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Perspectives on Glycosylation and its Congenital Disorders

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Abstract

Congenital disorders of glycosylation (CDG) are a rapidly expanding group of metabolic disorders that result from abnormal protein or lipid glycosylation. They are often difficult to clinically diagnose because they broadly affect many organs and functions and lack clinical uniformity. However, recent technological advances in next generation sequencing have revealed a treasure trove of new genetic disorders, expanded the knowledge of known disorders, and showed a critical role in infectious diseases. More comprehensive genetic tools specifically tailored for mammalian cell-based models have revealed a critical role for glycosylation in pathogen-host interactions, while also identifying new CDG susceptibility genes. We highlight recent advancements that have resulted in a better understanding of human glycosylation disorders, perspectives for potential future therapies and mysteries that continue to seek new insights for their solution.

Keywords

Glycosylation; Next generation sequencing

What 30 years of genetics has taught us about glycosylation

Glycosylation (see glossary) is an essential cellular process where individual monosaccharides are uniquely arranged to form the most expansive and diverse type of protein or lipid modifications across all species [1]. It is conservatively estimated that ~2% of all genes in the human genome encode for proteins involved in various aspects of glycosylation and that half of all cellular proteins are directly glycosylated in some form [2]. Thus, it is not surprising that more than 125 genetic disorders have been identified across several glycosylation pathways including N-linked, O-linked (O-Mannose, O-Glucose, O-Fucose, O-GlcNAc, O-GalNAc), glycosaminoglycan (GAG), glycosylphosphatidylinositol (GPI) and glycolipids [3,4] (Figure 1, Table 1). Adding further complexity are disorders with unanticipated roles in glycosylation, such as ER signal peptide recognition, vesicular transport, Golgi architecture and homeostasis to name a few [3,5] (Figure 2).

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Because glycosylation occurs in every cell in every organism, it is not surprising that patients with glycosylation defects show highly diverse clinical presentations that seldom generate genetic identity. This, plus the complexity of glycosylation itself, may account for the longevity and hesitation of working on glycobiology; what some have termed as “Glycophobia”. The recent arrival of **Next Generation Sequencing** (NGS) has rapidly expanded both the discovery of novel glycosylation-related disorders and the unraveling of many unsolved cases. Ultimately, the likelihood of a clinician encountering a patient with a glycosylation disorder has increased propelling glycobiology to a more pronounced position. Because of the rapid expansion of disorders across several unique pathways, we often use both congenital disorders of glycosylation (CDG) and glycosylation related disorders to describe a genetic disorder whose primary defect impacts one or more glycosylation pathways. Here we will explore new developments in the field of glycosylation disorders, perspectives on therapies and attempt to highlight unanswered questions that remain in this expanding field.

How Next-Generation sequencing propelled the CDG field

The first genetic disorder definitively linked to a specific gene via **Whole Exome Sequencing** (WES) occurred in early 2010 with the finding that Miller syndrome was caused by mutations in *DHODH*, a key enzyme in the pyrimidine *de novo* biosynthesis pathway [6]. Since then, broader accessibility and higher rates of conclusive diagnosis have made WES a preferred method for solving undiagnosed genetic disorders [7,8]. Prior to expansion by WES, there were approximately sixty glycosylation disorders with most having been solved by painstaking biochemical trial and error or individual gene sequencing (Figure 1). However, shortly thereafter, the first glycosylation-related disorder (PIGV-CDG) was solved using WES as the cause of Hyperphosphatasia with mental retardation syndrome 1 [9]. Fast forward to today, nearly half of the 128 glycosylation-related disorders discovered since 2010, have been identified by exome sequencing [10] (Table 1).

The emergence of pathogenic *de novo* mutations in CDG

Most glycosylation disorders follow an autosomal recessive inheritance pattern; however, exome sequencing using family based trios has helped to shed light on how *de novo* mutations can also result in various forms of CDG. The first gene to be identified with confirmed pathogenic *de novo* mutations was the X-linked *ALG13*, which is primarily associated with epilepsy [11]. Since the initial single case report, nearly all subsequently identified cases have been affected females with the same specific recurrent *de novo* mutation, c.320A>G (p.Asn107Ser) [12–18]. However, an affected male who was hemizygous for the c.320A>G (p.Asn107Ser) was recently identified with a clinical phenotype similar to previously reported affected females [19]. Since the Asn107 is critical for the binding of ALG13 to its substrate, UDP-GlcNAc, this mutation was thought to result in a complete loss of function [20], however the presence of an affected hemizygous male suggests that the p.Asn107Ser likely has some residual activity. Is it possible that this or other individuals with ALG13-CDG have varying degrees of mosaicism or chimerism and this accounts for why glycosylation is “normal” within these cases? This has yet to be addressed.

