# Functional diagnostics for congenital disorders of glycosylation from plasma glycopeptide PASEF-DDA data

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## Introduction

Molecular diagnostics is on the verge of implementing high-throughput functional Omics data in routine clinical practice for high-precision personalized healthcare. Glycoproteomics in blood plasma offers unique possibilities for clinical diagnostics by providing site-specific glycosylation data for up to hundreds of proteins by a single measurement. Since both biomarker discovery and diagnostics can be performed using the same holistic data, the use of PASEF-DDA effectively eliminates the tedious process of developing and applying different methods for untargeted biomarker discovery and target biomarker measurement. Here, we share preliminary results for diagnosis of Congenital Disorders of Glycosylation (CDG) by targeted data extraction from holistic PASEF-DDA data.

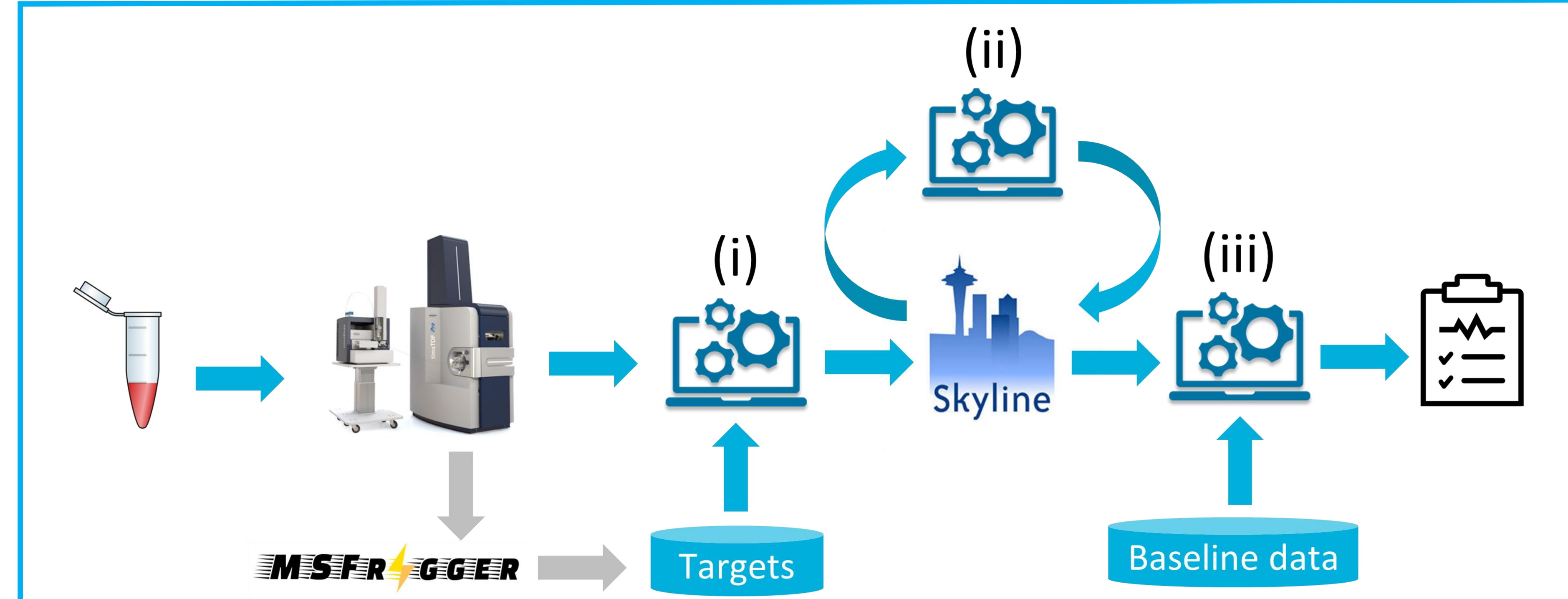


Fig. 1: Schematic overview of the diagnostic glycoproteomics workflow with indicated automation steps for (i) data transfer and workflow starter, (ii) Skyline peak extraction evaluation, and (iii) downstream data analysis and reporting.

