Detection of HNF1A MODY diabetes with glycan biomarkers

Introductions

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. Ablent glycosylation has been associated with various 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 glycans biomarker could be used in the identification and stratification of these patients.

This study focuses on 1) the identification of antenatal 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-PILOT-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 α1-3,4 fucose-specific recombinant exoglycosidase or α1-6,1-2,3,4 fucose-specific bovine kidney α1-fucosidase (BFK, Sigma) and 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, 1.5 x 150 mm) (AB Sciex) at 40 °C on a Dionex Ultimate 3000 UHPLC instrument with a fluorescence detector (exc = 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 Proteo发现e 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.

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 in 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 MODY 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 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 glycans biomarker in clinical practice.

References


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