Alterations in N-glycome composition during human fetal development

Martina Vinicki[1], Peng Liu[2], Gordan Lauc[1,3], Paul A. Fowler[2], Tamara Štambuk (tstambuk@genos.hr)[1*]

[1]Genos Ltd, Glycoscience Research Laboratory, Borongajska cesta 83H, Zagreb, Croatia
[2]The Institute of Medical Sciences, School of Medicine, Medical Sciences and Nutrition, University of Aberdeen, Aberdeen, UK.
[3]Faculty of Pharmacy and Biochemistry, University of Zagreb, Ante Kovačića 1, Zagreb, Croatia.

Introduction

Human fetal immune system development, particularly in the 1st and 2nd trimesters, is poorly understood. During pregnancy, in-utero exposure to drugs and chemicals through maternal smoking, alcohol consumption, drug abuse, prescription medications, and environmental pollutants is widespread. These exposures have potential lifelong consequences for:

- major health conditions
- fetal immune development
- fetal metabolic programming

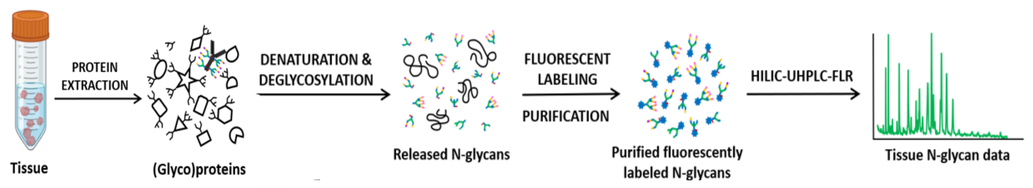
Understanding normal developmental processes and the impact of prenatal exposures on the fetal immune system and metabolism is essential.

The dynamics of protein glycosylation changes during fetal development are virtually unknown and may affect its response to exposures. The glycome, the complete set of glycans attached to proteins, affects their functioning and is shaped by genetic, epigenetic, and environmental factors. Altered glycosylation may indicate pathological changes and serve as biomarkers and functional regulators of physiological processes.

Materials and Methods

The composition of the N-glycome was analysed in 71 paired placenta and liver protein extracts (142 protein extracts in total) from fetuses at 7-20 weeks of gestation. These samples were collected from normally progressing, selectively terminated pregnancies as part of the Scottish Advanced Fetal Research Project (SAFeR) study (REC 15/NS/0123). Proteins were extracted from female (n=36) and male (n=35) placenta and liver samples and their glycosylation was investigated using hydrophilic interaction ultra-high-performance liquid chromatography (HILIC-UPLC) analysis. To make measurements across samples comparable, normalization by total area was performed. Batch correction was performed on log-transformed measurements using the ComBat method. Analyses of associations between clinical traits and glycan traits were performed using a general regression model. The false discovery rate (FDR) for analyses was controlled using the Benjamini-Hochberg procedure.

Workflow



Results

Analysis revealed extensive significant associations between gestational age and liver and placenta glycosylation patterns. Specifically, statistically significant differences in 6 out of 16 glycan-derived traits was observed in the liver tissue samples and in 10 out of 16 glycan-derived traits in the placenta tissue samples analysed. At the level of directly measured, initial glycan peaks, the differences in glycome composition related to gestational age were even more pronounced, with 20 out of 45 liver tissue glycans significantly altered and 40 out of 72 glycan peaks significantly altered in placenta tissue samples.

Our data also show more noticeable changes in the proportion of different glycan structures between different tissues in examined trimesters, than were observed within the same tissue during this period.

Figure 1. The differences in N-glycome composition in liver (L) and placenta (P) protein extracts related to gestational age from both male and female fetuses at first (red box) and second (green box) trimester with statistically significant changes (green square).
((LB - low branching, HB – high branching, G0 – agalactosylated, G1 – monogalactosylated, G2 – digalactosylated, G3 –trigalactosylated, G4 – tetragalactosylated, S1 – monosialylated, S2 – disialylated, S3 – trisialylated, B – bisected, CF – core fucose, AF – antennary fucose, HM – high manose, PM – paucimannose, Hy – hybrid) N-glycans).

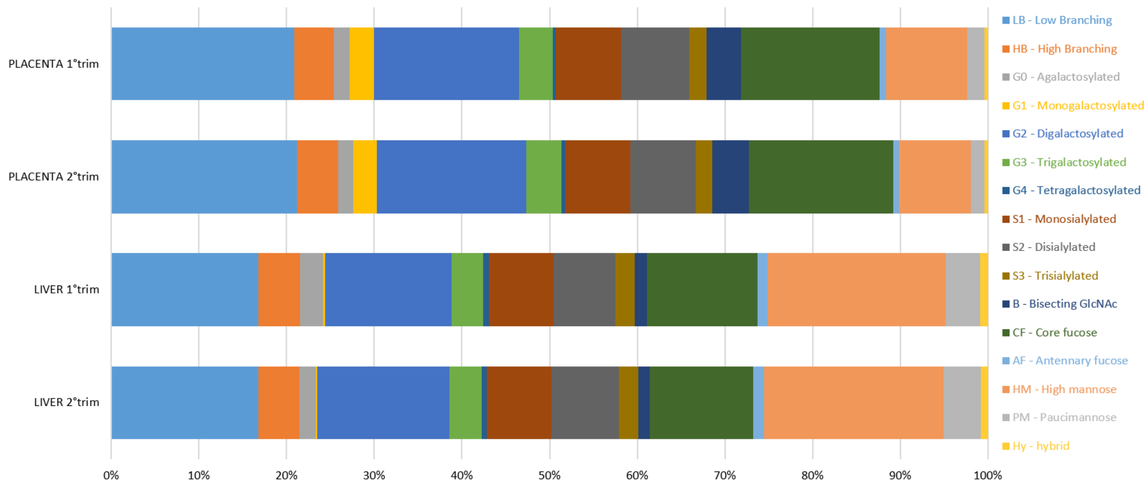


Figure 2. Proportion of different glycan structures in the total placenta/liver N-glycome in first and second trimester.
((LB - low branching, HB – high branching, G0 – agalactosylated, G1 – monogalactosylated, G2 – digalactosylated, G3 –trigalactosylated, G4 – tetragalactosylated, S1 – monosialylated, S2 – disialylated, S3 – trisialylated, B – bisected, CF – core fucose, AF – antennary fucose, HM – high manose, PM – paucimannose, Hy – hybrid) N-glycans).

Conclusion

The observed striking glycosylation changes could potentially reflect the fact that many new glycoproteins emerge as the tissue development progresses. Given the essential role of glycans in almost all biological processes, this area of research opens up new avenues to explore mechanisms of fetal programming effecting long-term health outcomes, and warrants further investigation.

Acknowledgement

This research has been supported by EU Horizon Europe Program grant INITIALISE (grant no: 101094099).

www.genos-glyco.com

SAFeR study