Surprisingly, when the abundant serum glycoprotein transferrin (Box 1), a biomarker for many types of CDG was tested in p.Asn107Ser ALG13-CDG females, it revealed normal glycosylation and only minor glycosylation abnormalities were seen in the affected male [15,19]. This is curious since *ALG13* encodes an essential glycosyltransferase required for the second step of **lipid linked oligosaccharide (LLO)** synthesis and has long been associated with N-linked glycosylation [21,22] (Figure 2). One explanation could be that in higher eukaryotes, *ALG13* may have functionally evolved since it differs significantly from lower eukaryotic organisms such as the yeast strain *Saccharomyces cerevisiae*. In *S. cerevisiae*, the *ALG13* gene encodes a small molecular weight protein of approximately 200 amino acids, however at some point in evolution *ALG13* underwent a fusion event to form an approximately 1100 amino acid protein that now contains several other functional domains outside the highly-conserved glycosyltransferase domain [23]. Could it be that in higher eukaryotes ALG13 has evolved additional functions outside its annotated role in glycosylation? Or could the several different isoforms expressed in humans possess vastly different functions? These are questions that remain a mystery. Interestingly, in zebrafish this fusion event did not occur since there are two separate genes that align to the single human gene (<https://zfin.org/>). This could make zebrafish a useful model for studying the function of ALG13.

It is of interest that a few reports have described individuals with a classical X-linked form of ALG13-CDG [24,25], however upon closer inspection these variants are present in the hemizygous state in several individuals from the **gnomAD-ExAC database** (<http://gnomad.broadinstitute.org/gene/ENSG00000101901>). Since the gnomAD-ExAC database has “removed individuals affected by severe pediatric disease” it seems unlikely that these variants are truly pathogenic. This finding underscores the importance of having an independent functional assay to assess pathogenicity.

De novo mutations in *SLC35A2*, which in humans encodes the major uridine diphosphate (UDP)-galactose transporter in the Golgi, are associated with another CDG originally described in three unrelated individuals (2 males and 1 female) [26] (Figure 2). What makes *SLC35A2*-CDG unusual is that, *SLC35A2* is essential for transporting UDP-galactose into the Golgi and ultimately affects several types of glycosylation, yet nearly all patients who were tested show normal glycosylation [27,28]. A few affected individuals have shown severely abnormal galactosylation early in life that later became completely normal [26]. Once again, the question of why the loss of a key glycosylation transporter results in such varied glycosylation status remains a mystery. It is possible that the UDP-GlcNAc transporter, *SLC35A3*, could have a broader specificity and potentially compensate for the loss of *SLC35A2* in certain tissue types. This substrate recognition and compensation by *SLC35A2*-*SLC35A3* chimera proteins has been seen in cell based models [29]. Alternatively, the normalization of transferrin and serum glycoproteins from the liver in some affected individuals could be explained by the positive selection for the wild-type *SLC35A2* allele [26].

Identification of CDG genes that cause divergent disorders

It is not unheard of for different mutations within a specific gene to cause distinct clinical disorders that can also vary in their inheritance patterns. Until recently, this was seldom seen for CDG. Perhaps the best example involves the *PIGA* gene, which encodes a protein required for the synthesis of N-acetylglucosaminyl phosphatidylinositol (GlcNAc-PI), the first step in GPI anchor biosynthesis [30]. Somatic mutations in *PIGA* can cause paroxysmal nocturnal hemoglobinuria, an acquired hematologic disorder characterized by hemolytic anemia, thrombosis and expansion of hematopoietic stem cells expressing the mutant *PIGA*. However, X-linked forms that present with severe neurological and frequent lethality also exist [31–35].

