Detection of HNF1A MODY diabetes with glycan biomarkers

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Introduction

Glycosylation is one of the most important co-/post-translational modifications. Glycans - oligosaccharide chains covalently attached to proteins through either N- or O-glycosidic bonds – are involved in most biological processes. Aberrant glycosylation has been associated to many pathological conditions in humans. Very often, glycosylation changes occur already before any symptoms of a disease are present. It proves the potential of using these changes as biomarkers for better prognosis, prediction and diagnosis.

Maturity onset diabetes of the young HNF1A (MODY 3) is the most frequent form of autosomal dominant monogenic diabetes. MODY is caused by mutations in the HNF1A gene and accounts for around 1–2% of all diabetes cases. Genetic testing is the most common form of testing for MODY, however the cost and its unavailability in some countries lead to over 80% of MODY subjects remaining misdiagnosed. As genome-wide association studies (GWAS) indicated, certain mutations in the HNF1A gene alter N-glycan outer arm fucosylation levels. Therefore, MODY 3 is an example of a disease where a glycan biomarker could be used in the identification and stratification of these patients.

This study focuses on 1) the identification of antennary fucosylated plasma N-glycans using an LC-MS/MS approach supported by exoglycosidase digestion; 2) further testing of these glycans and their various combinations for the diagnostic accuracy as potential diagnostic biomarkers. We have analyzed a cohort of 346 diabetes patients including 3 groups with different mutation types in the HNF1A gene.

Method

Automated N-glycan release, clean up and labeling. 4 µL of human blood plasma was used for glycan release by overnight treatment with PNGase F (NEB; UK). A protein binding plate (LC-PBM-96; Ludger Ltd; UK) was used to clean-up the samples which were later conjugated to a fluorescent procainamide label (LT-KPROC-24; Ludger Ltd, UK) by a reductive amination reaction. Unreacted procainamide dye was removed using a glycan labeling cleanup plate (LC-PROC-96; Ludger Ltd; UK).

Exoglycosidase digestions. The labelled glycans were treated with either a1-3,4>2 fucose-specific recombinant exoglycosidase or α1-6>2,3,4 fucose-specific bovine kidney a-L-fucosidase (BKF; Sigma) and were then purified using a HILIC membrane cleanup plate (LC-PROC-96; Ludger Ltd; UK).

HILIC-LC-MS/MS analysis of PROC-labelled glycans. The samples were injected onto a core-shell HALO 2 Penta-HILIC column [2.0 µm, 2.1 x 150 mm] (AMT; USA) at 40 °C on a Dionex Ultimate 3000 UHPLC instrument with a fluorescence detector (ex = 310 nm and em = 370 nm) attached to a Bruker Amazon Speed ETD (Bruker Daltonics; Bremen, Germany).

LC-MS/MS data processing. Bruker Compass DataAnalysis version 4.1 and Bruker ProteinScape version 4.0 software were used to analyse mass spectrometry data. HPLC data processing and quantitation was performed in a systematic and highly repeatable manner using an open source HappyTools software (version 0.0.2).

Results

LC-MS/MS and exoglycosidase digestions for glycan structure assignment

Retention times and mass detection were used for structure assignment **(figure 1).** Exoglycosidase digestions, together with MS/MS data examination, were applied to fully distinguish the presence of either outer-arm or core fucose.

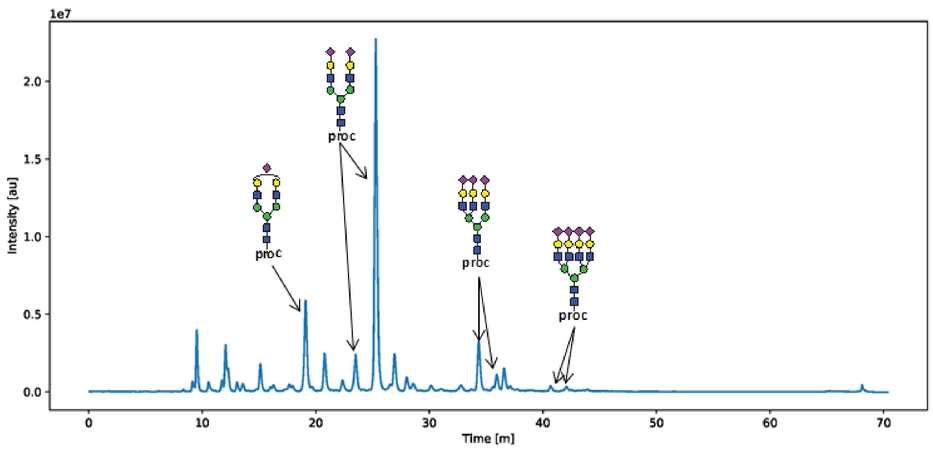

***Figure 1**: A typical fluorescence chromatogram of released procainamide-labelled plasma N-glycans. The Penta-HILIC column allows to separate glycans depending on their size and the degree of sialylation.*

