Plasma N-Glycan Profiling by Mass Spectrometry for Congenital Disorders of Glycosylation Type II

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BACKGROUND: Determination of the genetic defect in patients with a congenital disorder of glycosylation (CDG) is challenging because of the wide clinical presentation, the large number of gene products involved, and the occurrence of secondary causes of underglycosylation. Transferrin isoelectric focusing has been the method of choice for CDG screening; however, improved methods are required for the molecular diagnosis of patients with CDG type II.

METHODS: Plasma samples with a typical transferrin isoelectric focusing profile were analyzed. N-glycans were released from these samples by PNGase F [peptide-N-acetyl-β-glucosaminyl]-asparagine amidase digestion, permethylated and purified, and measured on a MALDI linear ion trap mass spectrometer. A set of 38 glycans was used for quantitative comparison and to establish reference ranges for such glycan features as the number of antennae, the level of truncation, and fucosylation. Plasma N-glycans from control individuals, patients with known CDG type II defects, and patients with a secondary cause of underglycosylation were analyzed.

RESULTS: CDGs due to mannosyl (α-1,6)-glycoprotein β-1,2-N-acetylgalactosaminyltransferase (MGAT2), β-1,4-galactosyltransferase 1 (B4GALT1), and SLC35C1 (a GDP-fucose transporter) defects could be diagnosed directly from the N-glycan profile. CDGs due to defects in genes encoding proteins involved in Golgi trafficking (COG7) and ATP6V0A2 (ATPase, H+ transporting, lysosomal V0 subunit a2) caused a loss of triantennary N-glycans and an increase of truncated structures. Secondary causes with liver involvement were characterized by increased fucosylation, whereas the presence of plasma sialidase produced isolated undersialylation.

CONCLUSIONS: MALDI ion trap analysis of plasma N-glycans documents features that discriminate between primary and secondary causes of underglycosylation and should be applied as the first step in the diagnostic track of all patients with an unsolved type II CDG.

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Plasma samples were examined by MALDI linear ion trap mass spectrometry for the presence of specific glycan features. The method is an efficient way to identify N-glycosylation defects and to discriminate a genetic cause involving the cytoplasm or endoplasmic reticulum (CDG type I) from one involving the Golgi apparatus (CDG type II).

Congenital disorders of glycosylation (CDGs) constitute a group of inherited metabolic defects caused by abnormalities in protein and/or lipid glycosylation. The clinical phenotype ranges from a severe multisystem presentation and early death, to relatively mild neurologic symptoms. Because the clinical features are not diagnostic and cannot be used as exclusion criteria, identification of CDG patients relies on the diagnostic screening by isoelectric focusing of plasma transferrin (TIEF) carrying 2 complex N-glycans. This method is an efficient way to identify N-glycosylation defects and to discriminate a genetic cause involving the cytoplasm or endoplasmic reticulum (CDG type I) from one involving the Golgi apparatus (CDG type II).

The elucidation of CDG type II defects is very challenging. O-Glycosylation analysis by isoelectric focusing of plasma apolipoprotein C-III allows differentiation between patients with an isolated N-glycosylation abnormality and patients with a combined N- and O-glycosylation defect. The clinical heterogeneity of these patients prevents straightforward identification of causative genes. An additional complicating factor is the presence of secondary causes with liver involvement.

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Nonstandard abbreviations: CDG, congenital disorder of glycosylation; TIEF, isoelectric focusing of plasma transferrin; HUS, hemolytic uremic syndrome; MS, mass spectrometry; MS5, multistage MS; GDP-Fuc, GDP-fucose; PNGase F, peptide-N-acetyl-β-glucosaminyl)-asparagine amidase; DHB, 2,5-dihydroxybenzoic acid; DMA, N,N-dimethylaniline; NeuNAc, complex N-glycans with at least 1 antenna without a terminal sialic acid; GlcNAc, N-acetyl glucosamine; Gal, galactose; COG, conserved oligomeric Golgi.
factor is the occurrence of secondary causes of type II TIEF profiles, including hemolytic uremic syndrome (HUS) with the presence of pneumococcal sialidase in plasma (6), or severe liver pathology (7–9). Given that some of the CDG type II defects present with severe hepatopathy as well, these factors may delay diagnosis. Finally, no proper diagnostic assay is available for Golgi glycosylation defects that produce a wild-type TIEF pattern, as is seen with CDG with defective fucosylation due to mutations in the SLC35C1 gene (solute carrier family 35, member C1) (10).