It was recently reported that recurrent *de novo* mutations within *DHDDS* and *NUS1* can cause developmental and epileptic encephalopathies [36]. *DHDDS* and *NUS1*, interact and play pivotal roles in the earliest part of the LLO pathway (Figure 2, Key Figure), and have already been shown to cause multiple recessive disorders [37–40]. In fact, *DHDDS* has now been shown to cause three clinically distinct disorders. The first, is due to a founder mutation c.124A>G (p.Lys42Glu) within the Ashkenazi Jewish population that causes an autosomal recessive form of retinitis pigmentosa [37,38], the second is a lethal autosomal recessive form that presents with a more traditional multisystem involvement CDG phenotype [39] and the third is due to *de novo* mutations causing a developmental and epileptic encephalopathy disorder [36]. These highly divergent presentations are unlikely to be isolated occurrences and more examples of this will likely appear as more affected individuals and trios undergo in-depth sequencing analysis.

In fact, we are already seeing this occur in other LLO pathway genes (*DPAGT1*, *ALG14*, *ALG2*) that can cause a severe form of CDG, but also a milder form of congenital myasthenic syndrome [41–45]. It remains unclear why mutations in this set of genes cause these two specific and very different disorders. It is known the early LLO biosynthesis enzymes do form specific protein complexes and we speculate that it may possible certain mutations can dramatically affect enzyme activity while other less severe mutations, alter complex formation.

Expanding the molecular toolbox for glycobiology

For decades, most genetic analysis of glycosylation was done in *Saccharomyces cerevisiae* using phenotypic mutants [46–48]. However, this work focused primarily on the early N-linked and Golgi processing pathways [46,49]. The major downside to using *Saccharomyces cerevisiae* is that it does not have machinery for N-glycan branching, galactosylation, sialylation or fucosylation pathways to name a few [46]. Therefore, a mammalian cell equivalent to *S.cerevisiae* was needed. Chinese hamster ovary (CHO) mutant cell lines have provided an immense wealth of knowledge about specific glycosylation pathways and their proteins, but isolating and identifying the mutated genes can often be painstaking [50].

Recently, technological advances have been made in developing comprehensive whole genome shRNA and CRISPR/CAS9 libraries for such screens. As a proof of principle,

several independent groups employed multiple screening methods (RNAi, Gene-Trap and CRISPR) to identify genes required for cellular resistance to the toxicity of the lectin ricin [51–54]. Importantly, while different methods were used, each study identified similar factors.

Since then, several genetic screens have been applied to identifying key host factors for several infectious agents. One example of such a genetic screen involved taking advantage of the pathogen, Lassa virus (LASV), which requires properly glycosylated α -dystroglycan (α -DG) as a ligand for viral entry [55]. α -DG is an extensively glycosylated extracellular peripheral protein that contains unique O-Mannose based glycans, which are critical for linking the extracellular matrix with the cytoskeleton [56]. An inability to properly assemble these O-Mannose glycans on α -DG results in a group of 17 glycosylation-related disorders termed “**Dystroglycanopathies**” [57–59]. Prior to the LASV screen several key steps in the assembly of the O-Mannose glycan had not been identified and in fact the complete structure of the glycan was still a mystery. Yet the screen identified, what is believed to be, every component required to make the functional O-Mannose glycans. Importantly, the screen identified established dystroglycanopathy genes (*DAG1, LARGE, FKRP, FKTN, POMT1, POMT2, POMGNT1, B3GALNT2*) but also revealed several potential candidate genes, that would later be found to cause various types of dystroglycanopathy (*ISPD, TMEM5, POMGNT2, B4GAT1, POMK*) [60–65] (Table 2). Since dolichol phosphate mannose (Dol-P Man) is required to generate O-Mannose glycans, it is not surprising that the several established CDG genes were identified (i.e. *PMM2, MPDU1, DPM1,3*) [56] (Table 2). Ultimately the identification of these novel genes led to the unraveling of these unique O-mannose glycans, using both traditional and state-of-the-art glycobiology tools [56,66–68].