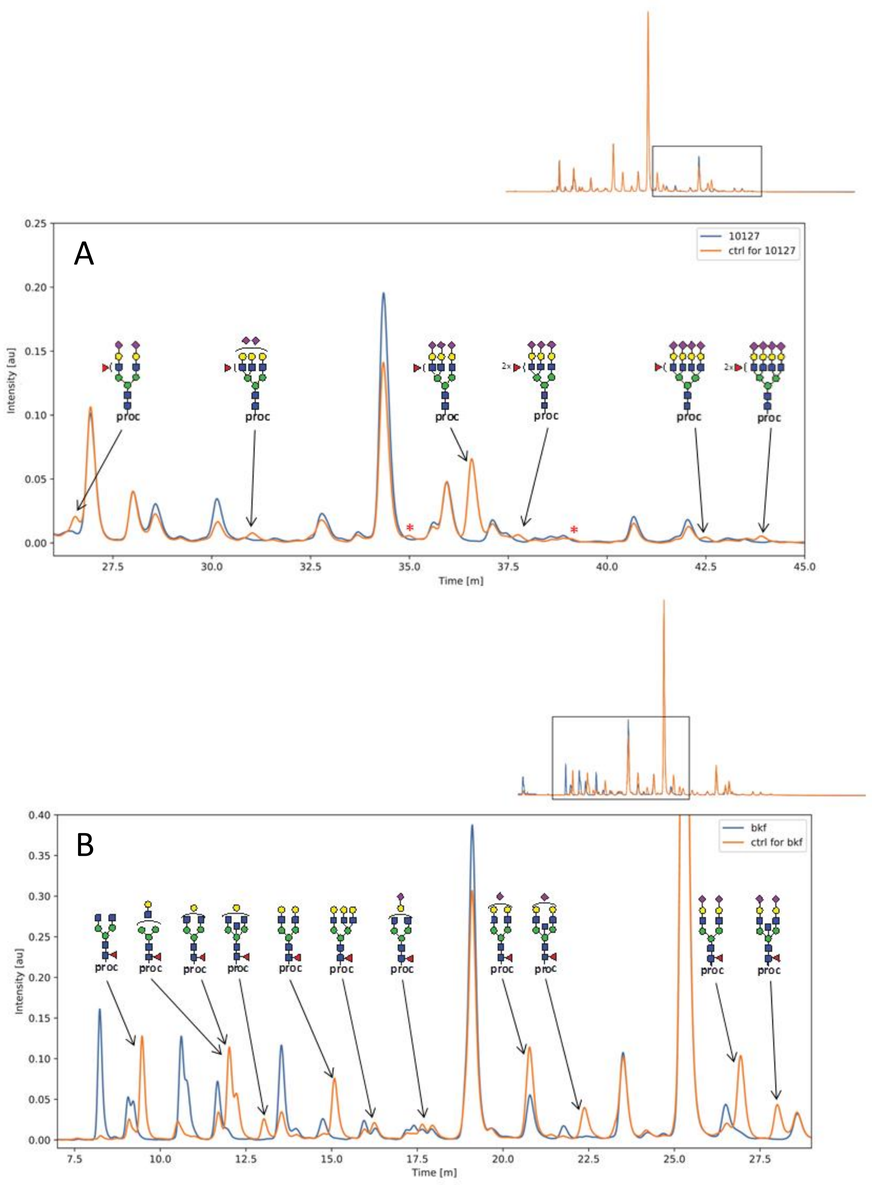

***Figure 2**: A fluorescence chromatogram obtained after 10127 exoglycosidase treatment presenting 6 identified antennary fucosylated procainamide-labelled N-glycan structures **(A)** and BKF exoglycosidase treatment presenting core fucosylated procainamide-labelled N-glycan structures **(B)**. Chromatograms of enzymatically treated samples (orange) were overlaid with control samples (blue) and normalized to the highest peak (Figure 2A and 2B). Zoomed areas show the glycan structure peak shifts.*

