GFPT1*, which is needed for UDP-GlcNAc synthesis (Engel et al. 2012, Senderek et al. 2011). Silencing the zebrafish ortholog (*gfpt1*) altered muscle fiber morphology and impaired neuromuscular junction development in embryos (Senderek et al. 2011). Muscle biopsy (Zoltowska et al. 2013) and cultured myotubes from patients had reduced cell-surface AChR and siRNA silencing of *GFPT1* also reduced AChR. Later other patients were found with mutations in *DPAGT1* (Belaya et al. 2012), a UDP-GlcNAc-requiring enzyme that initiates LLO synthesis and is known to cause a CDG (Wu et al. 2003) and more severe neurological features than CMS (Carrera et al. 2012). Muscle biopsies and cultured myoblasts from several cases showed reduction of AChR at the endplates. siRNA knockdown decreased expression and reduced three AChR subunits. *DPAGT1* and *GFPT1* mutations cause AChR instability pointing to faulty N-glycosylation of the receptors. More mutations were found in *ALG14*, a UDP-GlcNAc-requiring transferase used for LLO (Cossins et al. 2013). In yeast, Alg14 forms a multi-glycosyltransferase complex with Alg13 and Alg7 (*DPAGT1*) that carry out the first two steps in LLO synthesis (Gao et al. 2005, Lu et al. 2012). *ALG14* concentrates at the muscle motor endplates and siRNA knockdown of *ALG14* reduces cell-surface expression of muscle AChR expressed HEK293 cells (Cossins et al. 2013).

Mutations in *ALG2* encoding another LLO-mannosyltransferase also cause CMS (Cossins et al. 2013). In yeast Alg1 (first mannose in LLO) forms a complex with Alg2 and Alg11, which together add the next four mannose units. Their physical association of these enzymes in the ER membrane may improve the efficiency of LLO synthesis (Gao et al. 2004). Figure 1 illustrates these interactions. Why mutations in these genes manifest as CMS, rather than the severe CDG is unclear. Additional glycosylation genes will likely be associated with CMS (Houlden 2013). CMS cases responded well to anticholinesterase medication and drugs that increase acetylcholine release from the nerve terminals (Zoltowska et al. 2013). It is possible that CDG patients might benefit from such therapy.

CONGENITAL DISORDER OF DE-GLYCOSYLATION

Mutations in *NGLY1* interfere with the ERAD pathway that selects and degrades some misfolded N-glycosylated proteins exported from the ER causing the first “congenital disorder of de-glycosylation” (Anonymous 2014, Enns et al. 2014, Might & Wilsey 2014). Patients have global developmental delay, a movement disorder, hypotonia, and occasionally seizures, microcephaly and diminished reflexes. They also lack tears. *NGLY1* encodes the only known cytoplasmic enzyme that can strip bulky N-glycan chains from misfolded, retro-translocated glycoproteins prior to their proteasomal degradation. Most *NGLY1* mutations produce null alleles and are predicted to impair degradation of client proteins, yet free oligosaccharide chains, presumably derived from similarly

O-LINKED GLYCOSYLATION

O-linked protein glycosylation involves initial linkage between Serine/Threonine (Ser/Thr) and Man, Xylose (Xyl), N-acetylgalactosamine (GalNAc), Fuc, GlcNAc or Glc. We describe only those whose disruptions cause neurological defects.

O-MANNOSE GLYCOSYLATION—The O- α Man glycans contain GlcNAc, Gal, GalNAc, Xyl, Glucuronic acid (GlcA) and Sia in a surprisingly complex pathway (Stalnaker et al. 2011, Yoshida-Moriguchi et al. 2010) (Figure S2). The major carrier of these glycans is alpha-dystroglycan (α DG)—which has a crucial role in neuromuscular junctions and in linking skeletal muscle cell cytoskeleton to the extracellular matrix molecule, laminin. Defects in this pathway cause a group of disorders called α -dystroglycanopathies, which have been indispensable for working out both the structure and biosynthetic steps of this pathway (Praisman & Wells 2014). Recently, cadherins have also been identified as major carriers (Lommel et al. 2013, Vester-Christensen et al. 2013). Defects in this pathway often cause neurologic deficits, but some are restricted exclusively to muscle disorders.

α -Dystroglycan (α DG) is part of the dystrophin-glycoprotein complex, which links the extracellular matrix to the cytoskeleton. A functional complex requires α DG O-mannosylation to bind to laminin. The clinical spectrum of α -dystroglycanopathy is broad, ranging from very severe musculo-oculo-encephalopathies (including Walker Warburg syndrome [WWS], muscle-eye-brain, and Fukuyama congenital muscular dystrophy [FCMD]) to milder forms of limb-girdle muscular dystrophy (Godfrey et al. 2011). The brain shows cerebellar hypoplasia, white-matter changes on MRIs, and congenital brain malformations such as cobblestone lissencephaly, and hydrocephalus (Clement et al. 2008). The clinical course in the most severe form is rapidly progressive with early fatality (WWS). Surviving (mainly muscle-eye-brain) patients show profound developmental delay/intellectual disability, but some are mobile and can say a few words (Martin 2005).

Over twenty O-Man structures are found in mammals (Praisman & Wells 2014), employing a large group of biosynthetic enzymes. O-Man glycans can also play a traitorous role, as essential molecules for Lassa Virus entry into cells. This feature was cleverly exploited (Jae et al. 2013) to screen haploid libraries for genes used for virus entry. The method correctly predicted all previously unknown WWS-causing genes. Currently 16 genes have been proven to be disease causing (*POMT1*, *POMT2*, *POMGNT1*, *FKTN*, *FKRP*, *LARGE*, *ISPD*,

GTDC2, TMEM5, B3GALNT2, SGK196, B3GNT1, GMPPB, DPM1, DPM2, DPM3) of which three (*DPM1–3*) are involved in N-linked glycosylation as well. Some Lassa Virus tagged genes have not yet been linked to the disorder (Jae et al. 2013).

Deficiencies in any of these genes can potentially result in a severe or mild dystroglycanopathy. Clinical distinctions result more from the severity of the mutation than the gene identity (Cirak et al. 2013). The classification of the dystroglycanopathies is either based on a combination of severity and genetic cause (as suggested by OMIM (Amberger et al. 2011) or on the pure clinical phenotype (such as WWS). The severity/gene classification refers to the whole group as muscular dystrophy-dystroglycanopathies (MDDG). This is divided into three severity groups: A= severe (e.g., WWS); B = intermediate (e.g., MDC1D); C = mild (e.g., limb-girdle muscular dystrophy 2I). A number indicates the defective gene (e.g. 1=POMT1). Thus, a classic case of POMT1 deficient WWS is referred to as MDDGA1, whereas a case of SGK196-deficiency with an intermediate severity is referred to as MDDGB12.

While α DG is the predominant carrier of O-Man glycans, a brain specific knock out does not reduce the amount of these glycans; clearly other proteins carried them (Stalnakier et al. 2011). A clever application called “SimpleCell” was used to reduce the complex O-Man glycans to just a single O-Man at any site (Vester-Christensen et al. 2013). Proteolysis and isolation of the glycopeptides combined with mass-spectrometry identified 37 members of the cadherin family as the major carriers. Cadherins mediate cell-cell adhesion by forming trans homodimers between different extracellular domains and the O-Man modification appears at conserved sites in these molecules. Clustered protocadherins that contain these glycans are regulated during brain development and form larger oligomers. The glycosylation sites are not located at the trans binding domain, but they appear to help position the domains for the critical interactions. O-Man in clustered protocadherins is especially important since they may help explain ocular and brain malformations in the most severe disorders such as WWS. Plexins were also found to contain O-Man; they are also highly expressed in the brain. A separate study using a different approach found that O-Man is required for E-cadherin-mediated cell adhesion during embryonic development. Without them, E-cadherin was not localized to the adhesive sites and blocking O-Man addition prevents the morula to blastocyst transition (Lommel et al. 2013).

PROTEOGLYCANS—O- β -Xyl linked glycoaminoglycans (GAGs) attached to Ser generate proteoglycans such as heparan sulfate (HS), heparin, chondroitin (CS) and dermatan sulfates (DS) (Esko et al. 2009). The (20–100) repeating disaccharides of GlcA-GalNAc (CS and DS) or GlcA-GlcNAc (heparin and HS) are assembled on a common 5-sugar core glycan (Esko et al. 2009). Some GlcA is epimerized to iduronic acid (Ido) and multiple sulphate esters can be added to the amino groups of de-N-acetylated GlcNAc or to multiple OH-groups. The chain length plus diversity of sulfation patterns accounts for much of the enormous diversity and size and of the mammalian glycome.