## Methods

Samples: Full blood plasma samples from healthy donors and patients with congenital disorders of glycosylation (CDG) were analyzed by glycoproteomics and current CDG diagnostics using intact Transferrin immuno-purification mass spectrometry. For glycoproteomics, samples were subjected to tryptic digestion and enriched for glycopeptides using Sepharose CL-4B beads. LC-IMS-MS/MS: Glycopeptide fractions were analyzed by liquid chromatography with online tandem mass spectrometry (timsTOF pro2, Bruker Daltonics) using in-source supercharging with acetonitrile (nanoBooster). Peptides were separated using a 0.075 x 150mm C18 RP column (Bruker FIFTEEn) at 45° C with a 25 minutes linear gradient of 7-45% acetonitrile in 0.1% formic acid 0.02% trifluoroacetic acid. Data-dependent PASEF acquisition was performed using optimized instrument parameters for glycopeptide analysis. Samples were measured in randomized order in duplo with pooled controls samples of 5 random healthy donors at beginning, middle, and end of batch for quality control purposes. Data analysis: We have established an automated data analysis pipeline that uses Skyline to extract intensity data for a versioned list of glycopeptide targets based on available MS Fragger glycopeptide identification results. A graphical user interface is used for (i) secure data transfer and to start the computational workflow for batches of samples. Following Skyline processing an in-house developed algorithm is used to (ii) detect possible incorrect peak extractions by SkyLine for manual review and correction. Subsequent downstream processing (iii) generates a diagnostic report that summarizes analytical performance, site-specific glycosylation profiles & glycan trait distributions, and patient-specific differentials compared to baseline glycoproteome information from healthy donors.

## Results

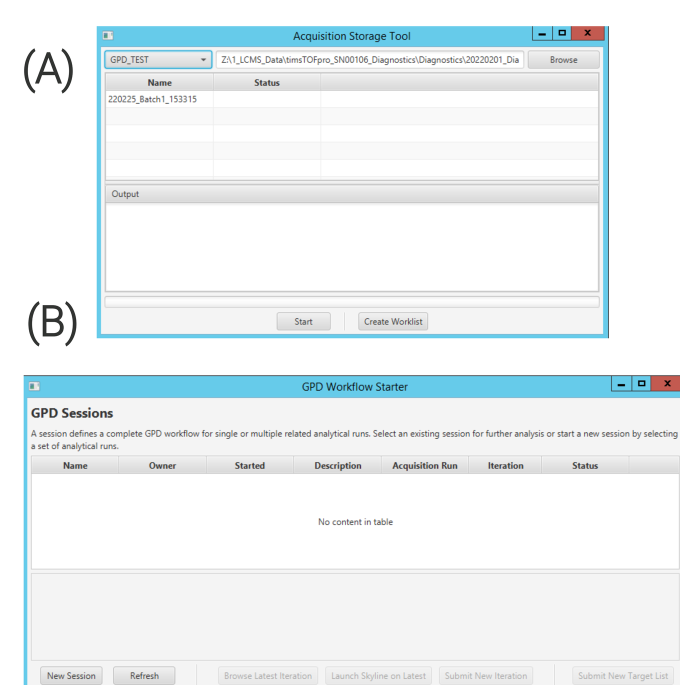


Fig. 2: Graphical user interfaces for automated digital workflow control. (A) GUI for secure data and metadata transfer from acquisition computer to network attached storage. (B) GUI for batch workflow control by the high-performance computing cluster.

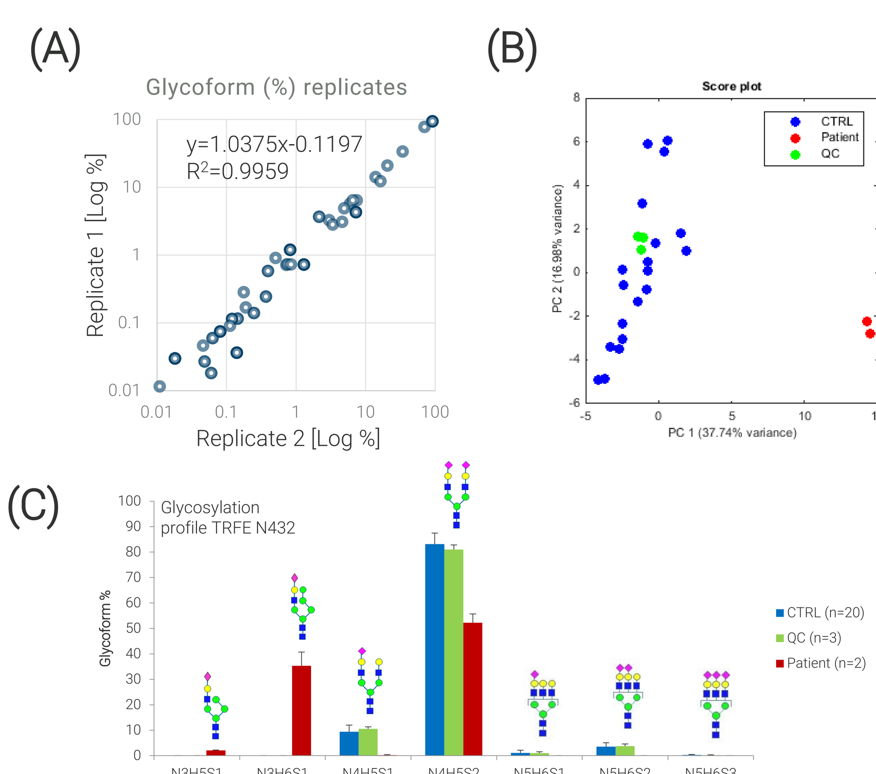


Fig. 3: Examples of clinical test report elements. (A) Glycoform % scatterplot between QC replicates. (B) PCA score plot of control samples, QC replicates, and MAN1B1-CDG patient replicates. (C) Site-specific glycoform distribution at N432 of Transferrin from a MAN1B1-CDG patient next to controls and QC replicates.