Performance of glycan biomarkers in differentiating HNF1A mutation types

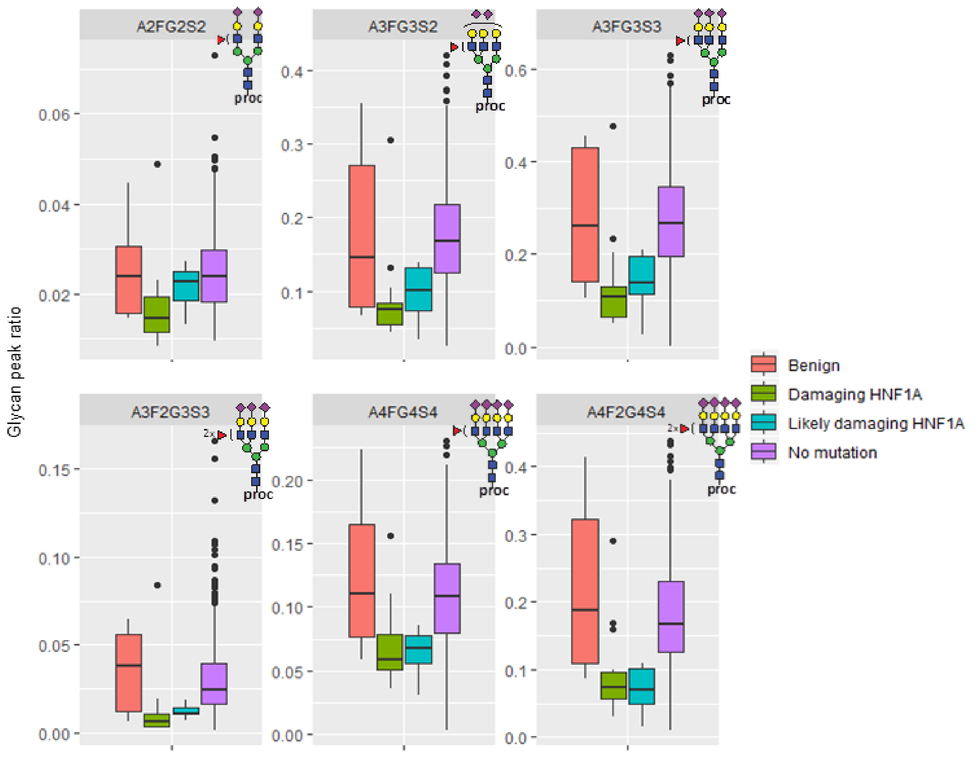

***Figure 3**: Box plots illustrating the performance of each antennary fucosylated glycan structure in differentiating subjects with different mutation types in HNF1A gene. Glycan peak ratio values were calculated as a ratio of an antennary fucosylated glycan peak to a sum of this peak and its equivalent non-fucosylated structural peak .*

