POSTER OF DISTINCTION

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SYNTHETIC HUMAN MILK OLIGOSACCHARIDES PREVENT EXPERIMENTAL NECROTIZING ENTEROCOLITIS VIA DIVERGENT TRANSCRIPTOMIC RESPONSES.

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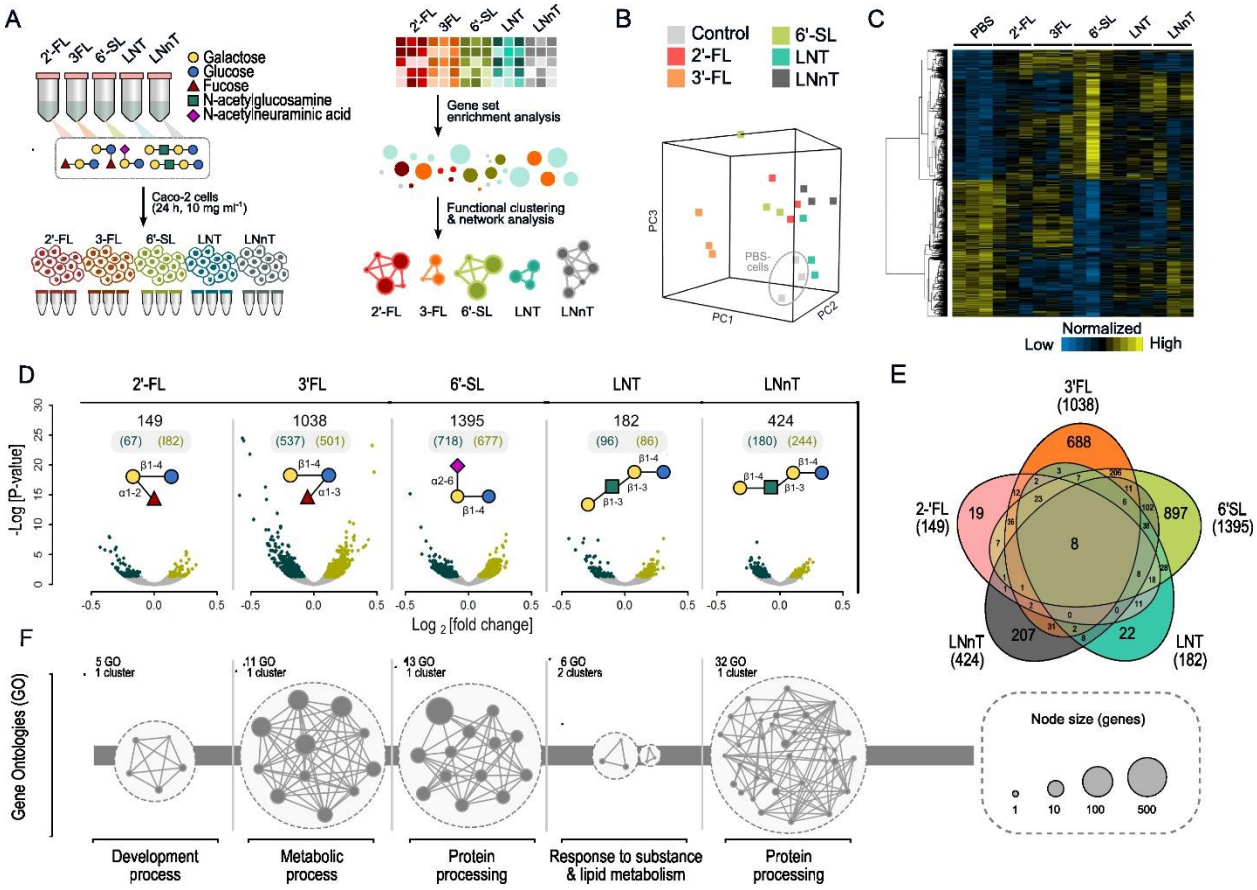
Background: Breastmilk reduces the risk of necrotizing enterocolitis (NEC) in preterm infants, but the bioactive components mediating this effect are not well understood. Human milk oligosaccharides (HMOs) reduce NEC both in humans and in relevant animal models. However, it is unclear if there are functional differences between individual oligosaccharides.
Aims: The objective of this study was to compare the intestinal transcriptome responses of individual HMOs using complementary *in vitro* and *in vivo* models of NEC.
Methods: RNA sequencing was performed on Caco-2Bbe1 gut epithelial cells after exposure to commercially-purified 2'-fucosyllactose (2'FL), 3-fucosyllactose, 6'-siallyllactose, lacto-N-tetraose (LNT) or lacto-N-neotetraose for 24hr at 37°C for 24 h (n=3). Signaling pathways were analyzed in murine- and human-derived NEC enteroids by qPCR. To validate these findings, five-day-old mouse pups were orally gavaged formula with or without individual HMOs, followed by NEC induction with hypoxia (5% O_2, 95% N_2) and lipopolysaccharide (4 mg/kg/day). Coded ileal sections (n=6-7/group) were analyzed for mucosal injury by histology, immune fluorescence, immunohistochemistry, and gene expression via qPCR.
Results: The HMO transcriptome clustered into divergent functional categories including metabolic process, protein processing and responses to external stimuli. Each synthetic HMO induced a unique transcriptome and exhibited varying effects on the intestinal epithelial functions and biological pathways. This was confirmed in the murine model of NEC, as both LNT and 2FL mitigated NEC injury with comparable recovery of intestinal cell proliferation (Ki67) and expression of stem cells (Lgr5+). Both qPCR and immunofluorescence staining showed differences between 2FL- and LNT-fed pups in host inflammatory and immune responses.
Conclusions: This study demonstrates that synthetic HMOs ameliorate intestinal injury in experimental NEC. However, the mechanisms by which individual oligosaccharides act on the intestine differ, suggesting that single synthetic HMOs

may not fully recapitulate the benefits of pooled HMOs. Future studies will further delineate structure-function relationships of synthetic HMOs on host intestinal innate and adaptive immune responses.



A. Experimental design of RNA-sequencing experiment using Caco-2Bbe1 cells. B-C. Raw heatmap and PCoA analyses comparing the cell transcriptome respones of individual synthetic HMOs. D-F. Individual synthetic HMOs modulate distinct intestinal gene signatures and molecular pathways.

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