National University Hospital

Global similarities and differences in bile acid biome and NASH progression

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Disclosures

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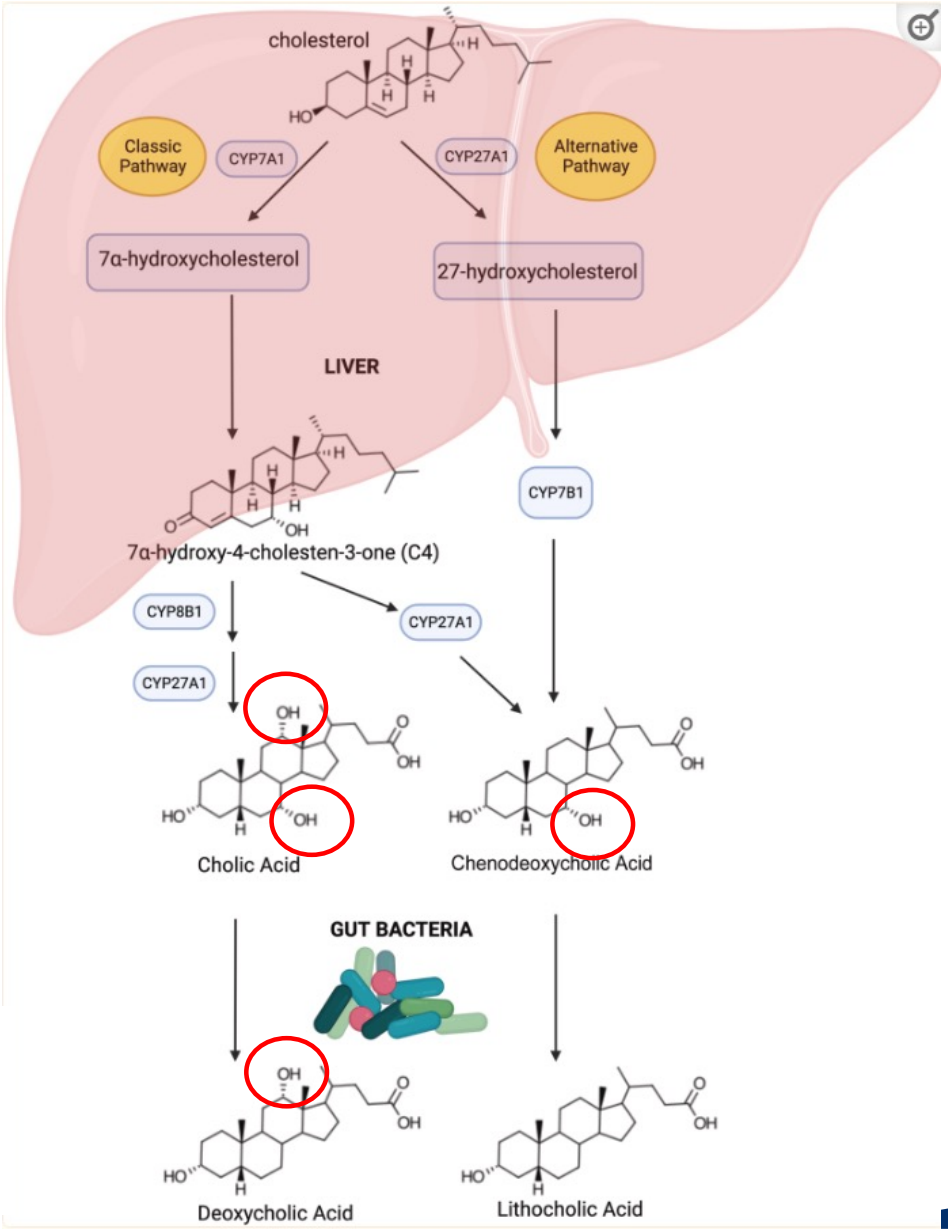
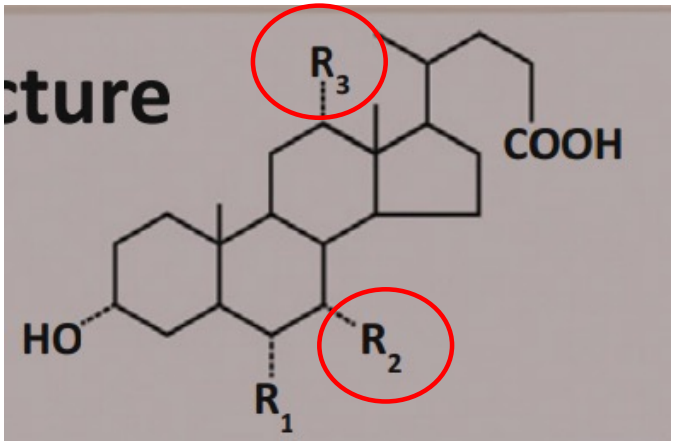
- Paid Speaking engagements: Boston Scientific, Olympus Medical, Roche, Astellas

Outline

- Physiology of bile acid synthesis
- Bile acid changes in NASH
- Biome changes that can drive changes seen in NASH
- Heterogeneity of the biome