GAG chains are only added to about 35 core proteins forming proteoglycans. For HS, this includes the GPI-anchored glypicans and transmembrane-anchored syndecans, which have been implicated in multiple signalling pathways. Cell surface HS chains bind growth factors

(*e.g.*, FGF family), cytokines, and morphogens during development to establish gradients of these molecules (Lander 2007, Zhang et al. 2007). Proteins with CS chains are often used to insure physical integrity and cushioning. Recent studies highlight the inhibitory action of CS in nerve regeneration and point to chondroitinases as potential therapeutics (Tennant 2014).

GLYCOSYLATED LIPIDS

GLYCOPHOSPHATIDYLINOSITOL (GPI) ANCHOR—Glycophosphatidylinositol (GPI) anchor glycans substitute for transmembrane regions of many signalling proteins. (Figure S3) They contain Man and Glucosamine (GlcN), and are assembled in the ER on a phosphatidylinositol backbone. The entire glycolipid is transferred to C-terminal regions of proteins with concomitant cleavage of a C-terminal peptide (Ferguson et al. 2009). The GPI-anchored proteins move to the Golgi where the lipid components are rebuilt before the proteins move to the surface where they can assemble into lipid rafts and influence membrane diffusion, intracellular protein sorting and signalling (Hancock 2004).

The first disease in the GPI anchor biosynthetic pathway was due to somatic mutations in the X-linked *PIGA* (Figure S3), causing a rare hematopoietic disease called paroxysmal nocturnal hemoglobinuria (PNH) (Parker 2012). Next generation sequencing revealed at least ten other GPI-AP deficiencies, including inherited deficiencies in *PIGA* that dramatically affect the CNS (Belet et al. 2014, Johnston et al. 2012, Kato et al. 2014, Swoboda et al. 2014, van der Crabben et al. 2014). These include epilepsy, hypotonia, micro- or macrocephaly, movement disorders, language disabilities and varying degrees of ID. Structural abnormalities of the brain are common (Table S1). *PIGA* appears to have the broadest neurological phenotype, but this may simply reflect the identification of more cases. Some GPI subtypes only have one or two reported families, as is the case with *PGAP1*, *PIGM* and *PIGT*.

There are few treatment options for GPI-AP deficiencies, although some patients' seizures respond to pyridoxine (Kuki et al. 2013, Thompson et al. 2006). One family presenting with venous thrombosis and intractable seizures, carried a promoter mutation *PIGM* that was effectively treated with butyrate (Almeida et al. 2006).

How the loss of GPI-AP results in such a broad neurological phenotype is unclear, and without GPI-deficient animal models we can only speculate about mechanisms. However, they likely reflect widespread dysfunction of the abundant GPI-AP within the brain/CNS. Such examples include the Nogo receptor (NgR), which is required for regulating plasticity, axonal regeneration and axonal growth inhibition in the adult CNS via its interactions with oligodendrocyte myelin glycoprotein and myelin-associated glycoprotein (Pernet & Schwab 2012). NCAM1 is required for neuron-neuron adhesion as well as outgrowth and fasciculation of neurites (Cremer et al. 1997, Hildebrandt et al. 2007, Schnaar et al. 2014). One of its N-linked glycans is a highly poly-sialylated. Both NgR and NCAM require GPI-anchoring to function (Atwal et al. 2008, Rosen et al. 1992, Wills et al. 2012).

GLYCOSPHINGOLIPIDS (GSL)—Glycosphingolipids (GSLs) link Glc (sometimes Gal) to ceramide (Glc-Cer), and addition of Gal to GlcCer makes lactosylceramide (LacCer). This core can be variably extended to more complex GSLs including sialylated gangliosides

(Schnaar et al. 2009). (Figure S4). The highest amount and concentration of GSLs occur in the brain and peripheral nervous system (Schnaar et al. 2014). GSLs assemble into lipid rafts and bind to each other or to proteins such as integrins, through which they affect signalling (Hakomori 2004).

Despite advances in NGS only three disorders in glycosphingolipid synthesis (Freeze et al. 2014) are known and all involve ganglioside biosynthesis. The initial step in the biosynthesis of the simplest ganglioside (GM3) uses the precursor intermediate, lactosylceramide, and sialyltransferase *ST3GAL5*, which is mutated in at least two distinct disorders. The first disorder was seen in an old order Amish family displaying infantile-onset symptomatic epilepsy syndrome (Simpson et al. 2004). The second family had “Salt and Pepper Syndrome” with altered dermal pigmentation along with severe intellectual disability, epilepsy, scoliosis, choreoathetosis and dysmorphic facial features (Boccuto et al. 2014). Importantly, analysis of patient samples from both studies confirmed a complete lack of GM3 ganglioside proving *ST3GAL5* was responsible for the disorder.

Mutations in *B4GALNT1* (also known as GM2/GD2 synthase) cause hereditary spastic paraplegia subtype 26 (Boukhris et al. 2013, Harlalka et al. 2013, Wakil et al. 2013). Patients have developmental delay and varying cognitive impairment with early-onset progressive spasticity due to the degeneration of axons. Cerebellar ataxia, peripheral neuropathy cortical atrophy and white matter hyper-intensities were also consistent across the disorder. A *B4galnt1*^{-/-} mouse recapitulates several of the neurological characteristics of SPG26, most prominently the progressive gait disorder (Takamiya et al. 1996).

ST3GAL3 can be used for the synthesis of more complex gangliosides as well as N- and O-glycans. It is required for development of high cognitive functions and is mutated in some individuals with West syndrome (Edvardson et al. 2013b, Hu et al. 2011). An *St3gal3*^{-/-} mouse model also exists, but these mice appear to have no overt neurological phenotype (Ellies et al. 2002, Kiwamoto et al. 2014).

Given that 80% of all brain glycans are found in GSL, many of which are complex gangliosides (Tettamanti et al. 1973), it is not surprising that disorders within GSL biosynthesis should give a neurological phenotype. Gangliosides can associate directly with ion transport proteins or indirectly with proteins that activate transport via signaling (Nowycky et al. 2014). The difficulty comes in the identification of these cases since screening for GSL is not routine, and there are no convenient biomarkers.

IDENTIFYING GLYCOSYLATION DISORDERS

BIOMARKERS

Altered glycosylation of selected biomarkers often helped identify the mutated pathway (not specific gene). Mass spectrometric analysis of serum transferrin is especially useful for the disorders affecting N-glycosylation (Tegtmeyer et al. 2014) or multiple pathways. Immunohistochemistry of muscle biopsies with monoclonal antibodies that recognize O-mannose based glycans confirm α -dystroglycanopathies (Lefeber et al. 2009). Loss of several leukocyte GPI-anchored proteins is easily assessed by routine flow cytometry (Ng &

Freeze 2014). Mammalian cell lines carrying mutations in specific glycosylation genes are also useful, and now the use of CRISPR/Cas9 technology allows selective mutation of any gene (Cai & Yang 2014). Still, a relevant glyco-biomarker is useful to measure the mutation's impact. One example is a GFP with an engineered N-glycosylation site that allows fluorescence only when the site is unoccupied (Losfeld et al. 2014). A similar rationale was used to design a glycosylation-dependent luciferase construct (Contessa et al. 2010). So, glycosylation-defective cells glow and cells complemented with the wild-type allele have reduced fluorescence.

BIASED DISCOVERY

Most of the major glycosylation pathways claim at least one disorder; some are much more highly represented than others. Why does the N-linked pathway account for most of the defects? This is probably because transferrin is a convenient biomarker and nearly all of the early steps in the pathway are encoded by single genes, functioning alone or as a part of protein complexes. Before the human genome was sequenced, mutant yeast and mammalian cell lines were readily available for biochemical readouts in complementation assays (Aebi & Hennet 2001). In fact, the LLO pathway is nearly saturated with a human defect at each known step. However, recent studies show that additional complexes with previously unknown functions, such as TRAP are functionally associated with the OST (Dejgaard et al. 2010, Shibatani et al. 2005). The latter portions of the N-linked pathway are not saturated, probably because of redundancy in some glycosyltransferases. Both the GPI anchor and O-mannosylation pathways have similar advantages of biomarkers, non-redundant genes, and, for GPI-anchors, mutant mammalian cell lines (Freeze 2013).

At the other end of the spectrum is the O-GalNAc pathway. In this pathway, 20 different gene products carry out the same reaction that initiates the pathway. The need for this apparent redundancy may lie in cell-type expression and a surprising degree of substrate specificity (Steentoft et al. 2014). The O-GalNAcT's have preferred acceptor sequence specificities, and some require recognition of nearby sites that are already glycosylated (Kong et al. 2014). The long-held impression was that these enzymes modified only a small group of highly selected mucins that contained dense clusters of short O-linked glycans (Brockhausen et al. 2009). It is now clear that hundreds of proteins carry only 1–2 isolated O-GalNAc glycans (Schjoldager et al. 2012). Some of those are critical for function including effects on proteolysis of selected growth factors (Schjoldager & Clausen 2012). However, a variety of GWAS studies implicate one transferase (GALNT2) in lipid metabolism (Holleboom et al. 2011). Critical breakthroughs in mass spectrometry and use of Simple Cell technology where all subsequent glycan modifications were eliminated enabled these discoveries. Now genome sequencing will likely reveal some candidate GALNT-based disorders using a clear, systematic approach to examine which proteins can be modified by each of these transferases. This is a significant breakthrough because the limitation in linking glycosylation deficiency with the specific target proteins.

