Metabolic profiling of tryptophan and kynurenine pathway in stool samples of newborns

Anne-Christine Aust [1*], Kateřina Coufalíková[1], Veronika Vidová [1],Sona Smetana [1], Vojtěch Thon [1], Lenka Micenková[1], Ivo Borek[2], Petr Janků[3], Zdeněk Spáčil [1]

1. Research Centre for Toxic Compounds in the Environment, Masaryk University, Brno, Czech Republic
2. Department of Pediatrics, University Hospital Brno and Masaryk University Medical School, Brno, Czech Republic
3. Department of Gynecology and Obstetrics, University Hospital Brno and Masaryk University Medical School, Brno, Czech Republic

* Correspondence: anne-christine.aust@recetox.muni.cz

Introduction

Many factors modulate the bacterial microflora diversity, such as mode of delivery. The different microbial composition, has an influence on the health of the newborn. Tryptophan and its metabolites, produced by many different bacterial species, have an important role in the mammalian gut immune homeostasis[1]. Stool samples were taken from participants in the CELSPAC Cohort Study. In the CELSPAC Study, in total 134 samples were collected. For the group comparison, 20 samples of CD were compared to 114 samples of VD. Further were metabolites compared in meconium and stool samples. A panel of tryptophan metabolites in neonatal stool samples were analyzed using the targeted SRM metabolic profiling in stool. The extracted tryptophan metabolites were quantified using labeled internal standards. Analytes were tested for significance with 95% confident intervals. Statistically significant differences between stool and meconium groups are Indole-3-aldehyde (IAld), Indole-3-lactic (ILA) acid and Tryptophan (TRP). These metabolites correspond to the difference in microbial composition, confirmed by metagenomics analysis.

1.) Material and Methods

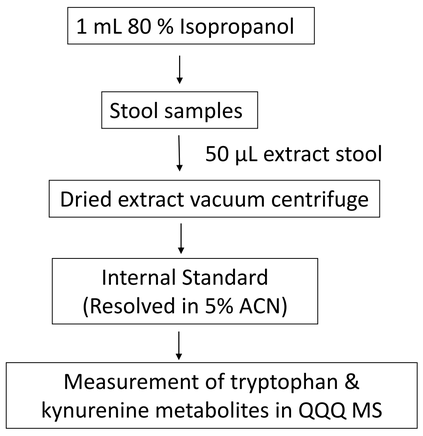

Figure 1. Sample Preparation

Stool samples were collected from 134 healthy children at the University Hospital Brno. The sample preparation was done according to Figure 1. Prior to extraction, samples were taken out of the -80 °C freezer and let at lab temperature for 30 min. A 1 mL of 80% isopropanol was added into a vial with a stool swab and extracted in an orbital shaker (5 min, 1600 rpm). Then the sample was centrifuged (2min, 12 000x g). A 50 µl of the extract was pipetted into a new vial and dried out in vacuum concentrator for 30 min (Savant SPD121 P SpeedVac, Thermo Fisher). Dried extract was resolved with solution of 5 % isopropanol contained isotopically labelled standards. Dissolution was supported with use of ultrasonic bath (1 min, 37 °C).
As quantification standard, also a quality control, a pool of all samples was measured. The samples were injected in 5% Acetonitrile with spiked-in internal standard. The extracted metabolites from stool were analysed in the Triple-Quad Mass Spectrometer. The mobile phase contained a gradient with 0.1 % formic acid, the samples were injected with a Waters Acquity UPLC analytical column (C18 Peptide, CSH Column, 1.7 µM, 2.1x 100 mm). The tryptophan and kynurenine metabolites were measured with the UHPLC-SRM-MS positive ion mode. All data were acquired in SRM mode. For calculation of the concentration of the analytes, the transition with the highest intensity for each metabolite, has been chosen. The highest intensity of each molecule was used, to control the SRM Assay method. For quantification of all samples, also mixed internal standards (labeled and unlabeled) were measured. The samples were semi-quantitative quantified with the Response Factor-method according to Pavlova *et al.*,2017[2].

Metabolites	Acronym	Chemical Formula	HMDB ID	Median (µg/mg of total protein)
Kynurenine	KYN	$C_{10}H_{12}N_2O_3$	HMDB0000684	3.91
Anthranilate	ATA	$C_7H_7NO_2$	HMDB0001123	0.27
Indole-3-acetic acid	IAA	$C_{10}H_9NO_2$	HMDB0000197	5.11
Tryptophan	TRP	$C_{11}H_{12}N_2O_2$	HMDB0000929	677.89
Indole-lactic acid	ILA	$C_{11}H_{11}NO_3$	HMDB0000671	48.32
N-acetyl-tryptophan	NAT	$C_{13}H_{14}N_2O_3$	HMDB0013713	6.43
Indole-3-aldehyde	IAld	C_9H_7NO	HMDB0029737	33.95