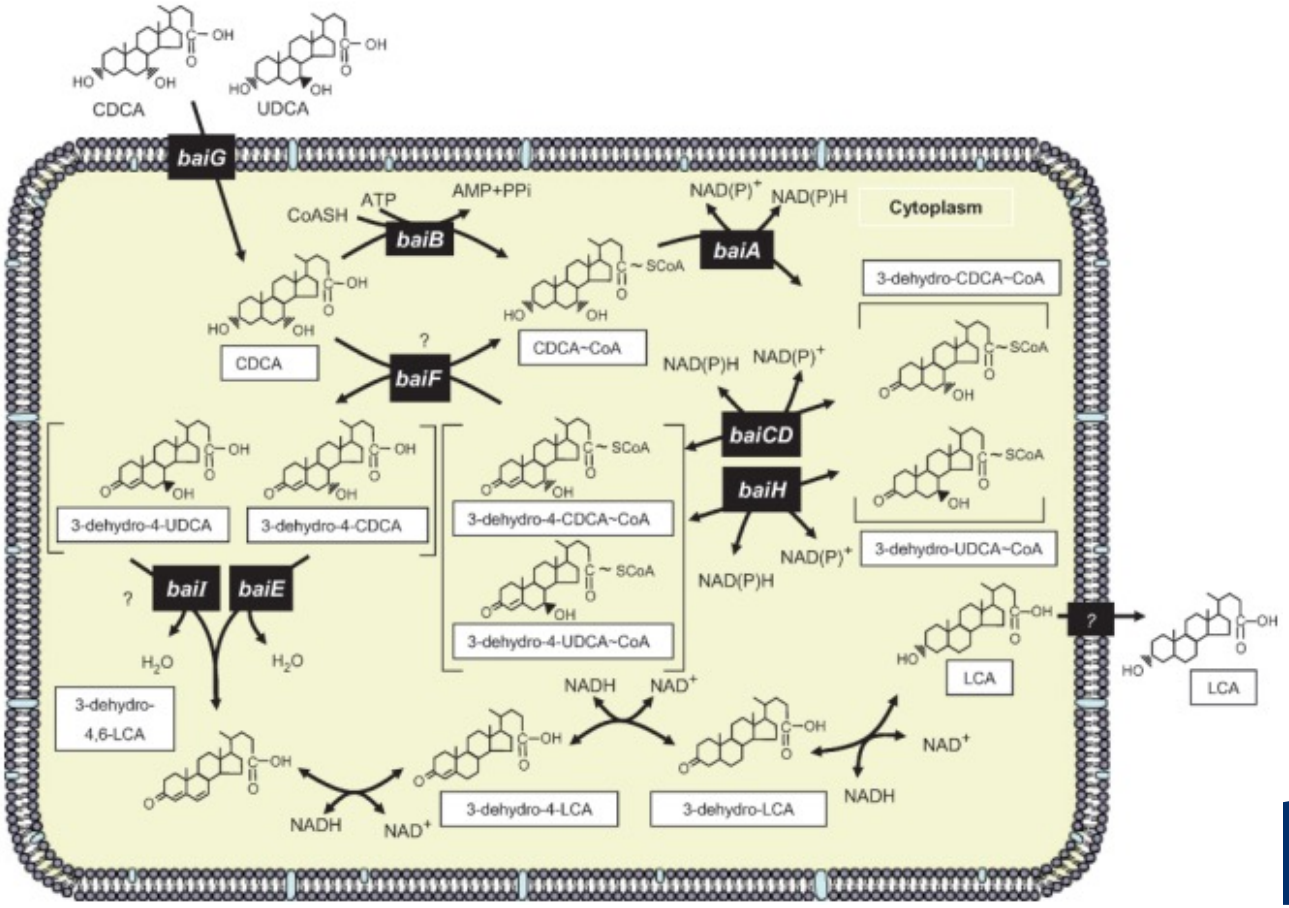
Bile acid metabolism and homeostasis





Ridlon et al, JLR 2023

Bile acids undergo multi-step 7-dehydroxylation in the gut under normal physiological states



Ridlon et al, JLR 2006

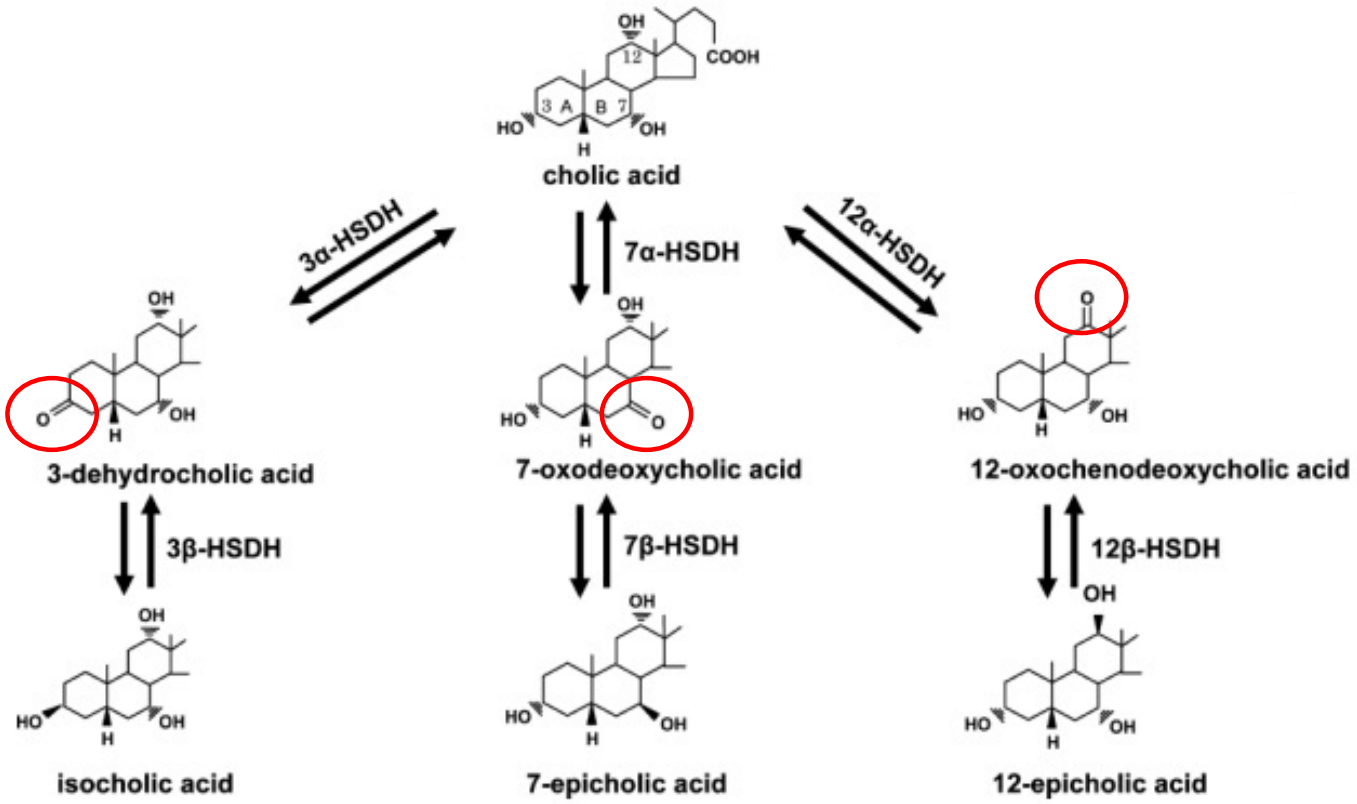
Bile acid inducible operons in 7 dehydroxylation