The paucity of defects in the GSL pathway is probably due to lack of simple biomarkers, but the predominance of GSLs in the nervous system will almost certainly mean that genome sequencing will reveal potential candidates. An especially valuable review of GSL,

ganglioside and sialic acid function illustrates the complexity found in the nervous system and systems available for analysis (Schnaar et al. 2014).

BASIS OF CLINICAL PRESENTATIONS

We cannot explain how altered glycosylation causes CDG neuropathology. That will likely require matching expression of the “neuro-glycome” with nervous system development and function. Defective glycosylation disrupts developmental pathways and alters brain structure (Freeze et al. 2012). Thus, early progressive pancerebellar atrophy correlates with severe ataxia in PMM2-CDG patients; the near absence of brain autopsy material from other CDG patients results in few other structural correlates (Aronica et al. 2005).

Epilepsy, intellectual disability and autism spectrum disorders result from network dysfunctions. Glycosylation insufficiencies in early development may manifest later clinically. Cortical malformations, microscopic and biochemical changes in synapses, receptors or ion channels, or disturbed neurotransmitter or energy homeostasis occur in varying combinations in different disorders. The clinical syndrome likely appears only when the network’s reserve capacity is exhausted, implying threshold effects, which could respond to therapeutic interventions without actually correcting the defect. Gross structural defects are largely untreatable, but predominantly functional disorders are more promising targets.

GLYCOSYLATION AND EPILEPSY

Deficiencies in several glycosylation pathways can cause pharmacologically manageable epilepsy and severe epileptic encephalopathies (Arranz et al. 2014, Kjaergaard et al. 2001, Martin et al. 2014, Morava et al. 2012, Wu et al. 2003). Glyco-genes associated with the latter are listed in Table 1. In dystroglycanopathies, epilepsy is likely caused by neuronal migration errors, e.g. lissencephaly (Vuillaumier-Barrot et al. 2012), since α -DG normally provides a neuronal migration stop signal. Impaired α -DG function in the developing cortex compromises the integrity of the superficial marginal zone, allowing neurons to migrate into the pial surface (Verrotti et al. 2010). Several MDDC genes have been associated with severe neuronal (over) migration disorders (Verrotti et al. 2010). However, O-mannosylation of other proteins, cadherins in particular, may also influence the cortical formation during development (Lommel et al. 2013). Furthermore, polysialic acid (PSA) is an important modification of N-glycans on NCAM, where it functions as negative regulator of NCAM during development. Abolishing PSA disturbs migration (Krocher et al. 2014, Schnaar et al. 2014) and synaptogenesis (Dityatev et al. 2004) and since the PSA is present on the N-glycans in NCAM, less N-glycosylation may yield less PSA.

Aside from cerebellar and cerebral atrophy, most CDG-patients with epilepsy do not have obvious brain malformations. The cause of epilepsy in these patients probably results from a disrupted balance of the excitatory (mainly glutaminergic) and inhibitory neuronal activity. Also, tightly regulated activation/deactivation of voltage-gated ion-channels in the cell membrane of excitable cells, including neurons, is vital for their proper function. Most of these channel proteins contain sialylated N-glycans, which are often vital for their proper function (Baycin-Hizal et al. 2014, Johnson & Bennett 2008). Lack of N-glycans can cause improper folding and transport of the proteins but changes in sialylation give a shifted gating

in the depolarized direction (Johnson & Bennett 2008). Furthermore, α -DG is upregulated in inhibitory synapses as a response to prolonged neuronal activity (Pribrag et al. 2014). RNAi-mediated knockdown of α -DG or one of the glycosyltransferases involved in the O-mannosylation of α -DG (LARGE) caused a block of the homeostatic increase in GABAergic activity, which may well explain the epilepsy seen in LARGE-deficient patients (Pribrag et al. 2014).

The nucleotide sugar transporters in the Golgi deliver activated substrates for addition of glycans in multiple pathways. Most of the transporters appear to be mono-specific while several in *C. elegans* and one in mammalian cells have a broader specificity (Caffaro et al. 2008, Hadley et al. 2014). Mutations in a UDP-GlcNAc transporter *SLC35A3* were found in one large kindred displaying epilepsy and autism spectrum disorders (Edvardson et al. 2013a). Direct assay showed reduced UDP-GlcNAc transport activity and a reduced multi-branched N-linked glycans. This is consistent with data from mammalian cells where *SLC35A3* was silenced (Maszczak-Seneczko et al. 2013). Surprisingly GAG chain heparan sulfate was not affected but keratan sulfate was. So the transporter may selectively supply UDP-GlcNAc to different pathways.

Seizures and brain malformations were also seen in a series of patients who have *de novo* mutations in the X-linked UDP-Galactose transporter, *SLC35A2* (2014, Kodera et al. 2013, Ng et al. 2013) and a review of early onset epileptic encephalopathies found that about 1% of patients examined had mutations in *SLC35A2* (Allen et al. 2013). The substrate specificity of many putative nucleotide sugar transporters remains uncharacterized.

A recent study shows that decreased localized production of the protein-free GAG hyaluronan (HA), causes spontaneous seizures. HA is very abundant in the extracellular matrix of the brain and knockout of one of the three biosynthetic genes, *Has3*, shows the greatest reduction of HA in the hippocampus. Epileptic activity in CA1 pyramidal neurons is enhanced, and a 40% reduction in volume occurs, resulting in more tightly packed neurons in the CA1 stratum pyramidale. Diffusion of fluorescent markers through the ECS of this layer was reduced, increasing the concentration of neuro transmitters. These results suggest that HA influences the size of the extracellular space. Perhaps altering the ECS volume may offer new approach for treating epilepsy (Arranz et al. 2014).

INTELLECTUAL DISABILITY AND GLYCOSYLATION

Most types of glycosylation are implicated at some point(s) during the development of the nervous system. It is not surprising that most patients with defective glycosylation, regardless of the deficient pathway, have ID (Freeze et al. 2012). In general, there is mounting evidence that defective synaptogenesis and synaptic plasticity are crucial in the development of intellectual disability (van Bokhoven 2011), especially when gross changes in the brain are absent.

Some examples where the role of glycans in ID is rather clear exist: the overmigration in α -dystroglycanopathies and importance of PSA in both neurogenesis (neurite outgrowth and axon pathfinding) and synaptogenesis/synaptic plasticity (Hildebrandt & Dityatev 2013) as described above. Interestingly, NCAM has many splice versions of which one of the three

most prominent ones, NCAM-120, is GPI anchored to the membrane (Senkov et al. 2012). A disrupted GPI anchor synthesis thus could cause a relative NCAM-120 deficiency and hence affect synaptogenesis and neurogenesis.

In *Drosophila*, N-glycosylation is vital for synaptogenesis, where the absence of hybrid and complex chains (due to MGAT1 deficiency) causes an imbalance in the bidirectional trans-synaptic signaling due to an imbalance in the lectin localization within the synaptomatrix (Parkinson et al. 2013).

GLYCOSAMINOGLYCANS IN AUTISM

A remarkable study in mice shows that an absence of GAG chains may contribute autistic behavior. The targeted loss of heparan sulfate in the brain of mice leads to autistic-like symptoms including poor social interaction, repetitive behavior and vocalization without causing morphological or cytological changes. Neuronal activation in the amygdala is attenuated after social stimuli and amygdala pyramidal neurons have reduced excitatory synaptic transmission. This is due to decreased localization AMPA-type glutamate receptors that normally bind to HS (Irie et al. 2012).

EYE DEFECTS

Neuroectoderm generates portions of the eye and visual defects are broadly represented across many different types of glycosylation disorders. Neuromuscular defects in the control of eye movements e.g., strabismus, are common in glycosylation disorders. Nystagmus, which is related to brain dysfunction or damage to the brainstem or cerebellum (Abadi 2002) is found in a high percentage of cases, e.g. *SRD5A3*-CDG have nystagmus (Cantagrel et al. 2010, Morava et al. 2010).

Retinal dystrophies and optic neuropathy sometimes present in glycosylation disorders. Interestingly, an Ashkenazi Jewish founder mutation in the dolichol synthesis gene *DHDDS* causes autosomal recessive retinitis pigmentosa as the solitary symptom (Zelinger et al. 2011, Zuchner et al. 2011).