Another genetic screen highlighting the importance of N-glycosylation in pathogen susceptibility was done by two independent groups studying host factors for *Flaviviridae* viruses (i.e. dengue virus (DENV) [69,70]. The screen specifically performed by Carette et al., used either a Gene-Trap mutagenized population of haploid cells or a stably infected HUH7 population of lentiviral CRISPR cells and took advantage of the cytopathic effects of DENV to identify critical host factors [69]. From this screen two critical pathways for DENV toxicity were identified, first was the oligosaccharide transferase complex (OST) (*STT3A, STT3B, RPN1, RPN2, OSTc, KRTCAP2, MAGT1, DAD1, OST4*) and second were factors in the translocon complex (*SSR1, SSR2* and *SSR3*), which has been shown to bind directly to and influence substrate specificity of the OST complex [71] (Table 2). As with the LASV screen, several of the identified factors from the DENV screen have been established as CDG genes including *STT3A, STT3B* and *MAGT1* [72,73] (Table 2). Interestingly, *SSR4* another core component of the TRAP complex was not identified in the screen, but does cause a form of CDG termed *SSR4-CDG* [74,75]. It is possible that unlike *SSR1–3, SSR4* could be an essential gene whose knock out phenotype is lethal to the cell type used in the screen.

These are just two examples of nearly a dozen screens performed to date, other screens have identified glycosylation factors critical for Influenza A virus, Enterovirus D68, cholera toxin and chikungunya virus to name a few [76–79] (Table 2). These findings reiterate the importance of glycosylation genes in infectious diseases.

The importance of the *Flaviviridae* viral screen is highlighted by a recent paper in which a small molecule inhibitor to the OST subunit, STT3b, was shown to have potent antiviral activity and a potentially high barrier of resistance [80]. This potentially opens the door to more glycosylation specific targeted therapies.

Perspective therapies

Over one-hundred glycosylation disorders exist, but there are relatively few treatment options for CDG's or glycosylation-related disorders in general. The first CDG to be efficiently treated was MPI-CDG, which simply required dietary supplementation with D-mannose [81]. However, MPI-CDG is unlike most CDG types since it lacks the severe neurological presentation [4]. Another disorder benefiting from "monosaccharide therapy" is SLC35C1-CDG which is a defect in the transport of GDP-fucose into the Golgi [82, 83]. Here individuals present with leukocyte adhesion deficiency (LADII) which, in some cases, can be quickly corrected by providing dietary L-fucose [83]. Interestingly, not all reported cases of LADII respond to fucose therapy, suggesting that certain patient mutations may act through an inability to directly bind GDP-fucose [84].

Over the last five years the field has seen increased success in finding treatments that either resolve most clinical issues or at least provide some measurable clinical benefits. Three such disorders (PGM1-CDG, SLC35A2-CDG and TMEM165-CDG) have been reported to show clinical benefit by simply providing the monosaccharide, D-galactose [85–88]. Another disorder, SLC39A8-CDG which is a defect in manganese transport can be effectively treated with both D-galactose and manganese supplementation [89–91]. In a few individuals with CAD-CDG, a defect in *de novo* pyrimidine biosynthesis, uridine supplementation has been shown to virtually reverse clinical symptoms in a surprisingly short period of time. One affected individual went from a minimal state of responsiveness to communicating and assisted walking within nine weeks of treatment [92]. While these therapies may not apply to all affected individuals, manipulating metabolic flux does appear to be a possible and a realistic option.

Still, a treatment for PMM2-CDG continues to garner the most attention given that it is the most common CDG type with > 900 reported cases [5,93]. Phosphomannomutase 2 (PMM2) is a 246-amino acid protein that is required for converting mannose-6 phosphate (Man6p) to mannose-1 phosphate (Man1p) within the cytoplasm. To date ~115 disease causing *PMM2* mutations have been identified and listed in the Human Gene Mutation Database (<http://www.hgmd.cf.ac.uk/ac/gene.php?gene=PMM2>) [94]. The primary glycosylation defect in PMM2-CDG arises because mannose-1 phosphate (Man1p) functions as the intermediate for making GDP-mannose, which then serves as a high-energy nucleotide sugar donor to various mannosyltransferases [95] (Figure 2).