Gene	Enzyme	Proposed function
baiB	Bile acid CoA-ligase	Facilitates formation of a bile acid-CoA thioester
baiCD	NAD+ dependent 3-oxo-Δ4-cholenoic acid oxioreductase	Catalyzes oxidation reaction to produce a 3-dehydro-Δ4-CA-CoA intermediate
baiE	Bile acid 7-α dehydrastase	Performs diaxial trans elimination of water in 3-oxo-
	CoA transferase	CoA conjugates
baiG	Bile acid membrane transport protein	Facilitates transport of bile acids across cell membranes
baiH	NADH:flavin oxioreductase activity	Catalyzes production of a 3-dehydo-Δ4-CA-CoA intermediate
baiI	Bile acid 7-ß dehydrastase; Δ5-ketosteroid isomerase	
BSH	Bile salt hydrolase	Deconjugation and reconjugation of bile salts

Deconjugation and 7 dehydroxylation lead to increased hydrophobicity
Can be more easily recovered from passive absorption across colonic epithelium
Increased hyprophobicity leads to greater "ease" to traverse membranes – increased toxic and metabolic effects

Adapted from Wise et al, Front Microbiol 2023

Keto / Oxo bile acids (at 3,7,12 positions)

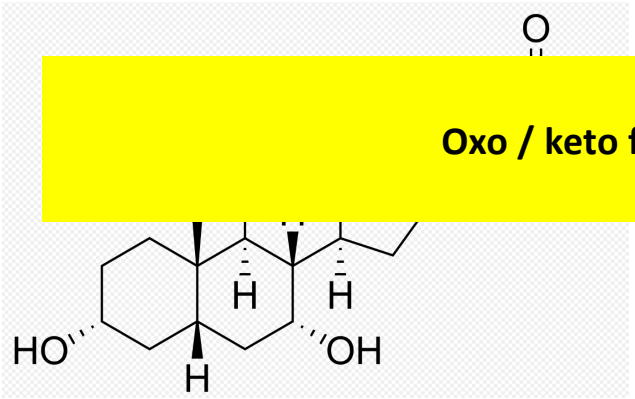


Ridlon et al, JLR 2006

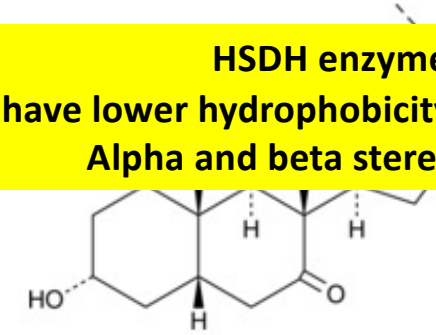
Stereoisomers

7 Hydroxysteroid dehydrogenase (HSDH)

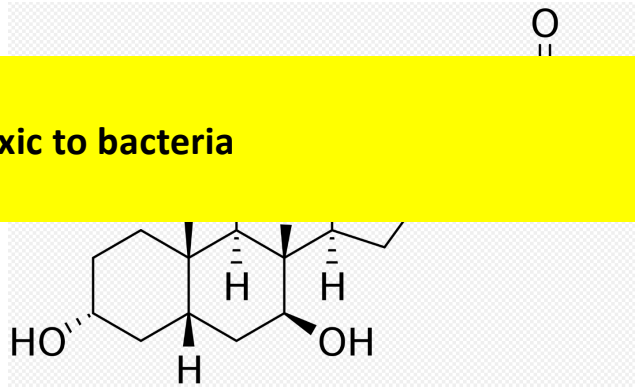
HSDH enzymes
Oxo / keto forms have lower hydrophobicity – may be less toxic to bacteria
Alpha and beta stereoisomers