Clearly, there is a need for additional diagnostic tools in a medical setting to facilitate gene identification in patients with CDG type II. HPLC and mass spectrometry (MS) provide structural information and have been applied in individual cases (11, 12). N-glycome fingerprinting by MS has been successful with other classes of disease, including liver fibrosis and ovarian cancer (13, 14). In the field of CDG, Butler et al. described HPLC analysis of purified glycoproteins and whole-serum N-glycans followed by MS confirmation of glycan structures (15). Mills et al. applied MALDI-TOF analysis of unmodified N-glycans in positive and negative ion modes to provide an overview of neutral and acidic glycans, respectively (16). Permethylated glycans have better ionization properties in MALDI MS, and additional structural information is obtained from fragmentation. In addition, their signal strengths reflect the relative amounts of glycans in the sample (17, 18).

Our goal was to develop a fast and robust method for plasma N-glycan profiling in a single measurement. The use of permethylated glycans and an appropriate matrix permitted all N-glycans to be measured together in positive ion mode, and relative quantification was achieved. In addition, the MALDI linear ion trap allowed multistage MS (MSn) glycan sequencing, which reveals detailed information on glycan structures (19). It was applied to samples from healthy individuals, patients with a Golgi-related CDG, and patients with a secondary cause of N-glycan hypoglycosylation. Through identification and quantification of the ions, we identified factors that were able to differentiate the patient groups.

Materials and Methods

All chemical reagents were of the highest level of purity available. Unless otherwise stated, they were purchased from Sigma-Aldrich.

PLASMA SAMPLES

Plasma samples were collected from EDTA- or heparin-treated blood by centrifugation and were stored at −20 °C. Plasma samples from healthy adults (>18 years, n = 10) and children (age, 0–14 years; n = 9) with a typical TIEF profile were used to establish reference ranges. We obtained samples from patients with known defects, including the following: 1 sample from a patient with a defect encoded by the MGAT2 [mannosyl (α-1,6-)–glycoprotein β-1,2-N-acetylgalactosaminyltransferase] gene [CDG type IIa; sample provided by Prof. J. Jaeken, University Hospital Gasthuisberg, Leuven, Belgium (11)]; 1 sample from a patient with a defect encoded by the B4GALT1 (β-1,4-galactosyltransferase 1) gene [CDG type IId (12)]; 1 sample from a patient with a defect in SLC35C1 [a GDP-fucose (GDP-Fuc) transporter; CDG type IIc (10)]; 1 sample from a patient with a defect encoded by the SLC35A1 [solute carrier family 35 (CMP-sialic acid transporter), member A1] gene [CDG type IIIf; provided by Dr. Mollicone, France (20)]; samples from 4 patients with a defect encoded by the COG7 (component of oligomeric Golgi complex 7) gene [CDG type IIe (21)]; and samples from 2 patients with a defect encoded by the ATP6V0A2 (ATPase, H+ transporting, lysosomal V0 subunit a2) gene (22). Samples with a secondary cause of underglycosylation were from 3 patients with severe liver failure of unknown etiology: a patient with citrullinemia type I with liver failure; a patient with hemophagocytic lymphohistiocytosis; and a patient with HUS due to Streptococcus pneumoniae infection and who was positive for plasma sialidase (specific activity, 41 100 pmol·h−1·mL−1 at pH 7.0; undetectable in controls).

TRANSFERRIN ISOFOCUSING

We incubated 5 µL plasma for 30 min with the same volume of 6.7 mmol/L ferric citrate and 0.17 mmol/L NaHCO3 (Fluka) in water and then diluted the mixture with 10 volumes of saline solution. We transferred 4 µL of each sample to a hydrated Immobiline dry gel (ampholyte: Servalyt, pH 5–7; GE Healthcare) and run on a PhastSystem (GE Healthcare) according to the manufacturer’s conditions and protocol. Transferrin isoforms were detected by immunoprecipitation in the gel with 60 µL rabbit antihuman transferrin antibody (8.5 g/L; Dako) per gel for 30 min, followed by overnight washing in saline, fixation with 200 g/L trichloroacetic acid, and Coomassie Blue staining.