2.) Results

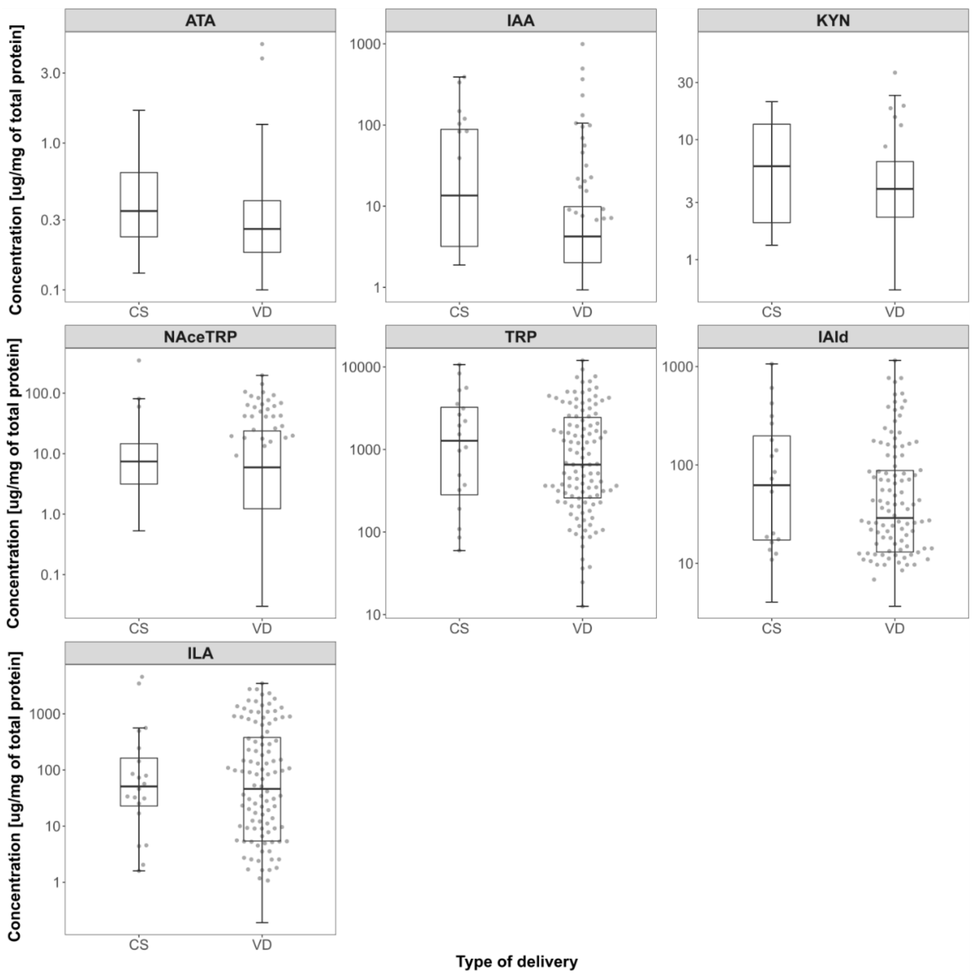

Figure 3. Boxplots of CD and VD samples. Only values above LOQ are shown. Grey spots indicate samples >LOQ.

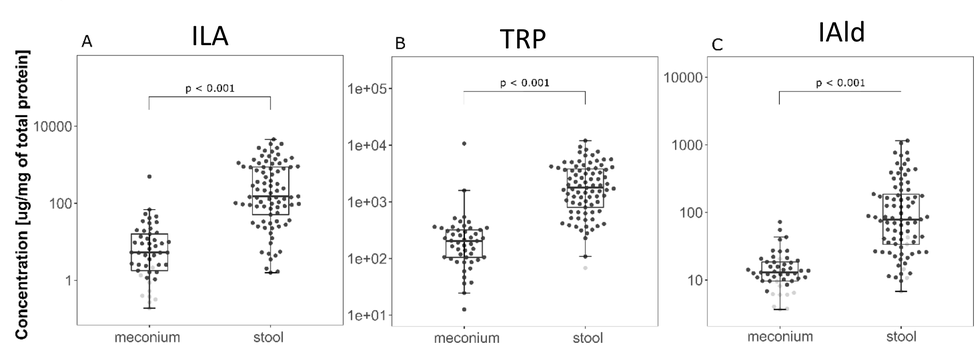

Figure 2. Box plots of metabolites quantified in meconium and stool of 134 newborns. Determined statistical difference of ILA (A), TRP (B) and IAld (C) in meconium and stool. Grey spots indicate values <LOQ.