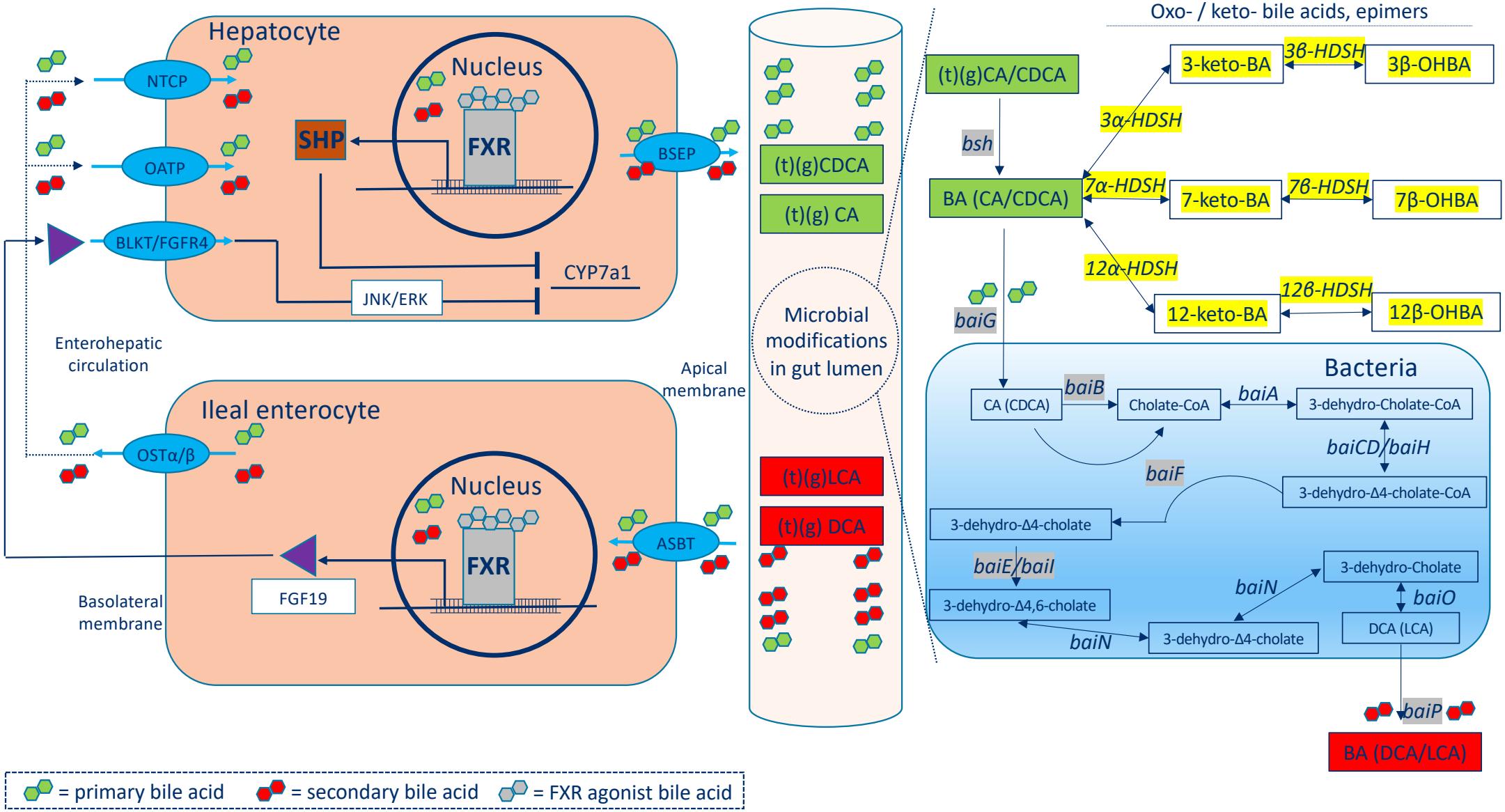
Chenodeoxycholic acid
(CDCA) - 3α 7α



7-oxodeoxycholic acid
- 3α **7oxo**

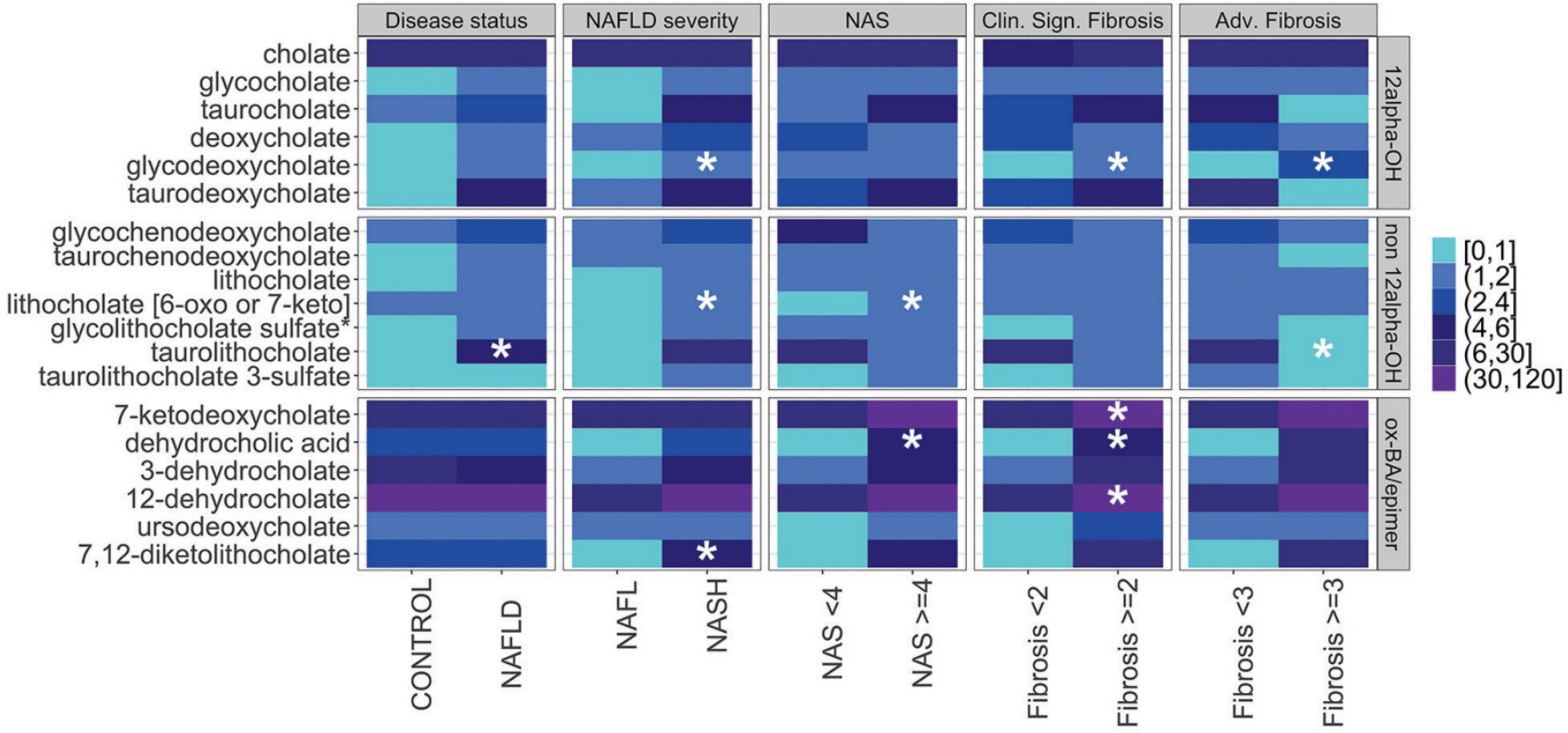


Ursodeoxycholic acid
(UDCA) - 3α **7β**


Hepatocyte
Nucleus
NTCP
OATP
BLKT/FGFR4
SHP
FXR
BSEP
CYP7a1
JNK/ERK
Enterohepatic circulation
Apical membrane
Ileal enterocyte
OSTα/β
Nucleus
FXR
ASBT
FGF19
Basolateral membrane
(t)(g)CDCA
(t)(g) CA
Microbial modifications in gut lumen
(t)(g)LCA
(t)(g) DCA
Oxo- / keto- bile acids, epimers
(t)(g)CA/CDCA
bsh
BA (CA/CDCA)
3-keto-BA
3β-HDSH
3β-OHBA
3α-HDSH
7α-HDSH
7-keto-BA
7β-HDSH
7β-OHBA
12α-HDSH
12-keto-BA
12β-HDSH
12β-OHBA
baiG
Bacteria
CA (CDCA)
baiB
Cholate-CoA
baiA
3-dehydro-Cholate-CoA
baiCD/baiH
baiF
3-dehydro-Δ4-cholate-CoA
3-dehydro-Δ4-cholate
baiE/baiI
3-dehydro-Δ4,6-cholate
baiN
3-dehydro-Δ4-cholate
baiN
3-dehydro-Cholate
baiO
DCA (LCA)
baiP
BA (DCA/LCA)
= primary bile acid
= secondary bile acid
= FXR agonist bile acid

