Protein glycosylation is increasingly recognized as a crucial modulator of protein function, offering a third layer of biological information over genomics and proteomics. Modern tools for analyzing released N-glycans from cells and body fluids, i.e., the glycobiome, have shown abnormal protein glycosylation in numerous human diseases. These include both genetic and acquired diseases, ranging from diabetes, cancer, and inflammatory disease to neurodegenerative and neuromuscular disease. Insights from this novel field in human medicine provide exciting perspectives toward understanding disease processes, identifying therapeutic targets, and designing individualized diagnostics based on protein concentrations and glycosylation status. However, the main question is how we can translate this information into concrete biomarkers in a clinical diagnostic setting, with high demands on technical robustness and the ability to interpret results within specific patient groups.

Unlike the genetic template for protein synthesis, the process of protein glycosylation is not directly encoded by the genome. Protein N-glycosylation, the best-studied ubiquitous type of glycosylation, follows a sequential pathway in the organelles of the secretory pathway. First, a dolichol-linked glycan composed of 14 monosaccharides is assembled in the endoplasmic reticulum (ER)\(^2\) and transferred to nascent proteins that enter the ER via the translocon complex. In the final stages in the ER, and further in the Golgi apparatus, the protein-bound glycan is remodeled to a mature glycan that is secreted in the Golgi apparatus or ER. Transferrin lacks the addition of complete glycans, resulting in unoccupied glycosylation sites. In patients with CDG-I subtypes, the genetic defect is located in the cytosol or Golgi apparatus, transferrin is observed with abnormal truncated glycans. Profiling of total plasma N-glycans, i.e., the release and analysis of N-glycans bound to all plasma proteins, provides information on glycan structures and therefore usually reflects Golgi glycosylation status (2, 3).

**Lessons from ALG1-CDG**

In this issue of *Clinical Chemistry*, Zhang et al. (4) report on the identification of a novel N-tetrasaccharide that is highly characteristic for a few very specific CDG-I subtypes: asparagine-linked glycosylation protein 1 (ALG1)-CDG, phosphomannomutase 2 (PMM2)-CDG, and phosphomannose isomerase (MPI)-CDG (Fig. 1). They clearly illustrate the biochemical and diagnostic lessons that can be learned by studying CDG. The authors applied total plasma and fibroblast N-glycan profiling to several CDG-I subtypes. Surprisingly, zooming in on the low molecular mass range highlighted the accumulation of several small, truncated glycan structures. These structures were visible only after PNGaseF digestion, indicating their protein-bound nature. In patients with ALG1-CDG, they identified a novel protein-linked N-tetrasaccharide (Neu5Acα2,6Galβ1,4GlcNAcβ1, 4GlcNAc), a trisaccharide (Galβ1,4GlcNAcβ1,4GlcNAc), and the chitobiose GlcNAcβ1,4GlcNAc. These glycans were also present in lower amounts in the PMM2-CDG and MPI-CDG patients tested. In PMM2-CDG and MPI-CDG, the Man\(_3\)GlcNAc\(_2\) and Man\(_4\)GlcNAc\(_2\) glycans were also increased. Mass spectrometry analysis of plasma transferrin indicated the presence of the N-tetrasaccharide in ALG1-CDG patients, but not in patients with PMM2- or MPI-CDG. In addition, the effects to derive important lessons on novel biochemical mechanisms and clinical diagnostic approaches with relevance to more common diseases. More than 100 different genetic defects in various glycosylation pathways are already known, approximately 50 of which affect the N-glycosylation pathway (1). Diagnostic screening for CDG with abnormal N-glycosylation is commonly carried out by isofoxing, capillary electrophoresis, or HPLC of plasma transferrin, an abundant liver-derived protein with 2 N-glycan structures. In patients with CDG-I subtypes, the genetic defect is located in the cytosol or ER. Transferrin lacks the addition of complete glycans, resulting in unoccupied glycosylation sites. In patients with CDG-II subtypes caused by a defect in the cytosol or Golgi apparatus, transferrin is observed with abnormal truncated glycans. Profiling of total plasma N-glycans, i.e., the release and analysis of N-glycans bound to all plasma proteins, provides information on glycan structures and therefore usually reflects Golgi glycosylation status (2, 3).

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accumulation of Man$_3$GlcNAc$_2$ and Man$_4$GlcNAc$_2$ glycans was not visible on the transferrin protein.

Previously, the presence of chitobiose has been reported on proteins in ALG1 mutant yeast. Interestingly, the protein-linked chitobiose was also observed in the T24 bladder carcinoma cell line grown under glucose deprivation, indicating a more general disease mechanism (5). ALG1 encodes the first mannosyltransferase, responsible for the elongation of dolichol-PP-GlcNAc$_2$. Apparently, deficiency of ALG1 results in accumulating GlcNAc$_2$ (chitobiose) structures that can be transferred onto nascent proteins. Subsequently, the GlcNAc$_2$ structure can be elongated in the Golgi apparatus by galactosyl and sialyl transferases to yield the N-tetrasaccharide. The low efficiency of this process accounts for the low abundance of these diagnostic markers.

In addition to the biochemical lessons, the study by Zhang et al. clearly shows the added value of highly sensitive and specific mass spectrometry techniques to identify unanticipated glycan-based biomarkers for disease. CDG-I subtyping has previously been based on the laborious analysis of lipid-linked oligosaccharides in fibroblasts, requiring a skin biopsy and radioactive labeling. More recently, next-generation sequencing has replaced several biochemical methods. The results from Zhang’s study, however, reveal that the ALG1-CDG subtype can already be identified in the initial screening for CDG-I if mass spectrometry of transferrin is performed. In addition, as suggested by the authors, PMM2-CDG might be diagnosed by total plasma N-glycan profiling by mass spectrometry.

**Fig. 1.** Mutations in ALG1 (ALG1, chitobiosylphosphodolichol beta-mannosyltransferase), PMM2 (phosphomannomutase 2), and MPI (mannose phosphate isomerase), 3 defects in the early steps of N-glycosylation in the endoplasmic reticulum, give rise to the presence of a unique N-tetrasaccharide on plasma proteins.

For ALG1, PMM2, and MPI, the N-tetrasaccharide can be seen on total plasma proteins, but only for ALG1 on transferrin. Total plasma glycan profiling shows additional accumulation of Man$_4$- and Man$_3$GlcNAc$_2$ glycans for PMM2 and MPI; however, they are not detectable on transferrin. These data from Zhang et al. (4) indicate that proteins other than transferrin contain diagnostic glycan markers, showing the potential for protein-specific glycan profiling in patient diagnostics.
can be used as biomarkers for CDG diagnostics and beyond. Quantification of such glycoproteins at the level of the intact protein, or by use of multiple reaction monitoring of glycopeptides (13), as commonly used in clinical chemistry laboratories, will allow the translation of glycomics information into useful clinical biomarkers by mass spectrometry.

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References


3 VPS13B, vacuolar protein sorting 13 homolog B (yeast); ALG1, ALG14, chitoosylidophospholipid beta-mannosyltransferase; PMM2, phosphomannomutase 2; MPI, mannose phosphate isomerase.