Whether directly related to CNS damage or neuromuscular weakness, the broad consequences of defective glycosylation on the visual system are clear. Finding eye abnormalities together with ID or seizures should prompt CDG testing.

FUTURE DIRECTIONS

Improved DNA sequencing technology, informatics and falling costs will soon make exome/genome analysis routine, yielding discovery of many new genetic disorders; glycosylation disorders will be among them. Candidate gene mutations require functional confirmation, but hope for therapy requires linking gene defects to pathology. At present, few glycosylation disorders occupy that rare ground. Enhanced alliances between glycosylation specialists and neuroscientists can begin to homestead that open territory. Timely opportunities are at hand.

In the next 5 years, the BRAIN initiative aims to identify neuronal and glial cell types and generate a “parts list” of the brain. An inventory of glycans and glycan-binding proteins could refine that analysis. Microfluidic methods are in development to display glycosylation of single living cells using glycan-binding lectins (O’Connell et al. 2014). On a larger scale, printed lectin arrays and a few antibodies can interrogate the cell surface glycans. Microarrays containing over 600 printed glycans are used to identify specificity of glycan-binding proteins, viruses, or pathogens (Cummings & Pierce 2014, Smith & Cummings 2014). These methods could be adapted to analyze tagged subpopulations of brain cells.

Two programs recently joined the National Institutes of Health Common Fund, which crosses medical specialties and serves the entire biomedical research community. The first, the NIH Undiagnosed Diseases Program (UDP), will make a concerted effort to solve the most puzzling disorders. It recently invested in six sites that will collect and share clinical expertise, uniform data collection and clinical laboratory data, including genomic information. Several glycosylation disorders are already among them and, in addition, a special program is now dedicated to clinical analysis of CDG patients. The second program in the Common Fund is Glycoscience, where the focus will be on the development of broadly applicable tools that enable participation by non-specialists. The three programs can synergize to spur fundamental developments in glycobiology that target the long term goals and needs of each program.

It is apparent from Table 1 that animal models of many glycosylation disorders are needed to understand their impact in neuroscience. The next phase after the “parts list” is an action plan to test functions in the appropriate vertebrate and invertebrate models.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This work was supported by NIH grants R01DK99551 (HHF) and U54NS065768 (MCP), The Rocket Fund (HHF) and The Bertrand Might Research Fund (HHF).

LITERATURE CITED

- Abadi RV. Mechanisms underlying nystagmus. *J. R. Soc. Med.* 2002; 95:231–234. [PubMed: 11983762]
- Aebi M, Hennet T. Congenital disorders of glycosylation: genetic model systems lead the way. *Trends Cell Biol.* 2001; 11:136–141. [PubMed: 11306275]
- Agrawal P, Kurcon T, Pilobello KT, Rakus JF, Koppolu S, et al. Mapping posttranscriptional regulation of the human glycome uncovers microRNA defining the glycode. *Proc. Natl. Acad. Sci. U.S.A.* 2014; 111:4338–4343. [PubMed: 24591635]
- Allen AS, Epi KC, Epilepsy Phenome/Genome P, Berkovic SF, Cossette P, et al. De novo mutations in epileptic encephalopathies. *Nature.* 2013; 501:217–221. [PubMed: 23934111]
- Almeida AM, Murakami Y, Layton DM, Hillmen P, Sellick GS, et al. Hypomorphic promoter mutation in *PIGM* causes inherited glycosylphosphatidylinositol deficiency. *Nat. Med.* 2006; 12:846–851. [PubMed: 16767100]
- Amberger J, Bocchini C, Hamosh A. A new face and new challenges for Online Mendelian Inheritance in Man (OMIM). *Hum. Mutat.* 2011; 32:564–567. [PubMed: 21472891]

- Anonymous. Mutations in *NGLY1* gene linked with new genetic disorder: parents' reports of children's symptoms help facilitate the discovery. *Am. J. Med. Genet. A.* 2014; 164:viii–ix.
- Aronica E, van Kempen AA, van der Heide M, Poll-The BT, van Slooten HJ, et al. Congenital disorder of glycosylation type Ia: a clinicopathological report of a newborn infant with cerebellar pathology. *Acta Neuropathol.* 2005; 109:433–442. [PubMed: 15714316]
- Arranz AM, Perkins KL, Irie F, Lewis DP, Hrabe J, et al. Hyaluronan deficiency due to Has3 knock-out causes altered neuronal activity and seizures via reduction in brain extracellular space. *J. Neurosci.* 2014; 34:6164–6176. [PubMed: 24790187]
- Atwal JK, Pinkston-Gosse J, Syken J, Stawicki S, Wu Y, et al. PirB is a functional receptor for myelin inhibitors of axonal regeneration. *Science.* 2008; 322:967–970. [PubMed: 18988857]
- Barone R, Fiumara A, Jaeken J. Congenital disorders of glycosylation with emphasis on cerebellar involvement. *Semin. Neurol.* 2014; 34:357–366. [PubMed: 25192513]
- Baycin-Hizal D, Gottschalk A, Jacobson E, Mai S, Wolozny D, et al. Physiologic and pathophysiologic consequences of altered sialylation and glycosylation on ion channel function. *Biochem. Biophys. Res. Commun.* 2014
- Belaya K, Finlayson S, Slater CR, Cossins J, Liu WW, et al. Mutations in *DPAGT1* cause a limb-girdle congenital myasthenic syndrome with tubular aggregates. *Am. J. Hum. Genet.* 2012; 91:193–201. [PubMed: 22742743]
- Belet S, Fieremans N, Yuan X, Van Esch H, Verbeeck J, et al. Early frameshift mutation in *PIGA* identified in a large XLID family without neonatal lethality. *Hum. Mutat.* 2014; 35:350–355. [PubMed: 24357517]
- Boccuto L, Aoki K, Flanagan-Steet H, Chen CF, Fan X, et al. A mutation in a ganglioside biosynthetic enzyme, *ST3GAL5*, results in salt & pepper syndrome, a neurocutaneous disorder with altered glycolipid and glycoprotein glycosylation. *Hum. Mol. Genet.* 2014; 23:418–433. [PubMed: 24026681]
- Boukhris A, Schule R, Loureiro JL, Lourenco CM, Mundwiller E, et al. Alteration of ganglioside biosynthesis responsible for complex hereditary spastic paraplegia. *Am. J. Hum. Genet.* 2013; 93:118–123. [PubMed: 23746551]
- Brockhausen, I.; Schachter, H.; Stanley, P. O-GalNAc Glycans. In: Varki, A.; Cummings, RD.; Esko, JD.; Freeze, HH.; Stanley, P., et al., editors. *Essentials of Glycobiology*. Cold Spring Harbor (NY): 2009.
- Caffaro CE, Luhn K, Bakker H, Vestweber D, Samuelson J, et al. A single *Caenorhabditis elegans* Golgi apparatus-type transporter of UDP-glucose, UDP-galactose, UDP-N-acetylglucosamine, and UDP-N-acetylgalactosamine. *Biochemistry.* 2008; 47:4337–4344. [PubMed: 18341292]
- Cai M, Yang Y. Targeted genome editing tools for disease modeling and gene therapy. *Curr. Gene Ther.* 2014; 14:2–9. [PubMed: 24665839]
- Cantagrel V, Lefeber DJ, Ng BG, Guan Z, Silhavy JL, et al. *SRD5A3* is required for converting polyprenol to dolichol and is mutated in a congenital glycosylation disorder. *Cell.* 2010; 142:203–217. [PubMed: 20637498]
- Carrera IA, Matthijs G, Perez B, Cerda CP. *DPAGT1*-CDG: report of a patient with fetal hypokinesia phenotype. *Am. J. Med. Genet. A.* 2012; 158A:2027–2030. [PubMed: 22786653]
- Chiyonobu T, Inoue N, Morimoto M, Kinoshita T, Murakami Y. Glycosylphosphatidylinositol (GPI) anchor deficiency caused by mutations in *PIGW* is associated with West syndrome and hyperphosphatasia with mental retardation syndrome. *J. Med. Genet.* 2014; 51:203–207. [PubMed: 24367057]
- Cirak S, Foley AR, Herrmann R, Willer T, Yau S, et al. *ISPD* gene mutations are a common cause of congenital and limb-girdle muscular dystrophies. *Brain.* 2013; 136:269–281. [PubMed: 23288328]
- Clement E, Mercuri E, Godfrey C, Smith J, Robb S, et al. Brain involvement in muscular dystrophies with defective dystroglycan glycosylation. *Ann. Neurol.* 2008; 64:573–582. [PubMed: 19067344]
- Cline A, Gao N, Flanagan-Steet H, Sharma V, Rosa S, et al. A zebrafish model of *PMM2*-CDG reveals altered neurogenesis and a substrate-accumulation mechanism for N-linked glycosylation deficiency. *Mol. Biol. Cell.* 2012; 23:4175–4187. [PubMed: 22956764]

