Characterising the impact of delivery mode on the biochemical signatures of neonatal brain cortex in mice

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Overview

- A metabolic phenotyping LC-MS/MS method was used to analyze brain cortical tissue of mice from 3 study groups: those born by caesarean-section (CS), spontaneous vaginal delivery (SVD) and cross fostered (CF, vaginally-born mice housed with a foster mother)
- Differences between CS and SVD were observed in choline-related metabolites and N-acetylglutamate (NAG) metabolism, associated with neurodevelopment.

1. Introduction

Delivery mode has been shown to modulate the early-life microbiome, a substantial contributor to neonatal brain development. The World Health Organization have reported that births by caesarean section continue to rise globally, accounting for more than 1 in 5 (21%) of all childbirths. Previous work in mice has demonstrated that caesarean-section birth altered the developing microbiota resulting in aberrant social behavior and increased anxiety-like behavior. Several lines of communication exist between the developing CNS and the intestinal microbiota, including immunological, nervous, and biochemical components. In this analysis, high resolution LC-MS/MS metabolic phenotyping was used to characterize the biochemical landscape of the neonatal cortex to determine the impact of delivery mode (vaginal vs caesarean section) on the neurobiochemical profiles.

2. Methods

A metabolomics study in mice considered the influence of caesarean-section births (CS, n=20; housed with a foster mother), cross fostering (CF, n=15, vaginally-born mice housed with a foster mother) and spontaneous vaginal delivery (SVD, n=13) . Cortical tissue was collected, extracted and analyzed by high resolution mass spectrometry using a LC-MS/MS QTOF (LCMS-9030 Shimadzu Corporation, Japan).

- **Sample Preparation**. Each cortical tissue sample weighing approximately 10 mg (between 5-15 mg) was homogenized using chloroform/methanol/H_2O, centrifuged and extracted. Aqueous and organic extracts were pooled for LC-MS/MS analysis.
- **LC Separation**. As previously described (Deda *et al.* 2020)
  - Nexera LC system (Shimadzu Corporation); flow rate 0.4 mL/min
  - Acquity BEH 1.7µm (2.1x100mm) at 50ºC
  - Binary gradient; Water/acetonitrile + 0.1% formic acid
- **Mass Spectrometry Detection**. QTOF LCMS-9030 using external mass calibration for positive and negative mode ESI;
  - MS mass scan m/z 100-1000; 100 msecs
  - DIA-MS/MS mass scans m/z 40-1000; 20 msecs for each mass scan; isolation width 20 Da; collision energy spread 5-55V; 45 mass scan events in total
  - Cycle time 1.0 second

2.1 Data Processing Workflows

The workflow for metabolite feature extraction considered Analyze component detection and searching for markers of neurodevelopment.

- **Component detection (metabolite feature detection)**. Analyze component detection algorithm was applied to all samples and pooled QCs in a batch driven workflow.
  - The Analyze algorithm loads all spectra into an array (no thresholds are applied; every scan is included), creates “ion groups” (ions that behave in a covariant manner and includes adducts, dimers, isotopes).
  - Noise and idiosyncratic ion behavior are subsequently removed from the array.
  - A clustering algorithm was applied to align each component within the batch of samples using a m/z tolerance of 1 mDa and a Rt tolerance of 0.1 min.
  - Features with a response variation less than 20% present in the pooled QC were considered for metabolite screening.

- **Metabolite pathway analysis**. Several metabolite pathways associated with neurodevelopment were considered in the data analysis. To normalize against a variable tissue weight metabolite ratio analysis was used to find statistical differences. Table 1 shows the metabolite pathways studied and highlights examples of the targets within each pathway. (In the Kennedy pathway 9 metabolite ratio’s were used for statistical analysis by ANOVA (all groups) and t-test (SVD:CS) taking into account peak area data for each tissue sample).

Pathway	Examples of targets within each pathway	
Kennedy pathway and glycerophospholipids metabolism [9 metabolite ratio’s studied]	Acetylcholine, choline, phosphocholine, CDP-choline, phosphatidycholine	Ethanolamine, CDP ethanolomine/ Phosphoethanolamine
Fatty acid methylation [4]	FAME 18:1 / FA 18:1 FAME 20:4 / FA 20:4	FAME 22:4 / FA 22:4 FAME 22:6 / FA 22:6
Fatty acid ratios [10]	18:1 / 20:3, 18:1 / 20:4 18:1 / 22:4, 18:1 / 22:6	20:3 / 22:6, 20:4 / 22:4, 20:4 / 22:6, 22:4 / 22:6
TCA cycle [4]	Malate / Fumarate Fumarate / Succinate	Citrate / Malate Succinate / Citrate
Lysine metabolism [3]	Lysine / 2-aminoadipate Trimethyllysine / Lysine	Pipecolate / Lysine
Histidine and Glutamate metabolism [4]	Histidine / Carnosine Glutamate / Histidine	Glutamine / Glutamate NAG / Glutamate
Aspartate metabolism [2]	NAA / Aspartate	NAAG / NAA
Methionine metabolism [2]	MTA / Methionine	SAH / Methionine
Phospholipid catabolism [6]	FA 18:1 / sum LPC 18:1 FA 20:4 / sum LPC 20:4	FA 22:4 / sum LPE 22:4 FA 22:6 / sum LPE 22:6
LPCs normalized to total LPC [16]	LPC 14:0 sn-1 / total LPC	Total of 16 LPCs from LPC 14:0 to LPC 22:6, sn-1 and sn-2
LPEs normalized to total LPE [11]	LPE 16:0 sn-1 / total LPC	Total of 11 LPEs from LPE 16:0 to LPE 22:6, sn-1 and sn-2
MAGs normalized to total MAG [12]	MG 14:0 (1) / total MAG	Total of 12 MAGs from MG 14:0 to MG 22:6, (1) and (2)

Table 1 Metabolite ratios and pathways considered for neurodevelopment (the number in brackets relates to the number of metabolite ratio’s studied within each pathway).