Initially, promising results utilizing primary fibroblast from various CDG cases (including PMM2-CDG) showed that when cells were incubated with mannose, temporary improvements to glycosylation could be achieved [96,97]. However, attempts to replicate this success in PMM2-CDG patients showed no significant benefit of oral mannose [96]. More recently, **pharmacological chaperons** have been used to stabilize specific patient

mutations [98]. However, one challenge that has plagued many therapy efforts is how to target such a large array of patient-relevant mutations. Since more than half of PMM2-CDG patients carry the common p.R141H mutation, efforts have focused on how to target this one specific mutation, which has a carrier frequency of ~1/90, but is lethal when homozygous [99,100]. However, none has been successful thus far.

Attempts have been made to directly provide hydrophobic Man1p derivatives that cross the plasma membrane into the cytoplasm of cultured fibroblasts. Penetration occurred, but compound toxicity related to the removal of the substituents covering the charged phosphate group was unacceptable [101]. Technological and chemical advancements have improved shuttling charged molecules, such as Man1P, across the plasma membrane and thus efforts have resurfaced at trying to facilitate Man1p penetration of the membrane [5].

Concluding Remarks

Over the last ten years the number of genetic disorders related to abnormal glycosylation has more than tripled from ~40 to over 125 today. This has been driven largely by advancements in next generation sequencing, specifically whole exome sequencing. (**See Outstanding Questions Box**) While WES has been incredibly successful in identifying causal candidate genes, the need for discriminating between benign and pathogenic variants is still an absolute necessity. Especially as an increased number of individuals from both healthy and diseased populations are sequenced revealing more rare variants. For many years, the complex world of glycosylation may have had a dampening effect on its embrace by the larger community, but the emergence of glycosylation pathways in genetic disorders, infectious diseases, cancer and metabolism has propelled it to the forefront of science requiring both new and continued collaborations between the medical and research communities.

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Glossary

Dystroglycanopathies

a group of genetic disorders that most often involve abnormal glycosylation of α -dystroglycan.

Glycosylation

a metabolic enzyme dependent process where sugar molecules (monosaccharides) are attached to an acceptor molecule, most often proteins or lipids.

Gnome-ExAC database

a free public database resource of unrelated individuals that excludes individuals with severe pediatric disease, as well as their first-degree relatives. It numbers 123,136 exomes and 15,496 genomes sequences.

Lipid linked oligosaccharide

also known as LLO refers to the glycolipid structure which is the typical precursor for N-glycans prior to transfer of the glycan to a nascent proteins.

Pharmacological chaperons

a group of molecules that are used to aid in the proper folding of proteins that are misfolded.

Next Generation sequencing

a general term for high throughput DNA sequencing.

Whole exome sequencing

a form of next generation sequencing that specifically targets the protein-coding regions of the genome termed “exons” that make up ~ 1% of the genome.

Highlights

- Over 125 Congenital disorders of glycosylation (CDG) are clinically diverse and cover all major glycosylation pathways.
- Next Generation Sequencing (NGS) enabled discovery of over 51 novel glycosylation disorders and identified *de novo* mutations in several disorders.
- Complex genetic screening of human cells shows glycosylation is critical for many pathogens, especially viruses. Targeting glycosylation pathways could become short-term treatments.
- Simple monosaccharide therapies and novel chemical approaches offer treatments for several glycosylation disorders.

Outstanding Questions Box

There are unanswered questions regarding glycosylation disorders on both the basic research and medical fronts. However, with the recent rise of next generation sequencing, more genetic screening methods and development of more sensitive biochemical instrumentation, specifically in mass spectrometry, the field of glycosylation has propelled into prominence. As these tools become increasingly sophisticated, mysteries will be answered and dogmas may be challenged.

- Will glycosylation-related disorders that are due to *de novo* mutations or those that display multiple inheritance patterns and diverse phenotypes become more frequent as more affected families are sequenced?
- Can monosaccharides alone or in combination with other means be used as a therapeutic strategy for additional glycosylation-related disorders or even in complex disorders like cancer?
- Knowing the critical roles of glycosylation in infectious diseases, will additional screens of viruses or other pathogens reveal additional glycosylation targets? Perhaps small molecules will be developed targeting those pathways.

