High-resolution mass spectrometry glycoprofiling of intact transferrin for diagnosis and subtype identification in the congenital disorders of glycosylation

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Diagnostic screening of the congenital disorders of glycosylation (CDG) generally involves isoelectric focusing of plasma transferrin, a robust method easily integrated in medical laboratories. Structural information is needed as the next step, as required for the challenging classification of Golgi glycosylation defects (CDG-II). Here, we present the use of high-resolution nano liquid chromatography-chip (C8)-quadrupole time of flight mass spectrometry (nanoLC-chip (C8)-QTOF MS) for protein-specific glycoprofiling of intact transferrin, which allows screening and direct diagnosis of a number of CDG-II defects. Transferrin was immunopurified from 10 μL of plasma and analyzed by nanoLC-chip-QTOF MS. Charge distribution raw data were deconvoluted by Mass Hunter software to reconstructed mass spectra. Plasma samples were processed from controls (n = 56), patients with known defects (n = 30), and patients with secondary (n = 6) or unsolved (n = 3) cause of abnormal glycosylation. This fast and robust method, established for CDG diagnostics, requires only 2 hours analysis time, including sample preparation and analysis. For CDG-I patients, the characteristic loss of complete N-glycans could be detected with high sensitivity. Known CDG-II defects (phosphoglucomutase 1 (PGM1-CDG), mannosyl (α-1,6-)-glycoprotein β-1,2-N-acetylglucosaminyltransferase (MGAT2-CDG), β-1,4-galactosyltransferase 1 (B4GALT1-CDG), CMP-sialic acid transporter (SLC35A1-CDG), UDP-galactose transporter (SLC35A2-CDG) and mannosyl-oligosaccharide 1,2-alpha-mannosidase (MAN1B1-CDG)) resulted in characteristic diagnostic profiles. Moreover, in the group of Golgi trafficking defects and unsolved CDG-II patients, distinct profiles were observed, which facilitate identification of the specific CDG subtype. The established QTOF method affords high sensitivity and resolution for the detection of complete glycan loss and structural assignment of truncated glycans in a single assay. The speed and robustness allow its clinical diagnostic application as a first step in the diagnostic procedure for CDG defects. (Translational Research 2015;166:639–649)

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Abbreviations: ApoCIII = apolipoprotein CIII; ATP6V0A2 = ATPase, H+ transporting, lysosomal V0 subunit a2; B4GALT1 = β-1,4-galactosyltransferase 1; CDG = Congenital Disorders of Glycosylation; CE = Capillary electrophoresis; CMP = cytidine monophosphate; COG1 = component of oligomeric Golgi complex 1; CV = Coefficient of variation; DPAGT1 = dolichyl-phosphate (UDP-N-acetylglucosamine) N-acetylglucosaminephosphotransferase 1; EDTA = Ethylenediaminetetraacetic acid; ESI = Electron spray ionization; GDP = guanosine diphosphate; HPLC = High pressure liquid chromatography; HUS = Hemolytic uremic syndrome; IEF = Isoelectric focusing; LC = Liquid Chromatography; MALDI = Matrix assisted laser desorption ionization; MAN1B1 = mannosyl-oligosaccharide 1,2-alpha-mannosidase; MGAT2 = mannosyl(α-1,6-)-glycoprotein β-1,2-N-acetylglucosaminyltransferase; MS = Mass spectrometry; NHS = N-Hydroxysuccinimidyl; PGM1 = Phosphoglucomutase 1; PMM2 = phosphomannomutase 2; QTOF = Quadrupole Time Of Flight; SLC35A1 = CMP-sialic acid transporter; SLC35A2 = UDP-galactose transporter; SLC35C1 = GDP-fucose transporter; Tf = transferrin; TIEF = Transferrin Isoelectric focusing; TMEM165 = transmembrane protein 165; Tris = 2-Amino-2-(hydroxymethyl)-1,3-propanediol; UDP = uridine disphosphate; VPS13B = vacuolar protein sorting 13 homolog B

AT A GLANCE COMMENTARY

van Scherpenzeel M, et al.

Background

Congenital disorders of glycosylation (CDG) have grown to an important group of metabolic disorders with challenging diagnostics. Screening is performed worldwide using rather nonspecific isofocusing of transferrin. The main current challenge involves the identification of gene defects in CDG-II, which requires analysis of glycan structures.

Translational Significance

We report about a high-resolution mass spectrometry method for detailed structural glycan assignment. The speed and robustness allowed its application as the first step in the diagnostic procedure for CDG in our laboratory. For unsolved CDG-II patients we could observe discriminating glycoprofiles, which will facilitate identification of the cause of disease.

INTRODUCTION

Transferrin is an abundant plasma protein of 679 amino acids, important for iron transport. It is synthesized in the liver and its primary structure consists of 2 similar lobes, each containing a single iron-binding site. The protein contains 2 sialylated, biantennary complex type N-glycans attached to the glycosylation sites at Asn-413 and Asn-611.[1] Abnormal transferrin glycosylation is known as a biochemical marker for the congenital disorders of glycosylation (CDG) and alcohol abuse.[2-4] CDG form a group of inherited metabolic disorders with defects in protein and lipid glycosylation. The protein N-glycosylation defects are classified into 2 groups. CDG-I is characterized by defects in the assembly or transfer of the dolichol-linked glycan in the endoplasmic reticulum, whereas CDG-II involves processing defects of the glycan in the Golgi. To date, more than 100 genetic disorders resulting from mutations in glycosylation-related genes have already been uncovered.[5] The clinical phenotypes are highly heterogeneous. They range from relatively mild symptoms limited to 1 organ,[6] to a severe multisystem presentation and early death.[7] The clinical features are rarely pathognomonic for the underlying gene defect, and the diagnosis usually requires extensive biochemical and genetic investigations.[8]

For diagnostic screening of CDG, isoelectric focusing (IEF) of plasma transferrin (TIEF) is most commonly used.[2] Other methods make use of high pressure liquid chromatography (HPLC)[9] or capillary electrophoresis (CE).[10-12] Separation of transferrin isoforms is based on their charge, typically dominated by the terminal sialic acid residues on N-glycan structures. Mass spectrometry (MS) methods have been exploited for the analysis of transferrin glycosylation using CE-MS,[12] matrix assisted laser desorption ionization (MALDI)-MS,[13] and LC-electron spray ionization (ESI)-MS.[14] With these methods a loss of complete glycans can sensitively be detected as characteristic of CDG-I.[15] For specific diagnosis of CDG-II defects, structural information on the N-glycan is required. This can be obtained by mass spectrometric analysis of transferrin glycopeptides[16,17] or released plasma N-glycans.[18-21] These methods, however, are time-consuming and lack information on the loss of complete glycans, as required for CDG-I diagnostics.

Here, we present a fast and high-resolution method to profile the glycosylation of intact transferrin in a