3. Results

- **Metabolite ratio analysis**. This approach was used to account for differences in cortical tissue weight (nominally each tissue weight was ~10 mg; ranging between 5-15 mg).
- **Significant metabolite pathways**. There were no statistical differences between SVD, CS, and CF groups for most metabolites in the screening list except for the Kennedy pathway, NAG production (N-acetylglutamate synthase is an essential allosteric activator of the first enzyme of the urea cycle) and polyunsaturated fatty acids (PUFAs) from the n-6 and n-3 families (considered crucial for brain development).

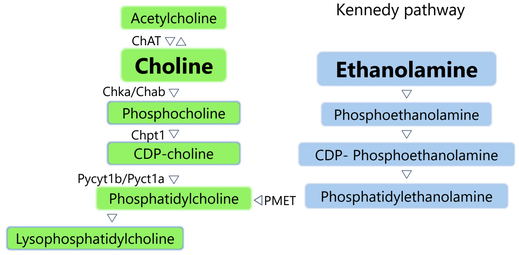

Figure 1 The CDP-choline pathway (Kennedy Pathway) is the principle mechanism for mammalian cells to synthesize phosphatidylcholine (PC) to build membranes or lipid-derived signaling molecules. The CDP-ethanolamine pathway is responsible for the biosynthesis of the phospholipid product phosphatidylethanolamine (PE).

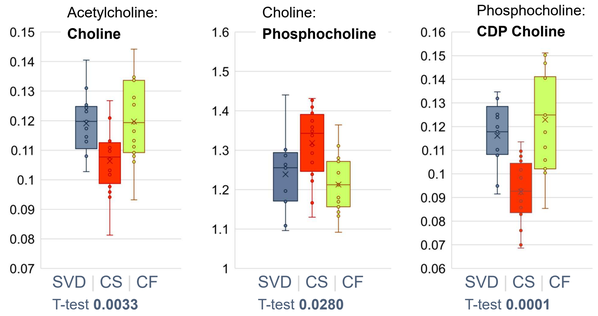

Figure 2 CDP choline pathway metabolite ratios for acetylcholine:choline, choline:phosphocholine and phosphocholine:CDP.choline.
(The t-test statistic was applied to SVD:CS in each metabolite ratio pathway).

Repeated injection and analysis of a pooled QC was used to assess the variance of ion signal throughout the batch (n=11; acetylcholine %RSD 5.89, choline %RSD 5.82, phosphocholine %RSD 3.82, CDP choline %RSD 1.09).

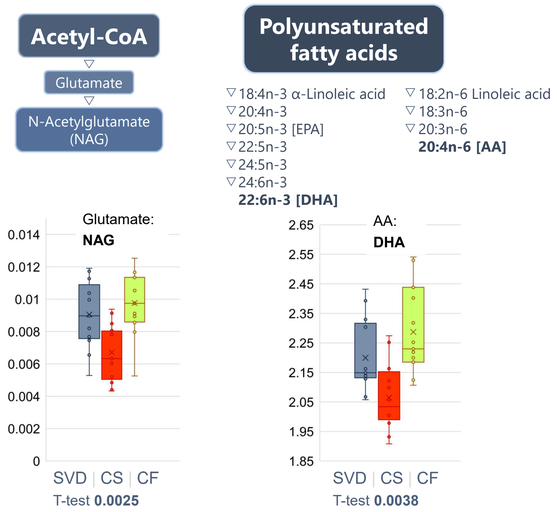

Figure 3 Metabolite pathways and ratios for glutamate:N-acetylglutamate (NAG) and arachidonic acid (AA):docosahexaenoic acid (DHA).
(The t-test statistic was applied to SVD:CS in each metabolite ratio pathway).
Pooled QC variance throughout the batch (n=11; glutamate %RSD 1.26, NAG %RSD 9.41, AA %RSD 1.96, DHA %RSD 2.42).

- Choline is considered to be a ‘neuroprotectant’ and ‘neurocognitive essential nutrient’ that is critical for healthy growth and the functioning of the infant brain.
- In the Kennedy metabolite pathway, choline:phosphocholine metabolite ratio was elevated in CS compared to SVD, acetylcholine:choline and phosphocholine:CDP-choline ratios were lower in CS compared to SVD.
- Glutamate:NAG and also AA:DHA metabolite ratios also showed statistically significant differences between CS and SVD.

4. Conclusions

- The aim of targeted metabolite ratio analysis was to study the influence of delivery mode on metabolic pathways specifically associated with brain development.
- We have previously shown that delivery mode alters gut microbial colonization patterns and modifies behavior and that gut microbial activity influences the neurobiochemical landscape of the mouse brain during this important developmental phase. However, in this work no microbiome related metabolite changes could be detected.
- Modest but statistically significant differences were detected in metabolite ratio analysis in a limited number of pathways. Most metabolite ratios failed to show any differences between CS and SVD groups.
- Attributing biological significance to such small changes remains challenging requiring further investigation.