Cholestasis alters the maturation of the extremely preterm neonatal gut microbiome

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BACKGROUND

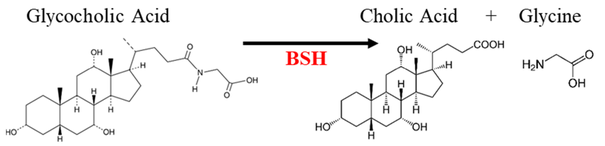
- Cholestasis (impaired bile flow from the liver to the intestine) affects ~1:2500 births, causes poor neonatal growth, and may progress to liver failure and death.
- Ursodeoxycholic acid (UDCA), a secondary bile salt, is used in the treatment of cholestatic liver disease. Its effects in preterm neonates are poorly understood.
- Normal bile flow requires an intact liver-gut-microbiome axis.
- Microbial bile salt hydrolase (BSH) enzymes transform primary bile salts into secondary bile salts.
- How the liver-gut-microbiome axis develops over time in preterm newborns, and whether cholestasis alters this development, is unknown.

PURPOSE

We aimed to test the hypotheses that:1) The gut microbiome of extremely premature neonates without cholestasis contains BSH genes and develops in a predictable manner over time; 2) Cholestasis interrupts this pattern of development; 3) The microbial alterations in cholestatic neonates are reflected in altered composition of the bile salt pool; 4) UDCA treatment quantitatively modifies bile salt profiles.

METHODS

- Stool samples (total n = 124) longitudinally collected from birth through hospital discharge. Samples were used for:
- Whole metagenome shotgun sequencing with pathway analysis
- Bile salt quantification by mass spectrometry
- In vitro bile salt hydrolase activity assay



	Control n=12	Cholestatic n=12	p-value
Gestational age (weeks)	27.2 ± 1.8	27.2 ± 1.9	0.975
Birth weight (g)	968.2 ± 287.5	924.1 ± 228.4	0.682
Male, *n (%)*	6 (50.0)	6 (50.0)	1.000
Small for gestational age at birth, *n (%)*	1 (8.3)	3 (25.0)	0.590
Mother's milk (>90%), *n (%)*	5 (41.7)	5 (41.7)	1.000
Days of antibiotics in first 14 d of life	3.8 ± 2.7	7.5 ± 5.7	0.058
Weight velocity,[3] g·d^{-1}	19.0 ± 4.1	15.5 ± 5.4	0.097
Length velocity,[2] cm·wk^{-1}	0.94 ± 0.17	0.80 ± 0.26	0.149
Head circumference velocity, cm·wk^{-1}	0.76 ± 0.140	0.63 ± 0.11	0.018*
Days to 140 mL·kg^{-1}·d^{-1}	14.5 ± 4.8	46.7 ± 45.6	0.041*
Days of initial TPN during study	10.7 ± 3.7	34.6 ± 25.7	0.008**
Days nil per os > 12 h after feeds initiated	4.3 ± 8.4	23.2 ± 21.4	0.013*
NEC – Stage ≥ IIA, *n(%)*	0 (0)	6 (50)	0.014*

Fig 1: Preterm cholestatic neonates required more days of parenteral nutrition and exhibited poor growth compared to preterm controls.

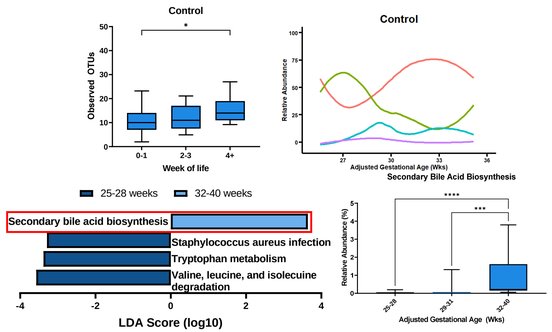


Fig 2: Cholestasis disrupts the maturation of the microbiome. The secondary bile acid biosynthesis pathway is the most significantly depleted pathway in cholestatic vs. control neonates (adj. GA 32-40 wks).