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9 Human genes: SLC35C1, solute carrier family 35, member C1; MGAT2, mannosyl (α-1,6-)–glycoprotein β-1,2-N-acetylgalactosaminyltransferase; B4GALT1, β-1,4-galactosyltransferase 1; SLC35A1, solute carrier family 35 (CMP-sialic acid transporter), member A1; COG7, component of oligomeric Golgi complex 7; ATP6V0A2, ATPase, H+ transporting, lysosomal V0 subunit a2.
** MASS SPECTROMETRY**

Glycan release by PNGase F [peptide-N4-(acetyl-β-glucosaminyl)-asparagine amidase] (New England Biolabs) was performed by adding 90 μL saline and 11 μL Denaturation Buffer to 10 μL plasma; incubating the mixture at 95 °C for 5 min; cooling; and adding 40 μL of 100 g/L NaOH (P40) (Roche), 15 μL Reaction Buffer, and 2 μL PNGase F. The mixture was then incubated overnight at 37 °C. Released glycans were purified on 3-mL graphitized carbon columns (Supelco) (23). All N-glycans were eluted in a single 2-mL fraction of an aqueous solution 250 mL/L acetonitrile (BioSolve) with 1 g/L aqueous trifluoroacetic acid and then dried. Permethylation was performed as previously described (17, 24), with the following improvements. No specific precautions were taken to prevent moisture. Four air-dried NaOH pellets (approximately 375 mg) were crushed in 10 mL anhydrous DMSO, 0.5 mL of this slurry and 0.2 mL CH3I were added to the dried glycans, and the mixture was shaken vigorously for 1 h. Permethylated glycans were extracted in a chloroform layer, which was washed 4 times with water and dried under nitrogen flow. Further purification was performed with C18 StageTips instead of with Sep-Pak cartridges (25). The tips were prewashed with 100 μL methanol (LabScan) and washed with 100 μL 20 mmol/L aqueous sodium acetate (Fluka). The glycans were diluted in 50 μL 500 mL/L aqueous methanol, loaded onto the tip, washed consecutively with 100 μL 20 mmol/L aqueous sodium acetate and 50 μL 150 mL/L aqueous acetonitrile, and eluted in 50 μL of 750 mL/L aqueous acetonitrile. The glycans were dried and then resuspended for spotting in 5–10 μL of a solution consisting of 1 volume of methanol and 1 volume of 20 mmol/L aqueous sodium acetate. The matrix consisted of 1 volume of methanol and 1 volume of 20 mL 500 mL/L aqueous methanol, and spotted each 4 times; we also measured 10 spots 4 times. The 10 major peaks were selected for further analysis (see Table 2 in the online Data Supplement). The reproducibility was assessed similarly to as described in our previous publication (19). We individually processed 10 aliquots of a control serum sample and spotted each 4 times; we also measured 10 spots 4 times. The 10 major peaks were selected for further analysis (see Table 2 in the online Data Supplement). Peak intensities were normalized to the sum of all peaks. Further details are provided in Table 2 in the online Data Supplement.

**Results**

**PLASMA N-GLYCAN ANALYSIS OF CONTROL SAMPLES**

For comparative purposes, we analyzed plasma N-glycans from 19 samples with a typical TIEF pattern. We optimized sample processing by using a smaller amount of NaOH with an increased reaction time to facilitate sample workup. The use of C18 StageTips instead of Sep-Pak cartridges greatly increased the throughput. Use of a DHB/DMA mixture as the matrix permitted good measurement reproducibility (see Table 2 in the online Data Supplement). The reproducibility of sample processing was comparable to that of our previous method, with the interassay CV varying from 13% to 34% (19).

Fig. 1 presents a representative N-glycan spectrum for the control individuals. We quantified 38
glycan ions (see Table 1 in the online Data Supplement) and confirmed assigned glycan structures by MS^n sequencing. Unless explicitly addressed, adult and pediatric controls were pooled for the establishment of reference ranges. The dominant ions were biantennary and triantennary fully sialylated complex N-glycans (m/z 2794 and 3605) with their fucosylated equivalents (m/z 2968 and 3779). The mean level of undersialylation was 18% (reference range, 12%–28%). The degree of fucosylation was higher for truncated structures than for fully assembled glycans. For example, the ratio of the major glycan at m/z 2794 to its fucosylated equivalent (m/z 2968) was about 8, whereas the ratio for the truncated structure, i.e., (m/z 2071)/(m/z 2245), was only about 0.5. A small fraction of oligomannosidic glycans was detected [see Table 1 in the online Data Supplement; sum of m/z 1581 and m/z 1785 (reference range, 0.0%–1.7%)].