- Contessa JN, Bhojani MS, Freeze HH, Ross BD, Rehemtulla A, Lawrence TS. Molecular imaging of N-linked glycosylation suggests glycan biosynthesis is a novel target for cancer therapy. *Clin. Cancer Res.* 2010; 16:3205–3214. [PubMed: 20413434]
- Cossins J, Belaya K, Hicks D, Salih MA, Finlayson S, et al. Congenital myasthenic syndromes due to mutations in *ALG2* and *ALG14*. *Brain.* 2013; 136:944–956. [PubMed: 23404334]
- Cremer H, Chazal G, Goridis C, Represa A. NCAM is essential for axonal growth and fasciculation in the hippocampus. *Mol. Cell. Neurosci.* 1997; 8:323–335. [PubMed: 9073395]
- Cummings RD, Pierce JM. The challenge and promise of glycomics. *Chem. Biol.* 2014; 21:1–15. [PubMed: 24439204]
- de Koning TJ, Toet M, Dorland L, de Vries LS, van den Berg IE, et al. Recurrent nonimmune hydrops fetalis associated with carbohydrate-deficient glycoprotein syndrome. *J. Inherit. Metab. Dis.* 1998; 21:681–682. [PubMed: 9762608]
- de la Morena-Barrio ME, Hernandez-Caselles T, Corral J, Garcia-Lopez R, Martinez-Martinez I, et al. GPI-anchor and GPI-anchored protein expression in PMM2-CDG patients. *Orphanet J. Rare Dis.* 2013; 8:170. [PubMed: 24139637]
- Dejgaard K, Theberge JF, Heath-Engel H, Chevet E, Tremblay ML, Thomas DY. Organization of the Sec61 translocon, studied by high resolution native electrophoresis. *J. Proteome Res.* 2010; 9:1763–1771. [PubMed: 20112977]
- Dennis JW, Nabi IR, Demetriou M. Metabolism, cell surface organization, and disease. *Cell.* 2009; 139:1229–1241. [PubMed: 20064370]
- Dityatev A, Dityateva G, Sytnyk V, Delling M, Toni N, et al. Polysialylated neural cell adhesion molecule promotes remodeling and formation of hippocampal synapses. *J. Neurosci.* 2004; 24:9372–9382. [PubMed: 15496673]
- Ednie AR, Bennett ES. Modulation of voltage-gated ion channels by sialylation. *Compr. Physiol.* 2012; 2:1269–1301. [PubMed: 23798301]
- Edvardson S, Ashikov A, Jalas C, Sturiale L, Shaag A, et al. Mutations in *SLC35A3* cause autism spectrum disorder, epilepsy and arthrogryposis. *J. Med. Genet.* 2013a; 50:733–739. [PubMed: 24031089]
- Edvardson S, Baumann AM, Muhlenhoff M, Stephan O, Kuss AW, et al. West syndrome caused by ST3Gal-III deficiency. *Epilepsia.* 2013b; 54:e24–e27. [PubMed: 23252400]
- Ellies LG, Sperandio M, Underhill GH, Yousif J, Smith M, et al. Sialyltransferase specificity in selectin ligand formation. *Blood.* 2002; 100:3618–3625. [PubMed: 12393657]
- Engel AG, Ohno K, Sine SM. Congenital myasthenic syndromes: recent advances. *Arch. Neurol.* 1999; 56:163–167. [PubMed: 10025421]
- Engel AG, Shen XM, Selcen D, Sine S. New horizons for congenital myasthenic syndromes. *Ann. N.Y. Acad. Sci.* 2012; 1275:54–62. [PubMed: 23278578]
- Enns GM, Shashi V, Bainbridge M, Gambello MJ, Zahir FR, et al. Mutations in *NGLY1* cause an inherited disorder of the endoplasmic reticulum-associated degradation pathway. *Genet. Med.* 2014
- Esko, JD.; Kimata, K.; Lindahl, U. Proteoglycans and Sulfated Glycosaminoglycans. In: Varki, A.; Cummings, RD.; Esko, JD.; Freeze, HH.; Stanley, P., et al., editors. *Essentials of Glycobiology*. Cold Spring Harbor (NY): 2009.
- EuroEPINOMICS-RES Consortium, Epilepsy Phenome/Genome Project, Epi4K Consortium. De Novo Mutations in Synaptic Transmission Genes Including *DNM1* Cause Epileptic Encephalopathies. *Am. J. Hum. Genet.* 2014
- Ferguson, MAJ.; Kinoshita, T.; Hart, GW. Glycosylphosphatidylinositol Anchors. In: Varki, A.; Cummings, RD.; Esko, JD.; Freeze, HH.; Stanley, P., et al., editors. *Essentials of Glycobiology*. Cold Spring Harbor (NY): 2009.
- Fragaki K, Ait-El-Mkadem S, Chaussonot A, Gire C, Mengual R, et al. Refractory epilepsy and mitochondrial dysfunction due to GM3 synthase deficiency. *Eur. J. Hum. Genet.* 2013; 21:528–534. [PubMed: 22990144]
- Freeze HH. Understanding human glycosylation disorders: biochemistry leads the charge. *J. Biol. Chem.* 2013; 288:6936–6945. [PubMed: 23329837]

- Freeze HH, Chong JX, Bamshad MJ, Ng BG. Solving glycosylation disorders: fundamental approaches reveal complicated pathways. *Am. J. Hum. Genet.* 2014; 94:161–175. [PubMed: 24507773]
- Freeze HH, Eklund EA, Ng BG, Patterson MC. Neurology of inherited glycosylation disorders. *Lancet Neurol.* 2012; 11:453–466. [PubMed: 22516080]
- Freeze HH, Ng BG. Golgi glycosylation and human inherited diseases. *Cold Spring Harb. Perspect. Biol.* 2011; 3:a005371. [PubMed: 21709180]
- Gao XD, Nishikawa A, Dean N. Physical interactions between the Alg1, Alg2, and Alg11 mannosyltransferases of the endoplasmic reticulum. *Glycobiology.* 2004; 14:559–570. [PubMed: 15044395]
- Gao XD, Tachikawa H, Sato T, Jigami Y, Dean N. Alg14 recruits Alg13 to the cytoplasmic face of the endoplasmic reticulum to form a novel bipartite UDP-N-acetylglucosamine transferase required for the second step of N-linked glycosylation. *J. Biol. Chem.* 2005; 280:36254–36262. [PubMed: 16100110]
- Garshasbi M, Hadavi V, Habibi H, Kahrizi K, Kariminejad R, et al. A defect in the *TUSC3* gene is associated with autosomal recessive mental retardation. *Am. J. Hum. Genet.* 2008; 82:1158–1164. [PubMed: 18452889]
- Godfrey C, Foley AR, Clement E, Muntoni F. Dystroglycanopathies: coming into focus. *Curr. Opin. Genet. Dev.* 2011; 21:278–285. [PubMed: 21397493]
- Hadley B, Maggioni A, Ashikov A, Day CJ, Haselhorst T, Tiralongo J. Structure and function of nucleotide sugar transporters: Current progress. *Comput. Struct. Biotechnol. J.* 2014; 10:23–32. [PubMed: 25210595]
- Hakomori S. Carbohydrate-to-carbohydrate interaction, through glycosynapse, as a basis of cell recognition and membrane organization. *Glycoconj. J.* 2004; 21:125–137. [PubMed: 15483378]
- Hancock JF. GPI-anchor synthesis: Ras takes charge. *Dev. Cell.* 2004; 6:743–745. [PubMed: 15177021]
- Harlalka GV, Lehman A, Chioza B, Baple EL, Maroofian R, et al. Mutations in *B4GALNT1* (GM2 synthase) underlie a new disorder of ganglioside biosynthesis. *Brain.* 2013; 136:3618–3624. [PubMed: 24103911]
- Helander A, Stodberg T, Jaeken J, Matthijs G, Eriksson M, Eggertsen G. Dolichol kinase deficiency (DOLK-CDG) with a purely neurological presentation caused by a novel mutation. *Mol. Genet. Metab.* 2013; 110:342–344. [PubMed: 23890587]
- Hildebrandt H, Dityatev A. Polysialic Acid in Brain Development and Synaptic Plasticity. *Top. Curr. Chem.* 2013
- Hildebrandt H, Muhlenhoff M, Weinhold B, Gerardy-Schahn R. Dissecting polysialic acid and NCAM functions in brain development. *J. Neurochem.* 2007; 103(Suppl 1):56–64. [PubMed: 17986140]
- Holleboom AG, Karlsson H, Lin RS, Beres TM, Sierts JA, et al. Heterozygosity for a loss-of-function mutation in *GALNT2* improves plasma triglyceride clearance in man. *Cell Metab.* 2011; 14:811–818. [PubMed: 22152306]
- Houlden H. Defective N-linked protein glycosylation pathway in congenital myasthenic syndromes. *Brain.* 2013; 136:692–695. [PubMed: 23436500]
- Hu H, Eggers K, Chen W, Garshasbi M, Motazacker MM, et al. *ST3GAL3* mutations impair the development of higher cognitive functions. *Am. J. Hum. Genet.* 2011; 89:407–414. [PubMed: 21907012]
- Irie F, Badie-Mahdavi H, Yamaguchi Y. Autism-like socio-communicative deficits and stereotypies in mice lacking heparan sulfate. *Proc. Natl. Acad. Sci. U.S.A.* 2012; 109:5052–5056. [PubMed: 22411800]
- Jae LT, Raaben M, Riemersma M, van Beusekom E, Blomen VA, et al. Deciphering the glycosylome of dystroglycanopathies using haploid screens for lassa virus entry. *Science.* 2013; 340:479–483. [PubMed: 23519211]
- Johnson D, Bennett ES. Gating of the shaker potassium channel is modulated differentially by N-glycosylation and sialic acids. *Pflugers Arch.* 2008; 456:393–405. [PubMed: 18043943]