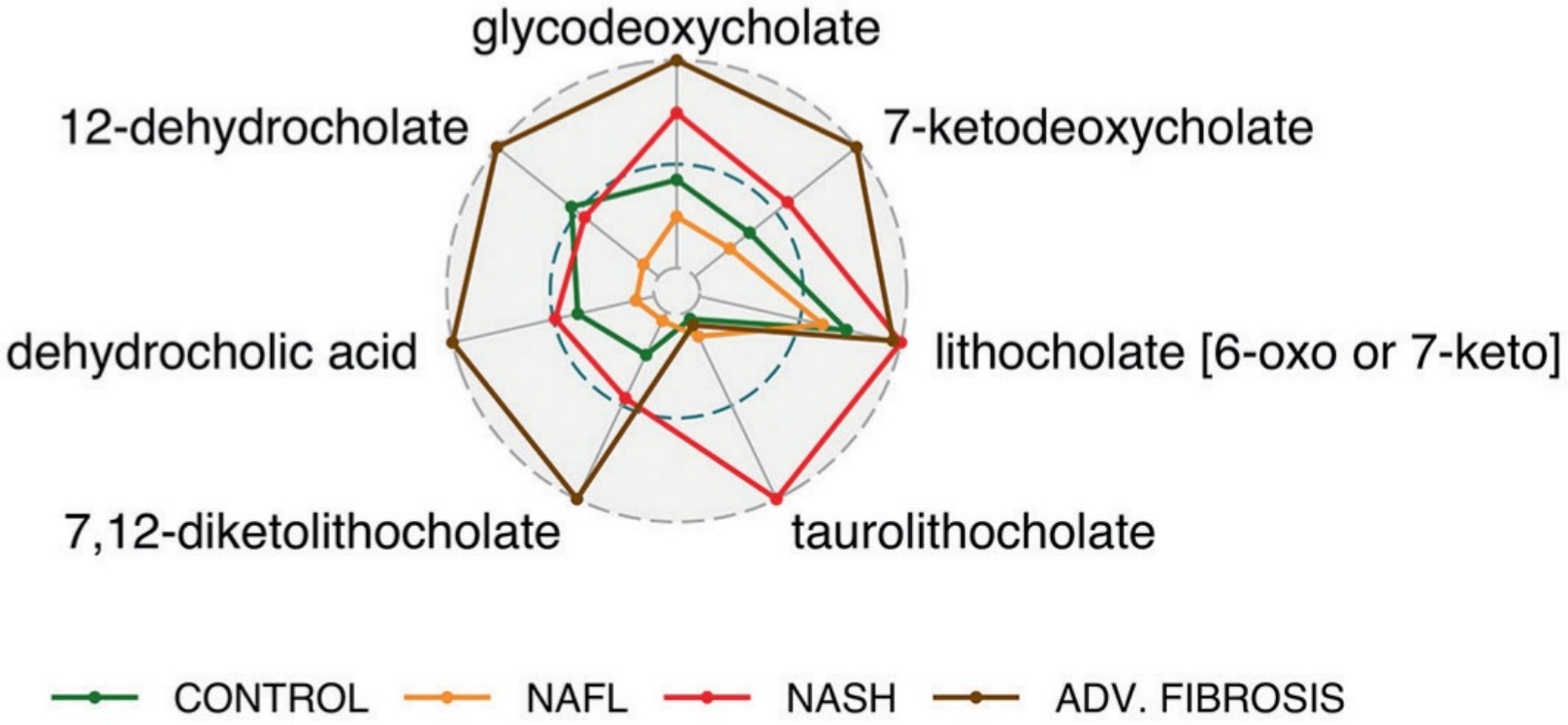
Bile acid profiles in NASH

Increase in secondary bile acids and keto bile acids

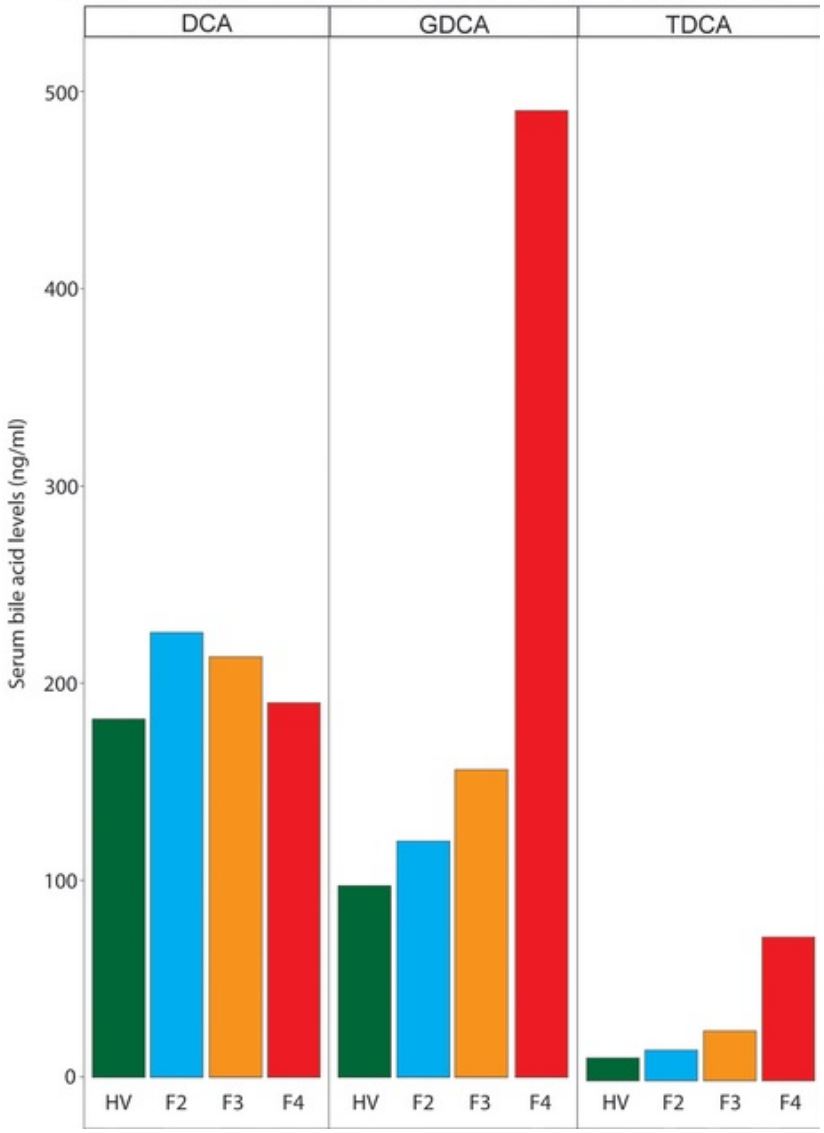


