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**Project abstracts:**

**Software for automated glycan assignments of unknown samples**

The aim of this project is to develop a software which would be able to automatically identify all *N*-glycans as well as the relative or absolute amount of each individual glycan present released from glycoprotein/mix of glycoproteins from unknown samples (the glycans will not necessarily be present in any database) using data from liquid chromatography (Hydrophilic interaction liquid chromatography - Ultra-performance liquid chromatography, HILIC – UPLC) and exoglycosidase digestions, validated with mass spectrometry (MS) data.

This will be based on already generated preliminary data in collaboration with Dr Alican Noyan from Ipsumio, where the basic script for the main steps involved in this process was generated. This preliminary work requires further refinement to enable more detailed glycans analyses, as only more common glycans were initially included. We will also include all exoglycosidase digestions as well as include the calculations of percentage areas of all glycans in the given peaks.

This software has the potential to not only automate very time consuming and laborious manual assignments, but also to deliver a solution to both academic and industrial laboratories which carry out glycan analyses which may significantly reduce the time required for data analyses. Detailed glycan analyses are important for the characterization of biopharmaceuticals. In-depth glycan analysis is also a critical consideration for Advanced therapy medicinal products (ATMPs) such as cell and gene therapies, which are certainly more complex and for which the glycan analyses are more challenging in comparison to monoclonal antibodies. After this software is developed for the *N*-glycans, it can be further modified to automatically analyse other classes of glycans, for example *O*-glycans, free glycans or glycans from glycolipids.

**The development of a high throughput platform to analyze Glycosphingolipid derived (GSL) glycans from human breast milk samples.**

Human milk (HM) is the preferred vector for delivering nutrition to a diverse gut microbiota. Human milk contains 3-5% fat, which provides 40-50% of total energy to the infant. The lipid fraction contains a low abundant group of bioactive glycosphingolipids (GSLs) that are positively associated with neurological development, immune modulation, and antimicrobial activity in infant development. Glycosphingolipids (GSLs) are a heterogeneous group of membrane lipids that comprise a ceramide backbone covalently linked to a glycan moiety. The diversified structure and composition of these glycan headgroups plays a significant role in how these molecules are utilized in the infant gut and potentially modulate gut colonization. Studying the impact of these glycosylated molecules on infant thriving and microbial transfer across large cohort studies is still at a very early stage.

The overall objective of this study is to develop, optimize and validate a semi-automated high throughput (HT) methodology for the detailed profiling of these conjugated glycans. This method will be used to structurally characterize the total glycan content of globosides, isoglobosides, lactose conjugated and cerebroside glycolipid molecules derived from HM samples collected from 81 breast feeding mothers as part of a trial designed to study the impact of probiotic supplementation on microbial transfer (ISRCTN53023014). This data would be analysed with respect to the Secretor and Lewis genotypes and the microbial transfer data available for each participant as part of the Microbemom Spokes research project.

The microbial seeding and colonization events in the first year of life determine an immune and metabolic infrastructure that have a profound, lifelong effect on host health. The results of this study will provide a much-needed platform for the analysis of GSL derived glycans and elucidate the diversity of the HM glycolipid content with respect to the development of a healthy gut microflora and further assess the potential for GSL derived glycans to be used as new additives for improved infant formula.

**Challenges here-** the samples we currently have are from MicrobeMom consortium, namely NMH- they do not allow to use the research on their samples to be used by companies to develop formulas. Therefore, we either need samples we could use for the formula purpose or academic funding.

We could also look at glycans from GSLs from cancer samples as potential cancer biomarkers, so the method would be really useful to develop.