- Johnston JJ, Gropman AL, Sapp JC, Teer JK, Martin JM, et al. The phenotype of a germline mutation in *PIGA*: the gene somatically mutated in paroxysmal nocturnal hemoglobinuria. *Am. J. Hum. Genet.* 2012; 90:295–300. [PubMed: 22305531]
- Kasper BT, Koppolu S, Mahal LK. Insights into miRNA regulation of the human glycome. *Biochem. Biophys. Res. Commun.* 2014; 445:774–779. [PubMed: 24463102]
- Kato M, Saitsu H, Murakami Y, Kikuchi K, Watanabe S, et al. *PIGA* mutations cause early-onset epileptic encephalopathies and distinctive features. *Neurology.* 2014; 82:1587–1596. [PubMed: 24706016]
- Kim S, Westphal V, Srikrishna G, Mehta DP, Peterson S, et al. Dolichol phosphate mannose synthase (DPM1) mutations define congenital disorder of glycosylation Ia (CDG-Ia). *J. Clin. Invest.* 2000; 105:191–198. [PubMed: 10642597]
- Kiwamoto T, Brummet ME, Wu F, Motari MG, Smith DF, et al. Mice deficient in the St3gal3 gene product alpha2,3 sialyltransferase (ST3Gal-III) exhibit enhanced allergic eosinophilic airway inflammation. *J. Allergy Clin. Immunol.* 2014; 133:240–247. e1-3. [PubMed: 23830412]
- Kizuka Y, Kitazume S, Okahara K, Villagra A, Sotomayor EM, Taniguchi N. Epigenetic regulation of a brain-specific glycosyltransferase N-acetylglucosaminyltransferase-IX (GnT-IX) by specific chromatin modifiers. *J. Biol. Chem.* 2014; 289:11253–11261. [PubMed: 24619417]
- Kjaergaard S, Schwartz M, Skovby F. Congenital disorder of glycosylation type Ia (CDG-Ia): phenotypic spectrum of the R141H/F119L genotype. *Arch. Dis. Child.* 2001; 85:236–239. [PubMed: 11517108]
- Kodera H, Nakamura K, Osaka H, Maegaki Y, Haginoya K, et al. De novo mutations in *SLC35A2* encoding a UDP-galactose transporter cause early-onset epileptic encephalopathy. *Hum. Mutat.* 2013; 34:1708–1714. [PubMed: 24115232]
- Kong Y, Joshi HJ, Schjoldager KT, Madsen TD, Gerken TA, et al. Probing Polypeptide GalNAc-Transferase Isoform Substrate Specificities by In Vitro Analysis. *Glycobiology.* 2014
- Kranz C, Sun L, Eklund EA, Krasnewich D, Casey JR, Freeze HH. CDG-Id in two siblings with partially different phenotypes. *Am. J. Med. Genet. A.* 2007; 143A:1414–1420. [PubMed: 17551933]
- Krocher T, Rockle I, Diederichs U, Weinhold B, Burkhardt H, et al. A crucial role for polysialic acid in developmental interneuron migration and the establishment of interneuron densities in the mouse prefrontal cortex. *Development.* 2014; 141:3022–3032. [PubMed: 24993945]
- Kuki I, Takahashi Y, Okazaki S, Kawawaki H, Ehara E, et al. Vitamin B6-responsive epilepsy due to inherited GPI deficiency. *Neurology.* 2013; 81:1467–1469. [PubMed: 24049131]
- Lander AD. Morpheus unbound: reimagining the morphogen gradient. *Cell.* 2007; 128:245–256. [PubMed: 17254964]
- Lefebvre DJ, Schonberger J, Morava E, Guillard M, Huyben KM, et al. Deficiency of Dol-P-Man synthase subunit DPM3 bridges the congenital disorders of glycosylation with the dystroglycanopathies. *Am. J. Hum. Genet.* 2009; 85:76–86. [PubMed: 19576565]
- Li H, Chavan M, Schindelin H, Lennarz WJ, Li H. Structure of the oligosaccharyl transferase complex at 12 Å resolution. *Structure.* 2008; 16:432–440. [PubMed: 18334218]
- Lommel M, Winterhalter PR, Willer T, Dahlhoff M, Schneider MR, et al. Protein O-mannosylation is crucial for E-cadherin-mediated cell adhesion. *Proc. Natl. Acad. Sci. U.S.A.* 2013; 110:21024–21029. [PubMed: 24297939]
- Losfeld ME, Ng BG, Kircher M, Buckingham KJ, Turner EH, et al. A new congenital disorder of glycosylation caused by a mutation in *SSR4*, the signal sequence receptor 4 protein of the TRAP complex. *Hum. Mol. Genet.* 2014; 23:1602–1605. [PubMed: 24218363]
- Lu J, Takahashi T, Ohoka A, Nakajima K, Hashimoto R, et al. Alg14 organizes the formation of a multiglycosyltransferase complex involved in initiation of lipid-linked oligosaccharide biosynthesis. *Glycobiology.* 2012; 22:504–516. [PubMed: 22061998]
- Martin HC, Kim GE, Pagnamenta AT, Murakami Y, Carvill GL, et al. Clinical whole-genome sequencing in severe early-onset epilepsy reveals new genes and improves molecular diagnosis. *Hum. Mol. Genet.* 2014; 23:3200–3211. [PubMed: 24463883]
- Martin PT. The dystroglycanopathies: the new disorders of O-linked glycosylation. *Semin. Pediatr. Neurol.* 2005; 12:152–158. [PubMed: 16584074]