Smirnova, Muthiah MD et al, Hepatology 2022

Increase in secondary bile acids and keto bile acids



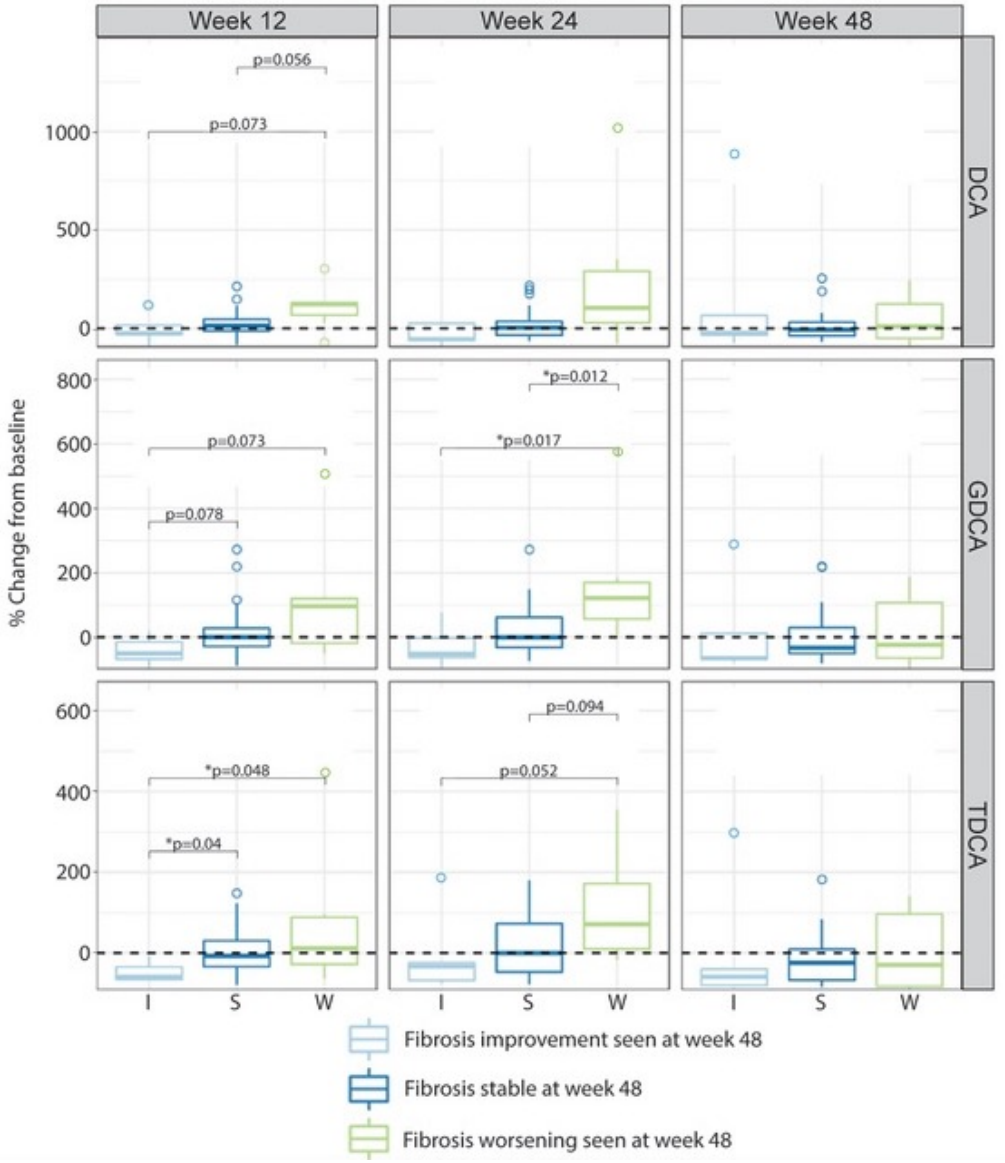
Smirnova, Muthiah MD et al, Hepatology 2022