Box 1**Serum transferrin, a biomarker for most CDG types**

The abundant serum glycoprotein transferrin is primarily synthesized in the liver and normally contains two N-glycan molecules. Glycosylation status can be analyzed using a number of methods including isoelectric focusing (IEF) and electrospray ionization mass spectrometry (ESI-MS) to name a few [105].

It can often be used to identify many, but not all types of N-linked glycosylation disorders [5,10] and while useful in identifying affected individuals, an abnormal Tf profile very rarely implicates a specific gene defect.

Abnormalities in Tf glycosylation can be described as either a type I or II profile [106]. A type I profile involves a Tf molecule that has an absence of either one or both of its N-glycan chains and is most commonly associated with defects in the synthesis or transfer of LLO molecules [106]. On the other hand, a type II is most often associated with processing and remodeling of the protein bound N-glycans and can result in a large degree of N-glycan structural diversity [106].

Abnormalities in sugar nucleotide metabolism, Golgi homeostasis and architecture, even pH maintenance within the different Golgi compartments can all contribute to type II disorders.

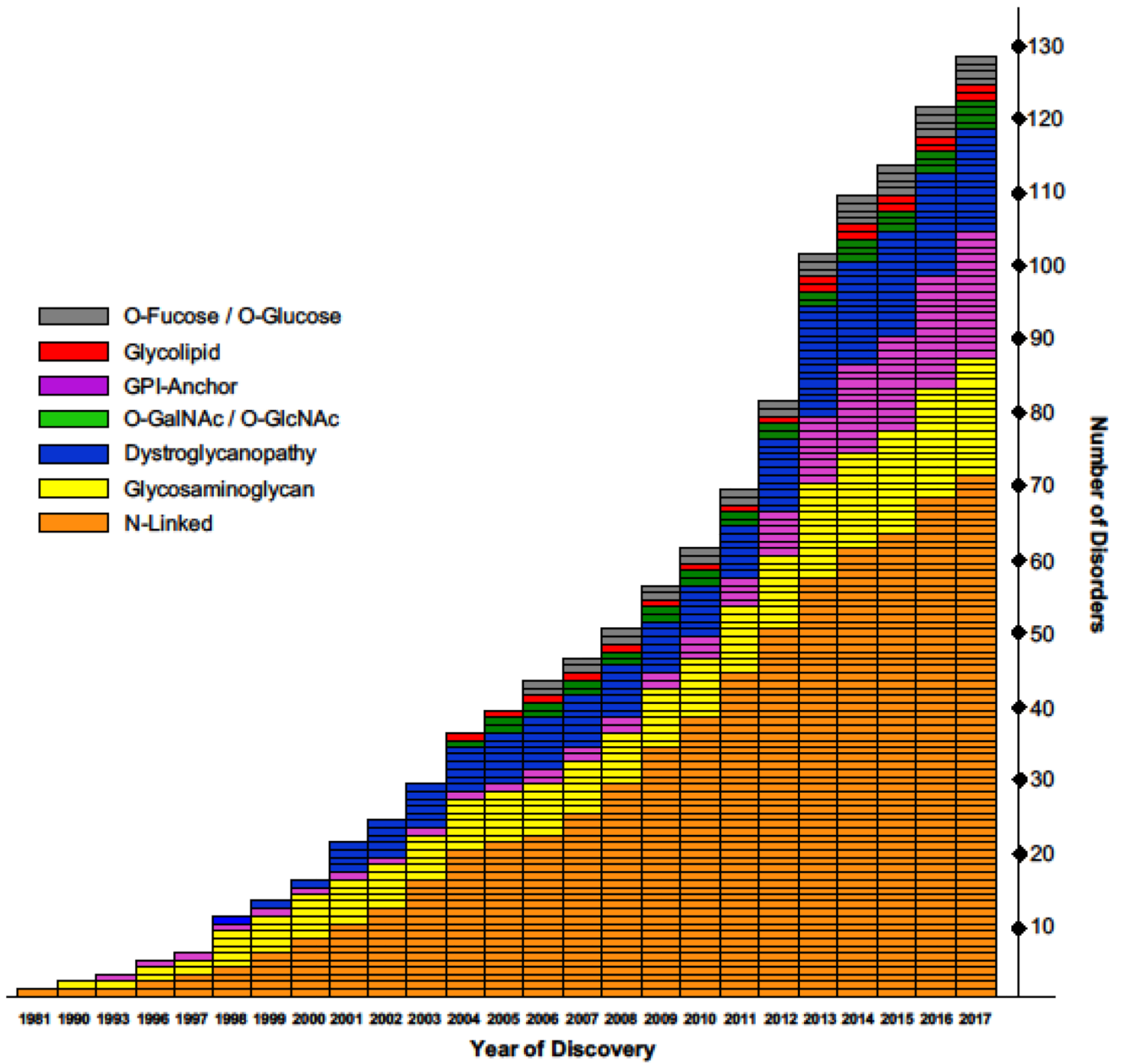


Figure 1. Discovery of glycosylation disorders

The distribution of glycosylation-related disorders by the year they were identified. They are grouped according to the glycosylation pathway which they affect and while most fall within a clear specific pathway, there are those that can affect multiple glycosylation disorders. This describes disorders identified up to the end of 2017. This figure is an updated version adapted from Freeze, H.H. et al. (2014) [10].