- Maszczyk-Seneczko D, Sosicka P, Olczak T, Jakimowicz P, Majkowski M, Olczak M. UDP-N-acetylglucosamine transporter (SLC35A3) regulates biosynthesis of highly branched N-glycans and keratan sulfate. *J. Biol. Chem.* 2013; 288:21850–21860. [PubMed: 23766508]
- Maydan G, Noyman I, Har-Zahav A, Neria ZB, Pasmanik-Chor M, et al. Multiple congenital anomalies-hypotonia-seizures syndrome is caused by a mutation in *PIGN*. *J. Med. Genet.* 2011; 48:383–389. [PubMed: 21493957]
- Michaud JL, Lachance M, Hamdan FF, Carmant L, Lortie A, et al. The genetic landscape of infantile spasms. *Hum. Mol. Genet.* 2014; 23:4846–4858. [PubMed: 24781210]
- Might M, Wilsey M. The shifting model in clinical diagnostics: how next-generation sequencing and families are altering the way rare diseases are discovered, studied, and treated. *Genet. Med.* 2014
- Mohorko E, Owen RL, Malojcic G, Brozzo MS, Aebi M, Glockshuber R. Structural basis of substrate specificity of human oligosaccharyl transferase subunit N33/Tusc3 and its role in regulating protein N-glycosylation. *Structure.* 2014; 22:590–601. [PubMed: 24685145]
- Molinari F, Foulquier F, Tarpey PS, Morelle W, Boissel S, et al. Oligosaccharyltransferase-subunit mutations in nonsyndromic mental retardation. *Am. J. Hum. Genet.* 2008; 82:1150–1157. [PubMed: 18455129]
- Morava E, Vodopitiz J, Lefeber DJ, Janecke AR, Schmidt WM, et al. Defining the phenotype in congenital disorder of glycosylation due to *ALG1* mutations. *Pediatrics.* 2012; 130:e1034–e1039. [PubMed: 22966035]
- Morava E, Wevers RA, Cantagrel V, Hoefsloot LH, Al-Gazali L, et al. A novel cerebello-ocular syndrome with abnormal glycosylation due to abnormalities in dolichol metabolism. *Brain.* 2010; 133:3210–3220. [PubMed: 20852264]
- Muppidi, S.; Wolfe, GI.; Barohn, RJ. *Pediatric Neurology.* Elsevier; 2012. Disease of the Neuromuscular Junction; p. 1549-1569.
- Ng BG, Buckingham KJ, Raymond K, Kircher M, Turner EH, et al. Mosaicism of the UDP-galactose transporter SLC35A2 causes a congenital disorder of glycosylation. *Am. J. Hum. Genet.* 2013; 92:632–636. [PubMed: 23561849]
- Ng BG, Freeze HH. Human genetic disorders involving glycosylphosphatidylinositol (GPI) anchors and glycosphingolipids (GSL). *J. Inherit. Metab. Dis.* 2014
- NIH. Brain Research through Advancing Innovative Neurotechnologies (BRAIN). <http://www.nih.gov/science/brain/>
- Nowycky MC, Wu G, Ledeen RW. Glycobiology of ion transport in the nervous system. *Adv. Neurobiol.* 2014; 9:321–342. [PubMed: 25151386]
- O'Connell TM, King D, Dixit CK, O'Connor B, Walls D, Ducree J. Sequential glycan profiling at single cell level with the microfluidic lab-in-a-trench platform: a new era in experimental cell biology. *Lab Chip.* 2014; 14:3629–3639. [PubMed: 25080365]
- Orr SL, Le D, Long JM, Sobieszczuk P, Ma B, et al. A phenotype survey of 36 mutant mouse strains with gene-targeted defects in glycosyltransferases or glycan-binding proteins. *Glycobiology.* 2013; 23:363–380. [PubMed: 23118208]
- Parker CJ. Paroxysmal nocturnal hemoglobinuria. *Curr. Opin. Hematol.* 2012; 19:141–148. [PubMed: 22395662]
- Parkinson W, Dear ML, Rushton E, Broadie K. N-glycosylation requirements in neuromuscular synaptogenesis. *Development.* 2013; 140:4970–4981. [PubMed: 24227656]
- Pedersen ME, Snieckute G, Kagias K, Nehammer C, Mulhaupt HA, et al. An epidermal microRNA regulates neuronal migration through control of the cellular glycosylation state. *Science.* 2013; 341:1404–1408. [PubMed: 24052309]
- Pernet V, Schwab ME. The role of Nogo-A in axonal plasticity, regrowth and repair. *Cell Tissue Res.* 2012; 349:97–104. [PubMed: 22588543]
- Praissman JL, Wells L. Mammalian O-mannosylation pathway: glycan structures, enzymes, and protein substrates. *Biochemistry.* 2014; 53:3066–3078. [PubMed: 24786756]
- Pribiag H, Peng H, Shah WA, Stellwagen D, Carbonetto S. Dystroglycan mediates homeostatic synaptic plasticity at GABAergic synapses. *Proc. Natl. Acad. Sci. U.S.A.* 2014; 111:6810–6815. [PubMed: 24753587]

- Rosen CL, Lisanti MP, Salzer JL. Expression of unique sets of GPI-linked proteins by different primary neurons in vitro. *J. Cell Biol.* 1992; 117:617–627. [PubMed: 1349305]
- Schenk B, Imbach T, Frank CG, Grubenmann CE, Raymond GV, et al. MPDU1 mutations underlie a novel human congenital disorder of glycosylation, designated type If. *J. Clin. Invest.* 2001; 108:1687–1695. [PubMed: 11733564]
- Schjoldager KT, Clausen H. Site-specific protein O-glycosylation modulates proprotein processing - deciphering specific functions of the large polypeptide GalNAc-transferase gene family. *Biochim. Biophys. Acta.* 2012; 1820:2079–2094. [PubMed: 23022508]
- Schjoldager KT, Vakhrushev SY, Kong Y, Steentoft C, Nudelman AS, et al. Probing isoform-specific functions of polypeptide GalNAc-transferases using zinc finger nuclease glycoengineered SimpleCells. *Proc. Natl. Acad. Sci. U.S.A.* 2012; 109:9893–9898. [PubMed: 22566642]
- Schnaar RL, Gerardy-Schahn R, Hildebrandt H. Sialic acids in the brain: gangliosides and polysialic acid in nervous system development, stability, disease, and regeneration. *Physiol. Rev.* 2014; 94:461–518. [PubMed: 24692354]
- Schnaar, RL.; Suzuki, A.; Stanley, P. Glycosphingolipids. In: Varki, A.; Cummings, RD.; Esko, JD.; Freeze, HH.; Stanley, P., et al., editors. *Essentials of Glycobiology*. Cold Spring Harbor (NY): 2009.
- Senderek J, Muller JS, Dusl M, Strom TM, Guergueltcheva V, et al. Hexosamine biosynthetic pathway mutations cause neuromuscular transmission defect. *Am. J. Hum. Genet.* 2011; 88:162–172. [PubMed: 21310273]
- Senkov O, Tikhobrazova O, Dityatev A. PSA-NCAM: synaptic functions mediated by its interactions with proteoglycans and glutamate receptors. *Int. J. Biochem. Cell Biol.* 2012; 44:591–595. [PubMed: 22300986]
- Sharma V, Ichikawa M, Freeze HH. Mannose metabolism: More than meets the eye. *Biochem. Biophys. Res. Commun.* 2014
- Shi Z, Ruvkun G. The mevalonate pathway regulates microRNA activity in *Caenorhabditis elegans*. *Proc. Natl. Acad. Sci. U.S.A.* 2012; 109:4568–4573. [PubMed: 22396595]
- Shibatani T, David LL, McCormack AL, Frueh K, Skach WR. Proteomic analysis of mammalian oligosaccharyltransferase reveals multiple subcomplexes that contain Sec61, TRAP, and two potential new subunits. *Biochemistry.* 2005; 44:5982–5992. [PubMed: 15835887]
- Simpson MA, Cross H, Proukakis C, Priestman DA, Neville DC, et al. Infantile-onset symptomatic epilepsy syndrome caused by a homozygous loss-of-function mutation of GM3 synthase. *Nat. Genet.* 2004; 36:1225–1229. [PubMed: 15502825]
- Smith DF, Cummings RD. Investigating virus-glycan interactions using glycan microarrays. *Curr. Opin. Virol.* 2014; 7C:79–87. [PubMed: 24995558]
- Spiro RG. Protein glycosylation: nature, distribution, enzymatic formation, and disease implications of glycopeptide bonds. *Glycobiology.* 2002; 12:43R–56R.
- Stalnakier SH, Aoki K, Lim JM, Porterfield M, Liu M, et al. Glycomic analyses of mouse models of congenital muscular dystrophy. *J. Biol. Chem.* 2011; 286:21180–21190. [PubMed: 21460210]
- Stanley, P.; Schachter, H.; Taniguchi, N. N-Glycans. In: Varki, A.; Cummings, RD.; Esko, JD.; Freeze, HH.; Stanley, P., et al., editors. *Essentials of Glycobiology*. Cold Spring Harbor (NY): 2009.
- Steentoft C, Bennett EP, Schjoldager KT, Vakhrushev SY, Wandall HH, Clausen H. Precision genome editing: a small revolution for glycobiology. *Glycobiology.* 2014; 24:663–680. [PubMed: 24861053]
- Sun L, Zhao Y, Zhou K, Freeze HH, Zhang YW, Xu H. Insufficient ER-stress response causes selective mouse cerebellar granule cell degeneration resembling that seen in congenital disorders of glycosylation. *Mol. Brain.* 2013; 6:52. [PubMed: 24305089]
- Swoboda KJ, Margraf RL, Carey JC, Zhou H, Newcomb TM, et al. A novel germline PIGA mutation in Ferro-Cerebro-Cutaneous syndrome: a neurodegenerative X-linked epileptic encephalopathy with systemic iron-overload. *Am. J. Med. Genet. A.* 2014; 164A:17–28. [PubMed: 24259288]
- Takamiya K, Yamamoto A, Furukawa K, Yamashiro S, Shin M, et al. Mice with disrupted GM2/GD2 synthase gene lack complex gangliosides but exhibit only subtle defects in their nervous system. *Proc. Natl. Acad. Sci. U.S.A.* 1996; 93:10662–10667. [PubMed: 8855236]