The quantitative analysis of glycan features in the control samples, as described in Materials and Methods, revealed the presence of 78% biantennary glycans (reference range, 67%–84%) and 16% triantennary glycans (reference range, 11%–26%) (Fig. 2; see Table 3 in the online Data Supplement). Truncated structures, such as ions with m/z values 1837, 2041, and 2245, accounted for one-third of all complex N-glycans (reference range, 21%–44%).

Among the controls, no appreciable distinction could be made between age groups, except for fucosylation status. In the pediatric group, <30% of the N-glycans were fucosylated (reference range, 17%–28%, with 1 sample at 34%), whereas >30% of the N-glycans were fucosylated in adults (reference range, 31%–37%). MS^n sequencing revealed the occurrence of both core and antenna fucosylation in both age groups.

PLASMA N-GLYCAN ANALYSIS OF PATIENT SAMPLES

After establishing reference ranges for glycan features, we compared the controls with a comprehensive panel of patient samples. Known defects included deficiencies in glycosyltransferases (MGAT2 and B4GALT1), nucleotide sugar transporters (SLC35C1 and SLC35A1), and Golgi transport proteins (COG7 and ATP6V0A2), whereas secondary causes included severe liver pathology and the presence of plasma sialidase.

GLYOSYLTTRANSFERASE DEFECTS

Samples from the 2 known CDG II subtypes with a glycosyltransferase defect, MGAT2-CDG and B4GALT1-CDG, were processed and measured (Fig. 3).

The N-glycan spectrum of the MGAT2-CDG patient was dominated by ions with m/z values of 1983 and 2157, corresponding to the monoantennary complex N-glycan and its fucosylated equivalent. Biantennary glycans were strongly reduced, and triantennary glycans were completely absent from the spectrum. This finding is in accordance with an enzymatic deficiency of MGAT2 (11).
The N-glycans of the B4GALT1-CDG patient showed a different profile, with a high proportion of structures with terminal GlcNAc (m/z values of 1663, 1823, 1837, 1908, and 2082) and truncated bi- and tri-antennary structures missing 1 or 2 NeuNAc–Gal moieties (m/z values 2228, 2403, 2474, and 2648). Overall, 88% of the quantified glycans had at least 1 terminal GlcNAc residue (12% in controls), corresponding to a deficient transfer of Gal to terminal GlcNAc residues by B4GALT1.

NUCLEOTIDE SUGAR TRANSPORTER DEFECTS

Nucleotide sugars must be actively transported from the cytosol to the Golgi lumen by highly specific nucleotide-sugar transporters. Malfunctioning of the GDP-Fuc transporter and UDP-NeuNAc transporter have been described for CDG patients with defects in SLC35C1 (10, 26) and SLC35A1 (20). Both types presented a typical TIEF pattern. Mass spectrometry of the CDG patient with an SLC35C1 defect revealed an evident decrease in fucosylation status to 11% (33% in controls; Figs. 2 and 3). In the MS profile, the prominent fucosylated ions at m/z 2606, m/z 2968, and m/z 3779 were not observed. MS^n fragmentation analysis showed that both core and antenna fucosylation were affected. This example illustrates the utility of MS for identifying glycosylation defects in patients with a wild-type TIEF pattern.

In accordance with the wild-type isofocusing pattern, the mass spectrum of whole plasma N-glycans of the SLC35A1-CDG patient was comparable to that of the controls.

DEFECTS IN GOLGI TRAFFICKING

Besides deficient transfer and transport of sugars, abnormal glycosylation may be caused by impaired Golgi trafficking, as seen in the CDG patients with defects in COG7 (27) and ATP6V0A2 (28). Plasma N-glycan profiling of 4 COG7-CDG patients and 2 ATP6V0A2-CDG patients showed a reduction in triantennary glycans along with an increase of truncated structures (Figs. 2 and 4). For both CDG types, the level of undersialylation was 2 to 3 times higher than the reference range. Overall, the total fraction of truncated structures was increased from 33% (reference range, 21%–44%) to 61% in COG7-CDG patients (reference range, 57%–67%) and 50% and 58% in the 2 ATP6V0A2-CDG patients (Fig. 2). The truncation occurred at various levels of the antenna: the fraction of glycans with terminal Gal (including m/z values 2041, 2433, and 2607) increased from 26% in the controls (reference range, 21%–34%) to 44% in the COG7-CDG patients (reference range, 39%–50%) and 36% and 40% in the 2 ATP6V0A2-CDG patients.