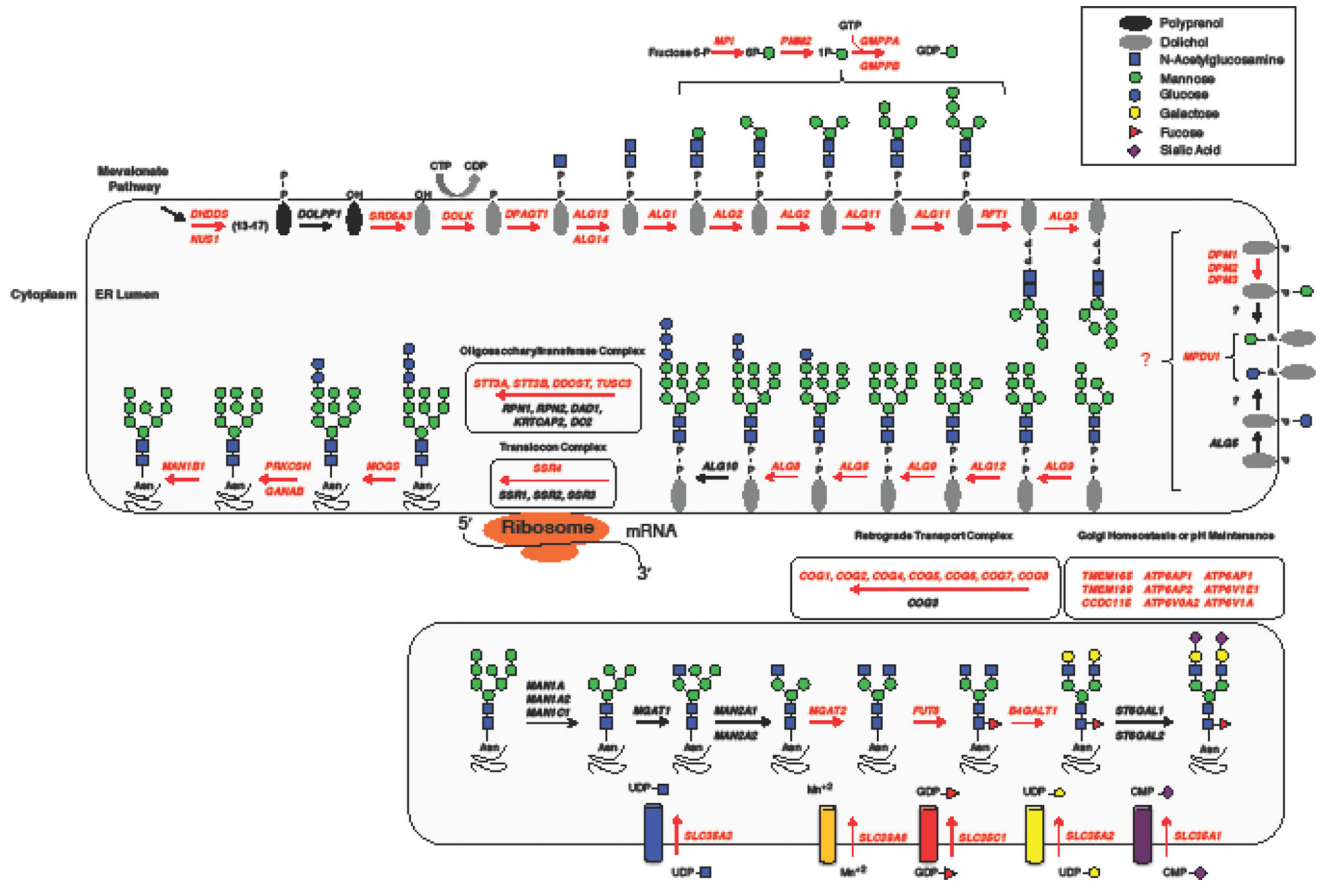


Figure 2, Key Figure. N-linked glycosylation pathway

A schematic of the N-linked pathway highlighting those genes required for the both the initial steps of lipid linked oligosaccharide synthesis as well as several key components for glycan processing within the Golgi. These genes highlighted in red represent known loci for glycosylation disorders. This figure is an updated version adapted from Freeze, H.H. et al. (2015) [4]

Table 1

Disorders Identified by WES from 2010 – 2017

| Glycosylation Pathway | Disorders identified from 2010 – 2017 | Disorders identified by WES from 2010 – 2017 | Total Number of Disorders |
|------------------------------|--|---|----------------------------------|
| N-Linked or Multiple | 33 | 25 | 71 |
| Glycosaminoglycan | 8 | 5 | 16 |
| Dystroglycanopathy | 7 | 4 | 14 |
| O-GalNAc / O-GlcNAc | 2 | 2 | 4 |
| GPI-Anchor | 14 | 12 | 17 |
| Glycolipid | 1 | 1 | 2 |
| O-Fucose / O-Glucose | 2 | 2 | 4 |
| Totals | 67 | 51 | 128 |

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Table 2

Glycosylation genes identified as critical host factors for various toxins or pathogens.

| Toxin or Pathogen | Genes identified | Glycosylation pathway utilized | Reference |