Smirnova, Muthiah MD et al, Hepatology 2022

Secondary bile acids associated with more severe disease

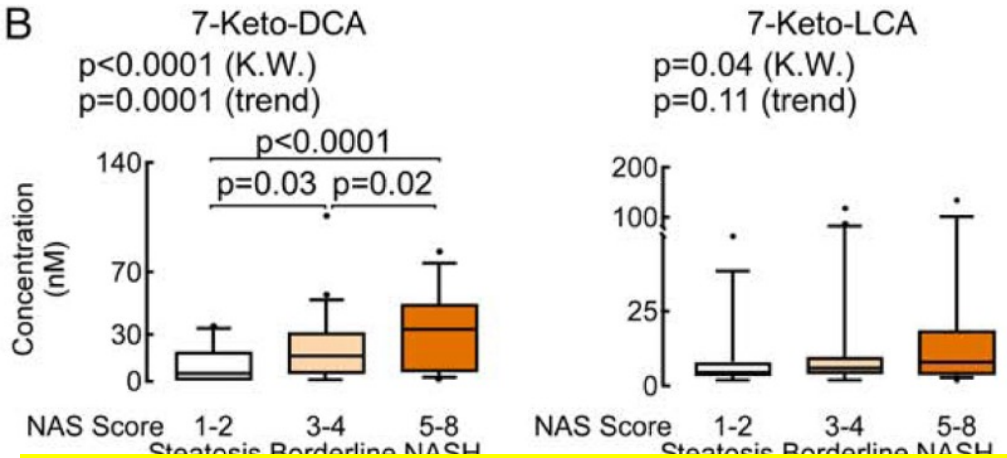
- Increased GDCA and TDCA at baseline from patients with advanced fibrosis or cirrhosis
- Patients from Selonsertib and Simtuzumab phase 2b stuides



Smirnova, Muthiah MD et al, Hepatology 2022

Secondary bile acids associated with more severe disease

- Patients from placebo arm of ATLAS study
- GDCA and TDCA levels at baseline and week 24 predicted fibrosis worsening at week 48

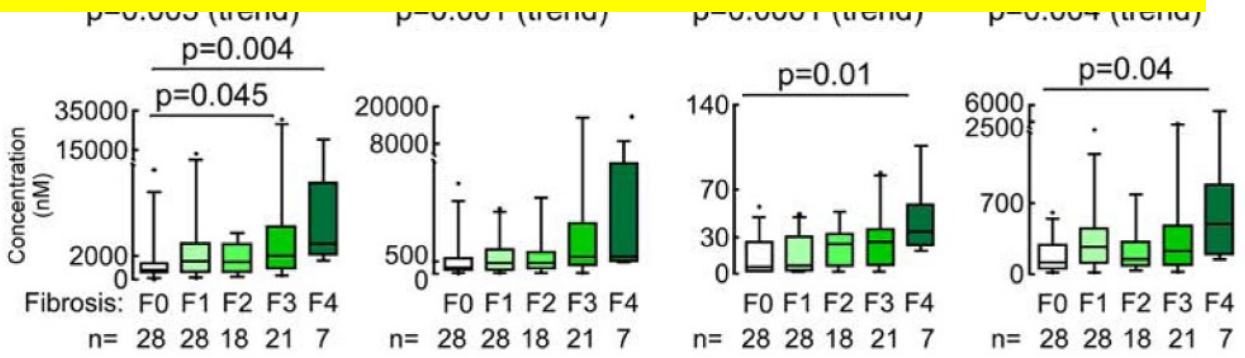


Increase in oxo- bile acids as disease progresses

Increase in gut modified secondary bile acids with fibrosis

Increase in glycine-conjugated primary bile acids with fibrosis

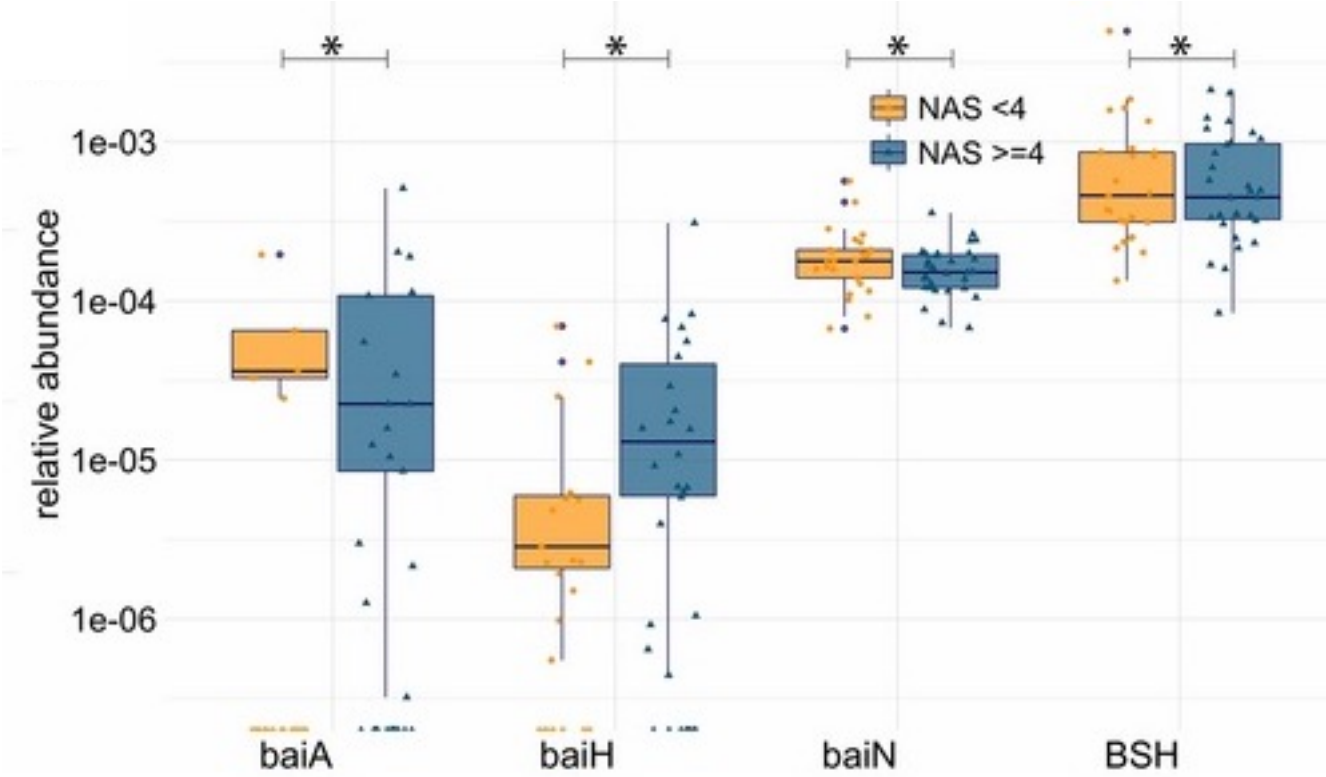
Decreased signalling via FXR leading to dysregulation of lipid and glucose metabolism



