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Bergström, Anders; Skov, Thomas; Bahl, Martin Iain; Roager, Henrik Munch; Fleischer Michaelsen, Kim; Licht, Tine Rask

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Introduction
We have developed a qPCR-based array (GUT Low Density Array, GULDA), which simultaneously determine the relative abundance of >30 different bacterial 16S rRNA gene targets in a given DNA-sample covering selected phylogenetic lines (Bergström et al., FEMS Microbiology Letters 337, 1, 36-47, 2012). GULDA was applied to fecal DNA from 330 healthy Danish infants, sampled at 9, 18 and 36 months after birth, enabling characterization of interbacterial relationships by multivariate data analysis (Principal Component Analysis), univariate data analysis (ANOVA, t-tests) and non-parametric pairwise Spearman correlations. Interpretation of these patterns in relation to previously determined nutritional, anthropometrical (growth indices), and blood sampled parameters was used to increase understanding of gut microbial physiology. Particular emphasis was given to possible early life microbiota biomarkers of obesity, given the correlation of early life overweight with adult obesity and related lifestyle diseases. Few studies have undertaken similar longitudinal and multiparametric analysis for such numerous participants. The concomitant measures on bacteria from all relevant phyla on multiple taxonomic levels give a unique possibility for recognition of gut bacteria clusters. Due to study dropout, non-compliance and failed fecal DNA purifications, at total of 648 fecal samples were available for the analysis (samples from each of the three age groups).

With exception of breastfeeding and certain obesity indices (Figures 6 and 7), we found quite few consistent correlations between the gut bacteria and the physiological parameters, hence the primary focus of the current presentation is on the interbacterial correlations.

Figure 1 – Principal component analysis (PCA) of the GULDA microbiota. Upper plot-Individuals (Scores); Lower plot-Bacteria (Loadings). This figure shows the two primary principal components, PC1 and PC2, which explain approximately 50% of data variation. Only individuals who completed all three fecal samplings were included, giving a total of 396 samples from 132 individuals. There is a strong temporal development moving along PC1 from left to right, moving from 9 to 36 months, and a moderate temporal development moving along PC2, from top and down. Higher density of 9 months samples with corresponding fewer bacterial species relative to 18 and 36 months evident. Bacteria codes (see Figure 3).

Figure 2 – Temporal development of the gut microbial composition log2(Fold change) of all GULDA bacteria from 9 to 18 months, 18 to 36 months, and 9 to 36 months. Overall the majority of changes in the gut composition take place between 9 and 18 months with less change occurring between 18 and 36 months. Specific increases were seen for most of the targeted Bacteroides, C. leptum, E. hallii, Roseburia spp., Desulfovibrio spp. and A. muciniphila, while decreases were observed for lactobacillus and bifidobacteria dominated microbiota with larger significant correlations(*) are shown. Only the phylum level targets and bacteria giving significant correlations(*) are shown. Positive correlation between ∆BMI and specific Clostridia from 9 to 18 months, 18 to 36 months, and 9 to 36 months with corresponding P values and corresponding False Discovery Rates (FDR). Numbers denote p value of Mann-Whitney statistical test between the gut microbiota fold changes. Only the phylum level targets and bacteria giving significant correlations(*) are shown. Positive correlation between ∆BMI and fold change increase of F16, F2, and F3 between 18 and 9 months suggest that, C. leptum and E. hallii both contributes of indigestible dietary carbohydrates (polysaccharides) to monosaccharide’s and short-chain fatty acids (SCFA) may be specifically relevant, considering the correlation of overweight in this life stage with adult obesity.

Conclusions
We found significant developments in the gut microbiota from 9 to 18 months, where cessation of breastfeeding and introduction of a Westernized diet induces replacement of a simpler, less diverse lactobacillus and bifidobacteria dominated microbiota with larger Clostridia (with polysaccharide preference) and Bacteroides (with animal fats, protein preference) targets. Moreover, we report the earliest signs of enterotype segregation as the development of microbiota characterized by either high or low relative levels of Bacteroidetes/Prevotella, seems to take place between 9 and 36 months. Correlations between △BMI and specific Clostridia from 9 to 18 months and △BMI and a tendency to a shift to the Prevotella-rich enterotype from 18 to 36 months may indicate specific carbohydrates to be of interest in relation to obesity development.

Figure 7: Temporal changes in abundance of bacterial groups and correlated changes in △BMI
Spearman regression analysis of the relative differences in △BMI from 9 to 18 months, 18 to 36 months, and 9 to 36 months with corresponding bacteria fold changers. Only the phylum level targets and bacteria giving significant correlations(*) are shown. Positive correlation between △BMI and fold change increase of F16, F2, and F3 between 18 and 9 months and 9 to 18 months suggest that, C. leptum and E. hallii both contributes of indigestible dietary carbohydrates (polysaccharides) to monosaccharide’s and short-chain fatty acids (SCFA) may be specifically relevant, considering the correlation of overweight in this life stage with adult obesity.

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Anners Bergström1, Thomas Skov2, Martin Iain Bahl1, Henrik Munch Roager1, Kim Fleischer Michaelsen2, Tine Rask Licht1
1DTU, National Food Institute, Denmark; 2University of Copenhagen, Faculty of Science, Denmark