Novel insights into fetal spina bifida pathogenesis through exploration of the maternal gut microbiome

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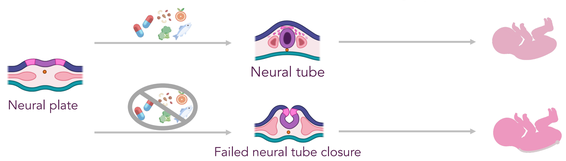
BACKGROUND

- Fetal neural tube defects (NTDs) are on the rise, driven by the most common NTD, **spina bifida**

1 in 2500 pregnancies are affected by NTDs per year in Canada

1500 fetuses per year are born with spina bifida in North America

- Low FA & B12 intake are associated with NTDs, including spina bifida



- Spina bifida can lead to developmental disorders in later life, including:

PROBLEM

- Current efforts to prevent spina bifida, including supplementation & fortification with FA/B12, are **not adequate**
- NTDs still occur when maternal folate/B12 levels are **sufficient**
- The mechanisms underlying metabolism, production, uptake & transport of folate are **poorly understood**…
  …this may explain, in part, why NTDs still occur

OTHER MECHANISMS?

- The *gut microbiome* is a key mediator of host micronutrient status
- Gut microbes are responsible for:

- Yet, the maternal gut microbiome has not been studied in the pathogenesis of spina bifida

HYPOTHESIS & AIMS

- We hypothesize that mothers carrying a fetus with spina bifida will have a dysfunctional gut metagenome, shown by altered expression of biosynthesis pathways key for neural tube closure
- We aimed to determine whether and how the gut metagenome of pregnant people carrying a fetus with spina bifida (cases; n=15) differed compared to those carrying a healthy fetus (controls; n=18)

METHODS

Maternal rectal swabs were collected for gut microbial composition at 25 weeks' gestation, bacterial DNA were sequenced (Illumina MiSeq) & reads were taxonomically & functionally classified.

Taxonomic quantification & analysis

Differences in relative abundances of bins between & within groups was assessed (ALDEx2) to identify significantly enriched taxa in cases & controls (effect size > [0.05]).

KEGG biosynthesis pathway analysis

Dereplicated bins were annotated (KEGG) and analyzed (Anvi'o v.7.0) to estimate the functional capabilities of bacterial taxa.

Dietary recall questionnaire & clinical data

Maternal medical history was obtained at recruitment & a 24-hour dietary recall questionnaire (ASA24™) was administered at time of sample collection.

Statistical analysis

Clinical & dietary data were analyzed & correlations between taxa relative abundance & clinical & dietary data were conducted (JMP v. 15.2.1; FDR *q* value < 0.05).

RESULTS

Cases reported lower dietary intakes of key micronutrients

Table 1. Maternal cohort characteristics & dietary intake data for cases and controls.

	Controls (n=18)	Cases (n=15)	p-value
BMI (kg/m²)	22.4	25.0	0.03
Gestational age at birth (weeks)	38.4	32.3	<0.0001
Birthweight (kg)	3.54	2.27	0.0004
Vitamin C (mg/day)	120	47.5	0.04
Vitamin B6 (mg/day)	1.73	1.27	0.03
Vitamin B12 (mcg/day)	5.36	2.95	0.04
Vitamin D (mcg/day)	3.69	2.71	0.04

Data are means ± SD (ANOVA; normal distribution/equal variance) or median (IQR; Kruskal-Wallis/Wilcoxon test for non-parametric data or outliers) with p value from one-way analysis of variance. BMI = body mass index.

Cases reported **lower dietary intakes of vitamins B6, B12, C and D,** but *not folate*, than **controls**

Cases & controls had similar phylum- & family-level gut microbiome composition…

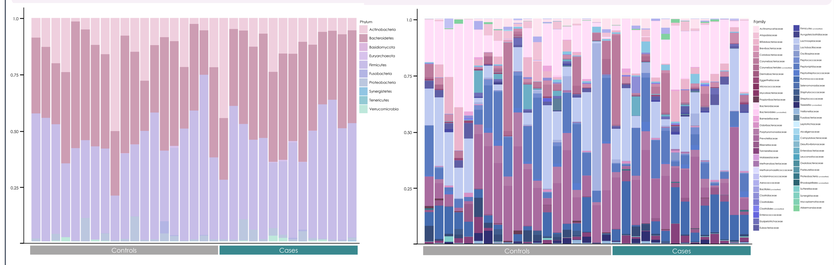


Figure 1. Gut microbial composition in cases and controls. Data shown are relative abundance levels of taxa at the phylum level (left) and family level (right). Each column represents the gut microbial composition of one participant. The colour of the bars represent distinct phyla or families.

…but cases & controls had different significantly enriched gut microbial taxa

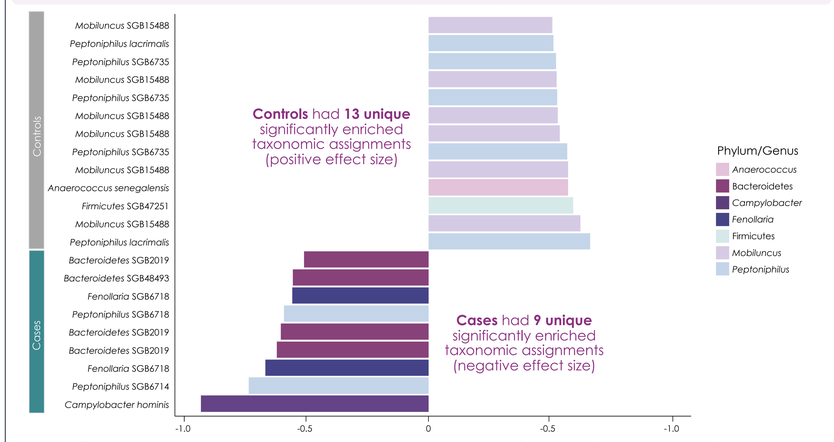


Figure 2. Most significantly enriched taxonomic assignments in cases and controls (both within and between groups). The colours of the bars represent the genus or phylum of each taxonomic assignment; Some taxonomic assignments have not yet been placed on the tree of life due to lack of information and thus, have not been previously named. The taxonomic assignment of each taxa is included in our visualization to demonstrate similarities and differences within and between groups. Bar length represents the ALDEx2 effect size (significant > [0.5]). Pie charts represent the genus or phylum of the enriched taxa in cases and controls.