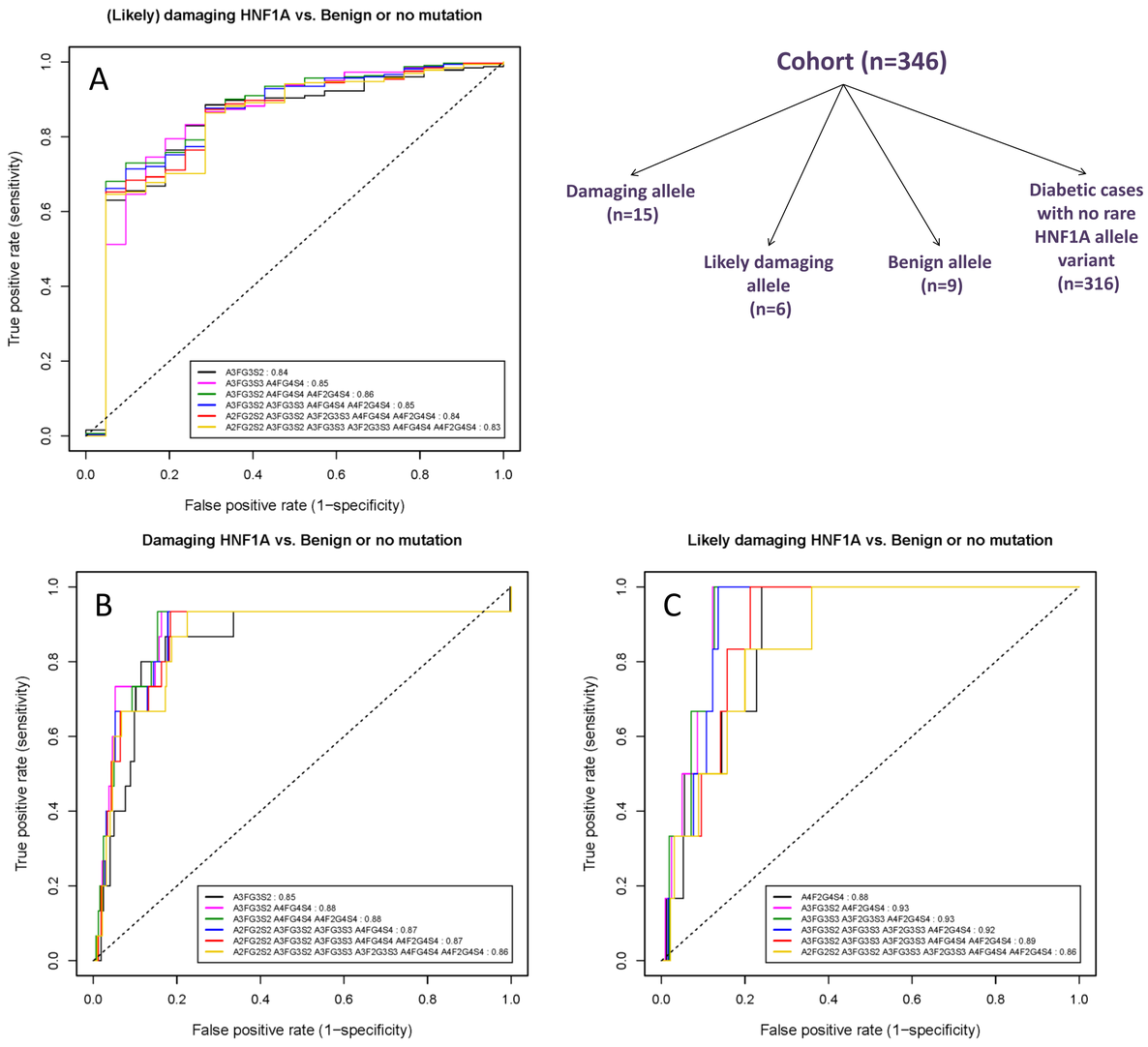

***Figure 4**: ROC curves illustrating the performance of glycan biomarkers in differentiating subjects with (likely) damaging **(A)**, damaging **(B)** and likely damaging **(C)** mutation type from diabetic subject group with benign or no mutation in HNF1A gene. The AUC values are displayed for the best performing single glycan structure and each 2 to 6 glycan length combination.*

Conclusions

A diabetes cohort including groups with different types of mutation in the HNF1A gene was used in order to evaluate differentiating performance of various glycan structures.

An improved HPLC separation using the HALO 2 Penta-HILIC column allowed for the identification of 6 antennary fucosylated glycan structures, in turn allowing better evaluation of glycan biomarkers for use in the differentiation of HNF1A mutation groups.

As shown (**figure 3 and 4)**, single antennary fucosylated glycan structures and their various combinations can be successfully used as biomarkers for discrimination of subjects with HNF1A mutations manifesting a decreased level of N-glycans with outer-arm fucosylation. A glycan combination with an AUC of 0.86 provides very good discrimination between (likely-) damaging and non-damaging / no mutation diabetic cases **(figure 4A)**.

We have presented an innovative way to test glycan features for potential biomarkers in discrimination of subjects with damaging mutations in the HNF1A gene. It was shown that a combination of glycan structures performs better than a single glycan as a diagnostic biomarker with an AUC of 0.86 vs. 0.84, respectively **(figure 4A).**

Simplifying the LC-MS assay to a microplate-based assay is the next step towards application of the glycan biomarker in clinical practice.

References

1. Shields BM, Hicks S, Shepherd MH, et al. “Maturity-onset diabetes of the young (MODY): how many cases are we missing?” Diabetologia. 2010;53(12):2504–2508
2. Lauc G, et al. “Genomics meets glycomics-the first GWAS study of human N-Glycome identifies HNF1α as a master regulator of plasma protein fucosylation.” PLoS genetics vol. 6,12 e1001256. 23 Dec. 2010

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