- Tegtmeyer LC, Rust S, van Scherpenzeel M, Ng BG, Losfeld ME, et al. Multiple phenotypes in phosphoglucomutase 1 deficiency. *N. Engl. J. Med.* 2014; 370:533–542. [PubMed: 24499211]
- Tennant KA. Thinking outside the brain: structural plasticity in the spinal cord promotes recovery from cortical stroke. *Exp. Neurol.* 2014; 254:195–199. [PubMed: 24518486]
- Tettamanti G, Bonali F, Marchesini S, Zambotti V. A new procedure for the extraction, purification and fractionation of brain gangliosides. *Biochim. Biophys. Acta.* 1973; 296:160–170. [PubMed: 4693502]
- Thompson MD, Killoran A, Percy ME, Nezarati M, Cole DE, Hwang PA. Hyperphosphatasia with neurologic deficit: a pyridoxine-responsive seizure disorder? *Pediatr. Neurol.* 2006; 34:303–307. [PubMed: 16638507]
- van Bokhoven H. Genetic and epigenetic networks in intellectual disabilities. *Annu. Rev. Genet.* 2011; 45:81–104. [PubMed: 21910631]
- van der Crabben SN, Harakalova M, Brilstra EH, van Berkestijn FM, Hofstede FC, et al. Expanding the spectrum of phenotypes associated with germline PIGA mutations: a child with developmental delay, accelerated linear growth, facial dysmorphisms, elevated alkaline phosphatase, and progressive CNS abnormalities. *Am. J. Med. Genet. A.* 2014; 164A:29–35. [PubMed: 24259184]
- Varki A. Biological roles of oligosaccharides: all of the theories are correct. *Glycobiology.* 1993; 3:97–130. [PubMed: 8490246]
- Varki, A.; Sharon, N. Historical Background and Overview. In: Varki, A.; Cummings, RD.; Esko, JD.; Freeze, HH.; Stanley, P., et al., editors. *Essentials of Glycobiology*. Cold Spring Harbor NY: The Consortium of Glycobiology Editors, La Jolla, California; 2009.
- Verrotti A, Spalice A, Ursitti F, Papetti L, Mariani R, et al. New trends in neuronal migration disorders. *Eur. J. Paediatr. Neurol.* 2010; 14:1–12. [PubMed: 19264520]
- Vester-Christensen MB, Halim A, Joshi HJ, Steentoft C, Bennett EP, et al. Mining the O-mannose glycoproteome reveals cadherins as major O-mannosylated glycoproteins. *Proc. Natl. Acad. Sci. U.S.A.* 2013; 110:21018–21023. [PubMed: 24101494]
- Vuillaumier-Barrot S, Bouchet-Seraphin C, Chelbi M, Devisme L, Quentin S, et al. Identification of mutations in TMEM5 and ISPD as a cause of severe cobblestone lissencephaly. *Am. J. Hum. Genet.* 2012; 91:1135–1143. [PubMed: 23217329]
- Wakil SM, Monies DM, Ramzan K, Hagos S, Bastaki L, et al. Novel B4GALNT1 mutations in a complicated form of hereditary spastic paraplegia. *Clin. Genet.* 2013
- Wang Y, Schachter H, Marth JD. Mice with a homozygous deletion of the Mgat2 gene encoding UDP-N-acetylglucosamine:alpha-6-D-mannoside beta1,2-N-acetylglucosaminyltransferase II: a model for congenital disorder of glycosylation type IIa. *Biochim. Biophys. Acta.* 2002; 1573:301–311. [PubMed: 12417412]
- Willett R, Ungar D, Lupashin V. The Golgi puppet master: COG complex at center stage of membrane trafficking interactions. *Histochem. Cell Biol.* 2013; 140:271–283. [PubMed: 23839779]
- Wills ZP, Mandel-Brehm C, Mardinly AR, McCord AE, Giger RJ, Greenberg ME. The nogo receptor family restricts synapse number in the developing hippocampus. *Neuron.* 2012; 73:466–481. [PubMed: 22325200]
- Wu X, Rush JS, Karaoglu D, Krasnewich D, Lubinsky MS, et al. Deficiency of UDP-GlcNAc:Dolichol Phosphate N-Acetylglucosamine-1 Phosphate Transferase (DPAGT1) causes a novel congenital disorder of Glycosylation Type Ij. *Hum. Mutat.* 2003; 22:144–150. [PubMed: 12872255]
- Yoshida-Moriguchi T, Yu L, Stalnaker SH, Davis S, Kunz S, et al. O-mannosyl phosphorylation of alpha-dystroglycan is required for laminin binding. *Science.* 2010; 327:88–92. [PubMed: 20044576]
- Zelinger L, Banin E, Obolensky A, Mizrahi-Meissonnier L, Beryozkin A, et al. A missense mutation in DHDDS, encoding dehydrodolichyl diphosphate synthase, is associated with autosomal-recessive retinitis pigmentosa in Ashkenazi Jews. *Am. J. Hum. Genet.* 2011; 88:207–215. [PubMed: 21295282]
- Zhang YT, Lander AD, Nie Q. Computational analysis of BMP gradients in dorsal-ventral patterning of the zebrafish embryo. *J. Theor. Biol.* 2007; 248:579–589. [PubMed: 17673236]

- Zhou H, Clapham DE. Mammalian MagT1 and TUSC3 are required for cellular magnesium uptake and vertebrate embryonic development. *Proc. Natl. Acad. Sci. U.S.A.* 2009; 106:15750–15755. [PubMed: 19717468]
- Zielinska DF, Gnad F, Wisniewski JR, Mann M. Precision mapping of an in vivo N-glycoproteome reveals rigid topological and sequence constraints. *Cell*. 2010; 141:897–907. [PubMed: 20510933]
- Zoltowska K, Webster R, Finlayson S, Maxwell S, Cossins J, et al. Mutations in *GFPT1* that underlie limb-girdle congenital myasthenic syndrome result in reduced cell-surface expression of muscle AChR. *Hum. Mol. Genet.* 2013; 22:2905–2913. [PubMed: 23569079]
- Zuchner S, Dallman J, Wen R, Beecham G, Naj A, et al. Whole-exome sequencing links a variant in *DHDDS* to retinitis pigmentosa. *Am. J. Hum. Genet.* 2011; 88:201–206. [PubMed: 21295283]

Figure 1. Protein complexes in the early steps of lipid linked oligosaccharide (LLO) synthesis
Initial steps in the synthesis of LLO glycan require UDP-GlcNAc generated from fructose-6-P. ALG7 (DPAGT1) adds the first GlcNAc-1-P to dolichol-P and a complex of ALG13 and ALG14 add the next GlcNAc. ALG7 and ALG13/14 exist as a complex and some myasthenia patients have mutations in these genes. The first five Man units are added by a complex of ALG1, ALG2 and ALG11 and other myasthenia patients have mutations in ALG2. In addition to the severity of gene mutations, the integrity, localization and distribution of these complexes may determine whether patients exhibit severe CDG or milder myasthenic condition.

Table 1**Human Glycosylation Disorders and Verified Model Systems**

Pathway *	Human Disorders	Defects Validated in Model Systems **			
		<i>M. musculus</i>	<i>D. rerio</i>	<i>D. melanogaster</i>	<i>C. elegans</i>
N-Linked	28	6	4	2	2
Multiple	33	12	8	2	1
GPI Anchor	12	5	2	0	1
O-Man	14	10	12	2	0
O-Xyl (GAG)	13	8	3	2	2
Other O-linked	7	4	2	4	0
GSL	3	3	2	0	0

* Total number of glycosylation disorders indicated by pathways affected.

** The number of models for each pathway and organism is based on functional analysis of an impaired gene. References were derived from OMIM and NCBI-PubMed.

Table 2

Early Onset Epileptic Encephalopathies (EOEE) associated with deficient glycosylation

	Glyco pathway	Relevant references
<i>Ohtahara</i>		
<i>PIGA</i>	GPI anchor	(Kato et al. 2014)
<i>PIGQ</i>	GPI anchor	(Martin et al. 2014)
<i>Early myoclonic encephalopathy/thies</i>		
<i>PIGA</i>	GPI anchor	(Kato et al. 2014)
<i>Epileptic spasms/West syndrome</i>		
<i>ALG13</i>	N-linked	(Allen et al. 2013, Michaud et al. 2014)
<i>DOLK</i>	Multiple	(Helander et al. 2013)
<i>DPAGT1</i>	N-linked	(Wu et al. 2003)
<i>SLC35A2</i>	Multiple	(Kodera et al. 2013)
<i>ST3GAL3</i>	Multiple	(Edvardson et al. 2013b)
<i>PIGA</i>	GPI anchor	(Kato et al. 2014)
<i>PIGW</i>	GPI anchor	(Chiyonobu et al. 2014)
<i>ST3GAL5</i>	GPI anchor	(Simpson et al. 2004)
<i>EOEE non-specified</i>		
<i>ALG1</i>	N-linked	(de Koning et al. 1998)
<i>ALG3</i>	N-linked	(Kranz et al. 2007)
<i>MPDU1</i>	N-linked	(Schenk et al. 2001)
<i>DPM1</i>	Multiple	(Kim et al. 2000)
<i>PIGA</i>	GPI anchor	(Kato et al. 2014)
<i>PIGN</i>	GPI anchor	(Maydan et al. 2011)
<i>ST3GAL5</i>	Glycosphingolipid	(Fragaki et al. 2013)