Overall, enriched taxa in cases & controls had similar biosynthesis pathway categories…

Enriched taxa in **cases** and **controls** had a similar number of **biosynthesis pathway categories** & the same number of **nucleotide & vitamin metabolism pathways** within these categories

Figure 3. Raw counts of KEGG biosynthesis pathways and pathway categories in controls and cases. Flow chart demonstrates breakdown of KEGG pathway categories and pathways in enriched taxa. Donut plots demonstrate pathway categories. The size of the category represents the number of pathways identified under each specific category across all samples, and the colour gradient represents percent of enriched taxa that had specific pathways identified under each category.

…but enriched taxa in cases had altered expression of NTD-related pathways

Cases had **lower** expression of **pyridoxal-p (vitamin B6)** & **cysteine biosynthesis** pathways compared to **controls**

There were **no differences** in expression of pathways related to **DNA synthesis** in **cases** compared to **controls**

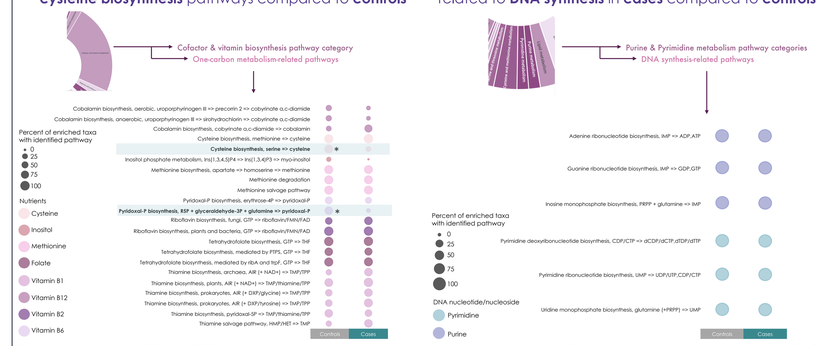


Figure 4. Bubble plots of putative NTD-related KEGG biosynthesis pathways in cases and controls. Key one carbon metabolism pathways (left) and DNA synthesis and metabolism pathways (right) were identified. The size of the bubble represents the percent of enriched species with the given pathway and the colour represents the nutrients involved in the pathways (left) and the DNA nucleotide/nucleoside involved in the pathways (right) . *Statistically significant, Fisher's exact test, p<0.05.

Limited associations between enriched taxa relative abundance & clinical/dietary intake data

Relative abundance of ***Mobiluncus* SGB15488** was positively associated with **vitamin D and B12 intake** in **controls,** but there were no associations between taxa relative abundance and clinical or dietary intake data in **cases**

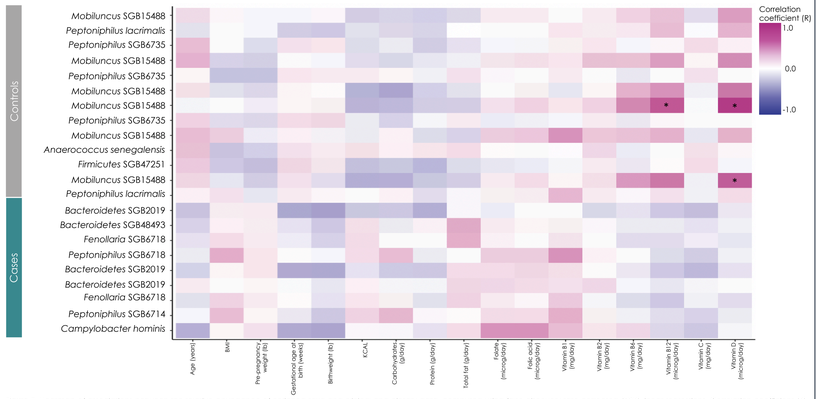
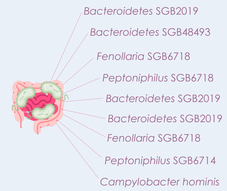


Figure 5. Heatmap of associations between the relative abundance of enriched taxa and clinical and dietary data, computed using false discovery rate-corrected (FDR) linear regressions. Correlation coefficient (R) is plotted, where pink represents a positive correlation and blue represents a negative correlation. *Statistically significant, FDR q<0.05.

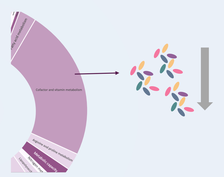
SUMMARY & CONCLUSIONS

- Mothers carrying a fetus with spina bifida had altered gut microbiome composition & function:

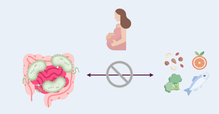
9 unique significantly enriched taxa



Lower expression of key pathways in enriched taxa



Taxa relative abundance does not associate with diet or clinical data



- These preliminary data suggest that *factors beyond folate intake/status* may be relevant for NTD pathogenesis
- Improved understanding of factors that influence NTD risk, **including the maternal gut microbiome**, may uncover interventions that target NTD prevention

ACKNOWLEDGEMENTS

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