The fraction of glycans with terminal GlcNAc was substantially higher in both patient groups (e.g.,...
from 15% in controls (reference range, 8%–24%) to 28% in the COG7-CDG patients (reference range, 25%–30%) and to 22% and 31% in the 2 ATP6V0A2-CDG patients. In 3 of the 4 COG7-CDG samples, oligomannosidic structures were increased compared with the controls (m/z 1581 and m/z 1785). These data confirm that trafficking defects affect several steps in the processing of N-glycans along the Golgi biosynthetic route.

SECONDARY CAUSES OF HYPOGLYCOSYLATION

One of the challenges in the diagnostics of patients with CDG type II is the exclusion of secondary causes of hypoglycosylation. We carried out a detailed analysis of the N-glycome to search for glycan features that discriminate between primary and secondary glycosylation abnormalities.

Plasma N-glycan profiling of HUS patients with sialidase in the blood was in agreement with the undersialylated transferrin isoforms revealed by isoelectric focusing; the most prominent ion was the monosialylated biantennary N-glycan (Fig. 3). The level of undersialylation was 10-fold higher than in the controls, and all observed ion peaks with m/z values >2800 represented undersialylated tri- and tetra-antennary structures, most of which were not observed in the controls (Fig. 4). In contrast to COG7- and ATP6V0A2-CDG glycomes, the percentage of triantennary glycans was not decreased, and there was no increase in ions with terminal GlcNAc or oligomannosidic glycans. This finding is in agreement with a typical N-glycan biosyn-

![Image](https://via.placeholder.com/150)

**Fig. 3.** N-glycan profile of CDG type II patients, with major glycans indicated.

All glycan structures were confirmed by MS^n. GlcNAc (■), mannose (●), Gal (○), NeuNAc (●), and Fuc (◆) moieties are indicated. The corresponding plasma TIEF profile is shown at the right of each panel; the numbers of sialic acids on transferrin (0–6) are indicated.
thesis followed by extracellular sialidase cleavage. TIEF and MS analysis of a sample from an HUS patient after treatment showed typical profiles (data not shown).

Three other patients presented a type II TIEF pattern, although their diagnoses were not directly related to a genetic glycosylation disorder. One patient had isolated liver failure, 1 patient had citrullinemia type I, and 1 patient had hemophagocytic lymphohistiocytosis. All patients had severe liver abnormalities according to their liver enzyme activities.

The patient with isolated liver failure presented a slightly abnormal type II TIEF profile. The mass spectrum revealed mainly undersialylation of tri- and tetra-antennary N-glycans, an increase in fucosylated glycans, and increased undergalactosylated biantennary structures (Figs. 2 and 4). The transferrin isofocusing

**Fig. 4.** MS	extsuperscript{-1} spectra of plasma N-glycans of 2 patients with a defect in Golgi trafficking (top 2 panels) and 4 patients with a secondary glycosylation defect (bottom 4 panels). GlcNAc (■), mannose (○), Gal (□), NeuNAc (▲), and Fuc (●) moieties are indicated. The corresponding plasma TIEF profile is shown at the right of the top 3 panels; the numbers of sialic acids on transferrin (0–6) are indicated.
pattern of the citrullinemia patient was also slightly abnormal, yet the mass spectrum of plasma N-glycans revealed a more prominent increase in truncated fucosylated structures than in the other patients with liver failure. The TIEF pattern of the hemophagocytic lymphohistiocytosis patient was clearly abnormal before treatment, a finding that was confirmed by an N-glycan profile with strongly increased truncated and fucosylated structures and a decrease of triantennary glycans. The TIEF profile and N-glycan spectrum normalized after 13 days of chemoimmunotherapy (data not shown). Our overall analysis of the glycan features in these 3 patients revealed that their fucosylation status was substantially different from that of patients with a Golgi-trafficking defect.

Discussion

The lack of proper diagnostic tools to exclude secondary causes of underglycosylation in patients with CDG type II and to highlight the genetic defect stimulated us to test the diagnostic potential of MALDI ion trap profiling of plasma N-glycans. Particular glycan features, such as fucosylation, the terminal monosaccharide, and level of undersialylation, were used to compare patient groups.