|--------------------------------|---|----------------------------------|-----------|
| Ricin Toxicity | <i>FUT9, SLC35C1, GALNT2, B4GALT1, B4GALNT3, ST3GAL4</i> | Fucosylation and galactosylation | [51,102] |
| Dengue Virus | <i>MAGT1, STT3A, STT3B, DDOST, RPN1, RPN2, OSTC, OST4, KRTPAP2, DAD1, B3GALT6, EXT1, SLC35B2, SSR1, SSR2, SSR3, SRP9, SRP14, HMI3, SPCS3, SEC61A1</i> | OST and translocon complexes | [69,70] |
| Lassa Virus | <i>LARGE, ISPD, B3GALNT2, TMEM5, B4GAT1, POMK, DAG, POMT1, POMT2, FKTN, FKRP, ST3GAL4, SLC35A1, GNE, CMAS, SLC35A2, PTAR1, COG5, 7, 8, MAN1B1, MGAT1, MAN1A1, ALG5, ALG6, ALG8, MPDU1, DPM1, DPM3</i> | O-Mannose | [64] |
| Cholera Toxin | <i>ST3GAL5, B3GALT4, SLC35A2</i> | Galactosylation | [77] |
| Enterovirus D68 | <i>SLC35A1, GNE, NANS, ST6GAL1, MGAT5, B4GALT1, ST3GAL4</i> | Sialic Acid | [78] |
| Chikungunya Virus | <i>EXT1, EXT2, PTAR1, TMEM165, COG 1– 8, NDST1, EXTL3, B3GAT3</i> | GAG synthesis | [79] |
| Rift Valley fever virus | <i>SLC35B2, EXT1, EXT2, EXTL3, XYLT2 UXS1, UGDH, NDST1, B3GAT3, B3GALT6, B4GALT7, COG1, COG2, COG3, COG4, COG5, COG7, COG8, PTAR1</i> | GAG synthesis | [103] |
| Monkeypox Virus | <i>EXT1, B3GAT3, B4GALT7, B3GALT6, SLC35B2, XYLT2, COG3, COG4, COG7, COG8, PTAR1, TRAPPC13, PIGL, DPM3, MPDU1</i> | GAG synthesis | [104] |