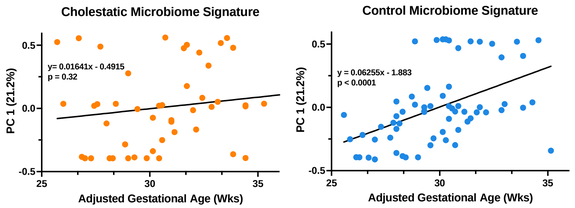


Fig 3: Cholestasis disrupts the maturation of the microbiome. The secondary bile acid biosynthesis pathway is the most significantly depleted pathway in cholestatic vs. control neonates (adj. GA 32-40 wks).

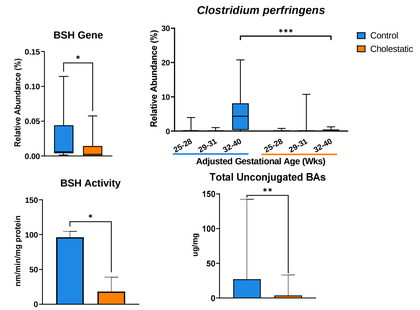


Fig 4: Cholestasis decreases the abundance of BSH and BSH carrying *Clostridium perfringens*. BSH enzymatic activity and its products – unconjugated bile salts – were reduced in cholestasis.

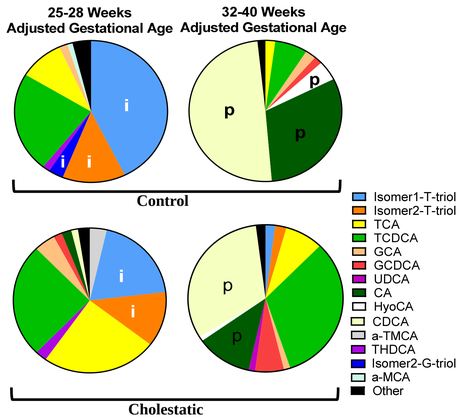


Fig 5: Isomer (i) bile salts dominate the preterm BA pool (adj. GA 25-28 wks). Primary (p) unconjugated bile salts are reduced in cholestatic preterm neonates (adj. GA 32-40 wks).

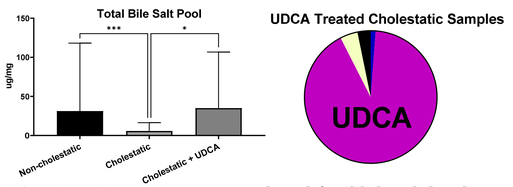


Fig 6: UDCA treatment restored total fecal bile salt levels. Cholestatic samples from neonates on UDCA had higher quantities of fecal UDCA compared to untreated cholestatic neonates.

RESULTS

- Gut microbiota from extremely preterm neonates without cholestasis develop in a predictable manner with increasing postconceptional age.
- Increasing secondary bile salt biosynthesis is the most distinctive metagenomic feature of preterm development.
- Control neonates had increasing abundance over time of BSH genes and of the BSH carrier *Clostridium perfringens*; this pattern of development is absent in cholestatic neonates
- BSH enzymatic activity and its products – unconjugated bile salts – were reduced in cholestatic neonates compared to gestational age matched controls.
- Total fecal bile salts were reduced in cholestasis but completely restored by UDCA treatment.
- Compared to the controls, cholestatic neonates required more days of parenteral nutrition and antibiotics.
- Cholestatic infants exhibited poor neonatal growth, indicated by lower head circumference and weight velocities.

CONCLUSION

- The preterm neonatal gut microbiome develops in a predictable manner over time.
- Cholestasis alters the maturation of the gut microbiome and metagenome.
- Microbial BSH enzyme abundance and activity are reduced in cholestatic neonates.
- UDCA makes up > 90% of fecal bile salts in neonates receiving enteral UDCA.
- Ongoing studies are exploring the liver-gut-microbiome axis as a therapeutic target to enhance neonatal growth and improve clinical outcomes.

REFERENCES

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