Glycosyltransferase defects (MGAT2-CDG and B4GALT1-CDG) produced very distinct plasma N-glycan profiles that pointed directly to the defect. Plasma transferrin analysis by 1H nuclear magnetic resonance and electrospray MS of purified disialotransferrin from the MGAT2-CDG patient had previously revealed 2 monoantennary N-glycans on the protein (11). MALDI MS showed that the fucosylated equivalent was also present. HPLC and MS analysis of the transferrin glycans of the B4GALT1-CDG patient has revealed 3 glycans: the fully sialylated biantennary N-glycan and truncated equivalents missing 1 or 2 NeuNAc-Gal moieties (12). Whole-plasma profiling showed a much wider spectrum of N-glycans, including fucosylated N-glycans and glycans with a bisecting GlcNAc or truncated third antenna. A minor fraction of fully sialylated mono- and biantennary glycans was still present, in line with the transferrin isomeric distribution on TIEF. These N-glycan MALDI fingerprints are directly indicative of the genetic defect. The 2 transporter defects do not produce an abnormal TIEF profile. In the GDP-Fuc transporter defect (SLC35C1-CDG), the lack of fucose residues does not influence the isoelectric point of transferrin. Our MS data confirmed a decrease in fucosylated structures compared with the controls and other CDG patients. Thus, a clinical suspicion of SLC35C1-CDG as leukocyte adhesion deficiency type II should trigger clinicians to request plasma N-glycan profiling for diagnostics. In the patient with a deficient Golgi CMP-NeuNAc transporter, the typical sialylation of transferrin was attributed to the leakiness of the mutation or by sample contamination owing to frequent transusions (20, 29).

Defects in the Golgi secretory pathway can affect both N- and O-glycosylation, as seen in the COG7-CDG and ATP6V0A2-CDG defects. The conserved oligomeric Golgi (COG) complex is involved in the control of the intracellular trafficking and stability of Golgi-associated glycosylation enzymes. Defects have been found in various subunits of the complex (COG1 and COGs 4–8) (27, 30). Previously published serum N-glycan spectra for 4 different COG defects were mainly characterized by decreased sialylation (31). CDGs caused by COG1 and COG7 defects showed the most severe underglycosylation, with strongly decreased galactosylation and increased oligomannosidic glycans at m/z 1582. Our findings with the COG7-CDG sample confirm the reduction of sialylation and galactosylation. We also observed an increase in oligomannosidic glycans from 0.7% (reference range, 0.0%–1.7%) to 2.0%, 2.8%, and 2.9% in 3 of the COG7-CDG patients. The fourth COG7-CDG patient showed a typical percentage (1.2%). The increased occurrence of oligomannosidic glycans has been explained by mislocalization of the 1,2-N-acetylgalcosaminyltransferase I and mannosidase II enzymes in the medial Golgi (32). Mutations in the a2 subunit of the vacuolar H+-ATPase (ATP6V0A2) (33, 34) cause impaired Golgi trafficking via altering the intracellular pH gradient and produce glycosylation abnormalities at several levels. Unlike MGAT2 and B4GALT1 mutations, Golgi-trafficking defects affect multiple steps in the N-glycan processing pathway, as can be observed by the wide range of truncated glycan structures.

The exclusion of secondary causes of underglycosylation still presents a major challenge in the diagnosis of CDG type II. Separate measurement of plasma sialidase in cases of HUS or repetitive blood sampling in cases of liver failure are required to obtain insight into the nature of an abnormal TIEF profile. Therefore, we searched glycan profiles for specific ions or general glycan features that could discriminate between primary and secondary defects. None of the individual glycan ions showed a marked difference; however, our analysis of general glycan features revealed that the level of fucosylation carries such a discriminating power. In patients with a glycosyltransferase or Golgi-trafficking defect, the fucosylation status was increased (31% to 40%), compared with the pediatric controls (reference range, 17%–34%). This result can be explained by the increase in truncated structures, for which the fucosy-
In summary, our improved method for plasma N-glycan profiling allows distinguishing between primary genetic defects in the N-glycosylation process, Golgi-trafficking disorders, and secondary causes of underglycosylation. Therefore, plasma N-glycan profiling by MS should be applied as a first diagnostic step for all samples from patients presenting with a type II TIEF profile. This step should greatly facilitate further biochemical and genetic confirmations for cases of type II CDGs.

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