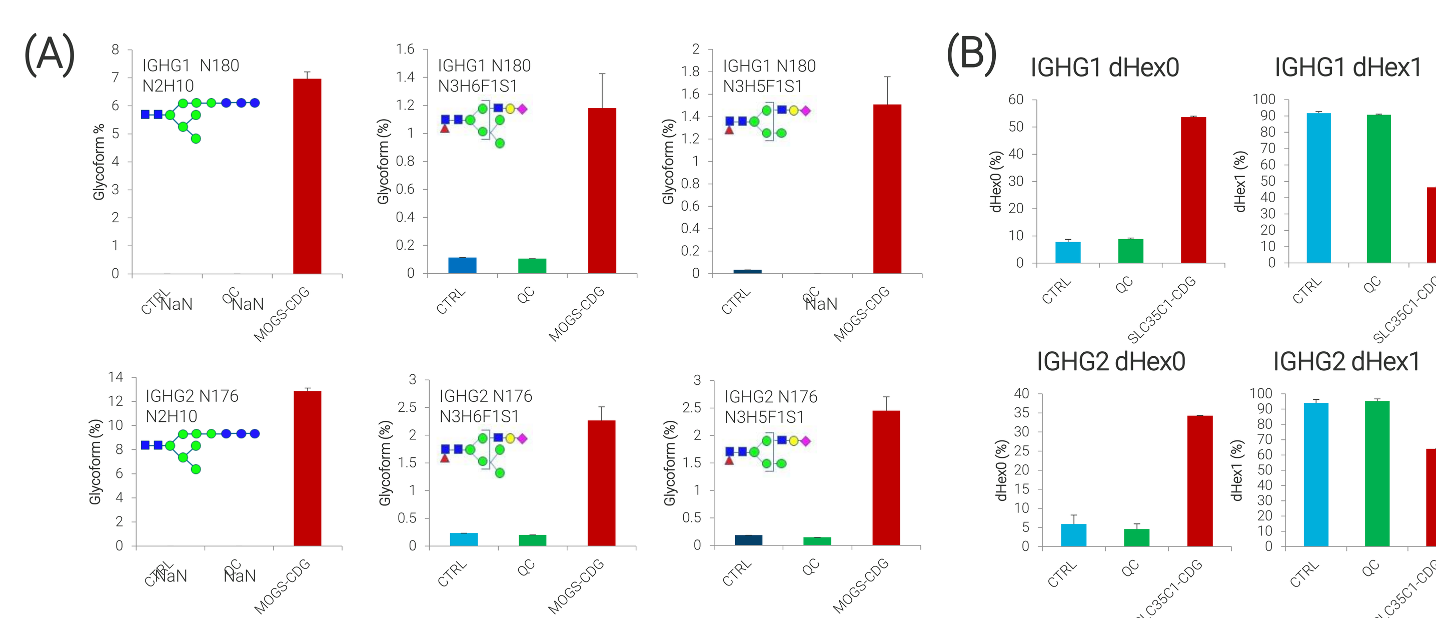


Fig. 4: Glycoproteomics enables functional diagnosis for CDG patients with normal Transferrin glycosylation that are missed by current diagnostics. (A) Diagnostic HexNAc2Hex10 – HexNAc3Hex6dHex1NeuAc1 - HexNAc3Hex5dHex1NeuAc1 glycoform percentages at Immunoglobulin heavy constant gamma 1 & 2 from a MOGS-CDG patient next to baseline and QC data. Mannosyl-oligosaccharide glucosidase (MOGS) is responsible for trimming glucose from GlcNAc2Man9Glc3 at newly synthesized N-glycoproteins. (B) IGHG1 and IGHG2 dHex0 and dHex1 levels (%) from a SLC35C1-CDG patient next to baseline and QC replicate levels. Solute carrier family 35 member C1 (SLC35C1) transports GDP-fucose into the Golgi for subsequent fucosylation (dHex) of N-glycan biosynthesis products.

## Conclusion

- Holistic PASEF-DDA approaches can be implemented for patientcare in routine clinical environments
- Site-specific multi-protein glycosylation data minimizes the risk of missed diagnoses due to tissue-, protein-, or site-specificity in disease
- Analytical and clinical validation are ongoing to finalize implementation of this glycoproteomics workflow as ISO15189 certified clinical test in CDG patientcare
- In 2021 the EnFORCE project started to develop an integrated glycopeptide identification, quantification, and diagnostic test reporting workflow via real-time data processing on the PaSER platform

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Glycoproteomics

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