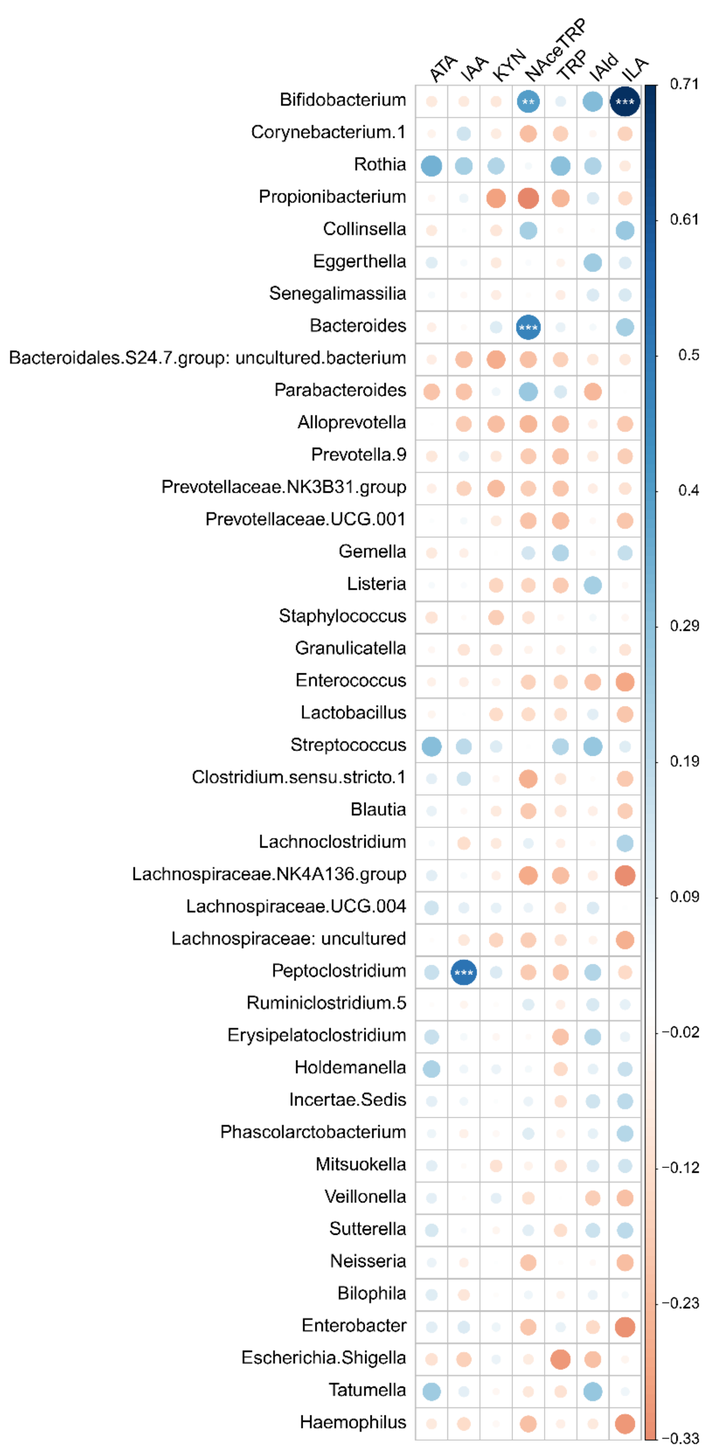

Figure 4. Spearman correlation of TRP metabolites and microbiome abundance in the stool. The colour indicates Spearman correlation coefficients. Statistical significance is marked with * for FDR<0.1, ** for FDR<0.05 and *** for FDR<0.01.

Conclusion

- Significant altered metabolites measured with SRM-Tandem-MS out of the participants: Indole-3-aldehyde, Indole-3-lactic acid and Tryptophan
- Through significant altered metabolites the development from meconium to stool can be seen as gradual development in the gut barrier homeostasis with the metabolites IAld and ILA
- In the spearman correlation plot bacterial producer of TRP metabolites in stool samples can be identified

Perspective

- Extraction and measurement of Tryptophan and Kynurenine metabolites in stool samples from older newborns in a follow-up study
- Quantification of Tryptophan metabolites in other tissue samples from patients in CELSPAC Study

Reference

[1]Zelante, Teresa; Iannitti, Rossana G.; Cunha, Cristina; Luca, Antonella de; Giovannini, Gloria; Pieraccini, Giuseppe et al. (2013): Tryptophan catabolites from microbiota engage aryl hydrocarbon receptor and balance mucosal reactivity via interleukin-22. In: *Immunity* 39 (2), S. 372–385. DOI: 10.1016/j.immuni.2013.08.003.

[2]Pavlova, Tereza; Vidova, Veronika; Bienertova-Vasku, Julie; Janku, Petr; Almasi, Martina; Klanova, Jana; Spacil, Zdenek (2017): Urinary intermediates of tryptophan as indicators of the gut microbial metabolism. In: Analytica chimica acta 987, S. 72–80. DOI: 10.1016/j.aca.2017.08.022.

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