Nimer et al, Metabolism 2022
Hrncir et al, Microorganisms 2021

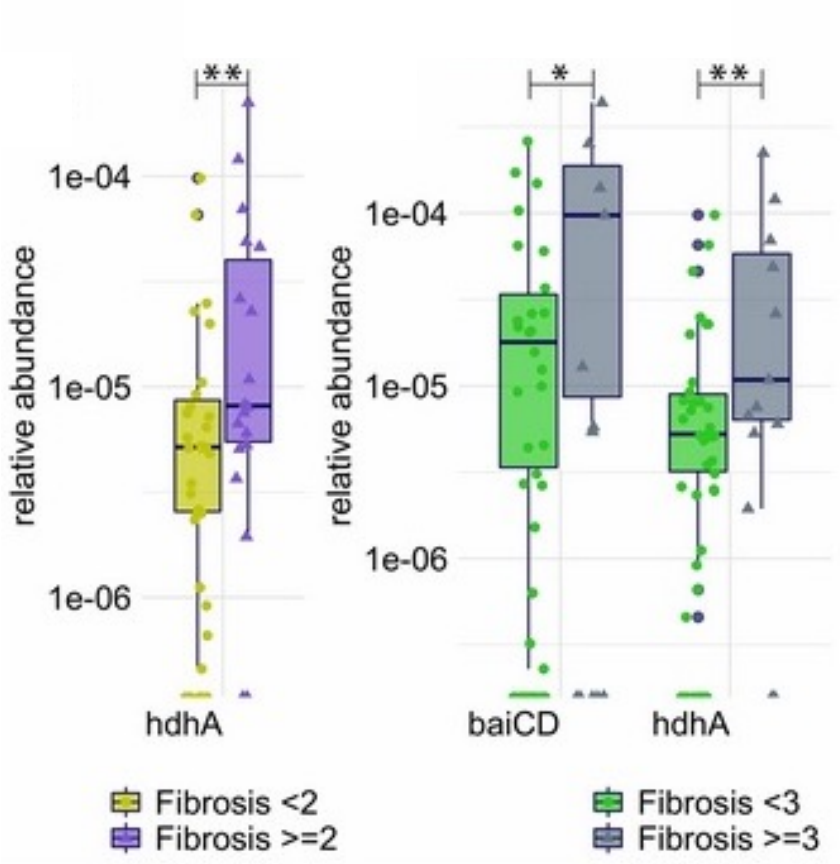
Biome changes driving changes in BA profiles

Increase in genes of enzymes involved in gut biotransformation of bile acids with activity



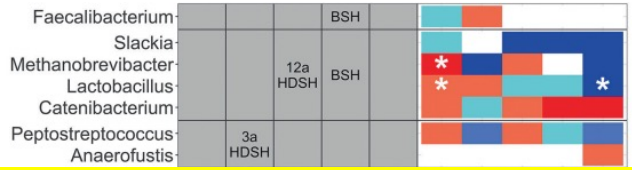
Smirnova, Muthiah MD et al, Hepatology 2022

Increase in genes of enzymes involved in gut biotransformation of bile acids with fibrosis



Smirnova, Muthiah MD et al, Hepatology 2022

Changes in specific taxa account for changes in gene expression and bile acids

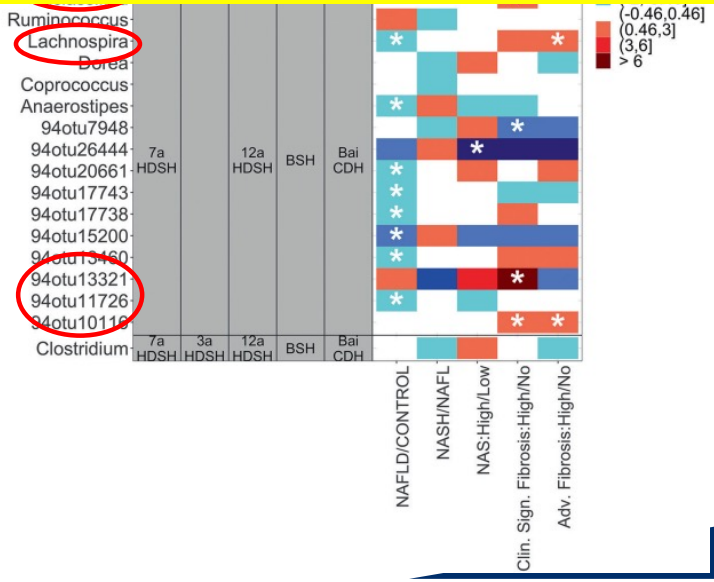


***Bacteroides* (7α, 12α, BSH) increased in clinically significant fibrosis**

***Eggerthella* (3α, 12α, BaiCD, BaiH) increased in advanced fibrosis**

***Lachnospira* (7α, 12α, BaiCD, BaiH, BSH) increased in clinically significant fibrosis and advanced fibrosis**

No changes in *clostridium*



Smirnova, Muthiah MD et al, Hepatology 2022

Effect of bile acids on bacteria

- Bile acids can function as antibiotics
- Unconjugated bile acids have stronger anti-bacterial activity
- Secondary bile acids further limit the growth of bacteria via breaking cell membrane integrity

Stacey et al, Proc R Soc Med 1947
Guzior et al, Microbiome 2021
Kanmani et al, Biomedicines 2020

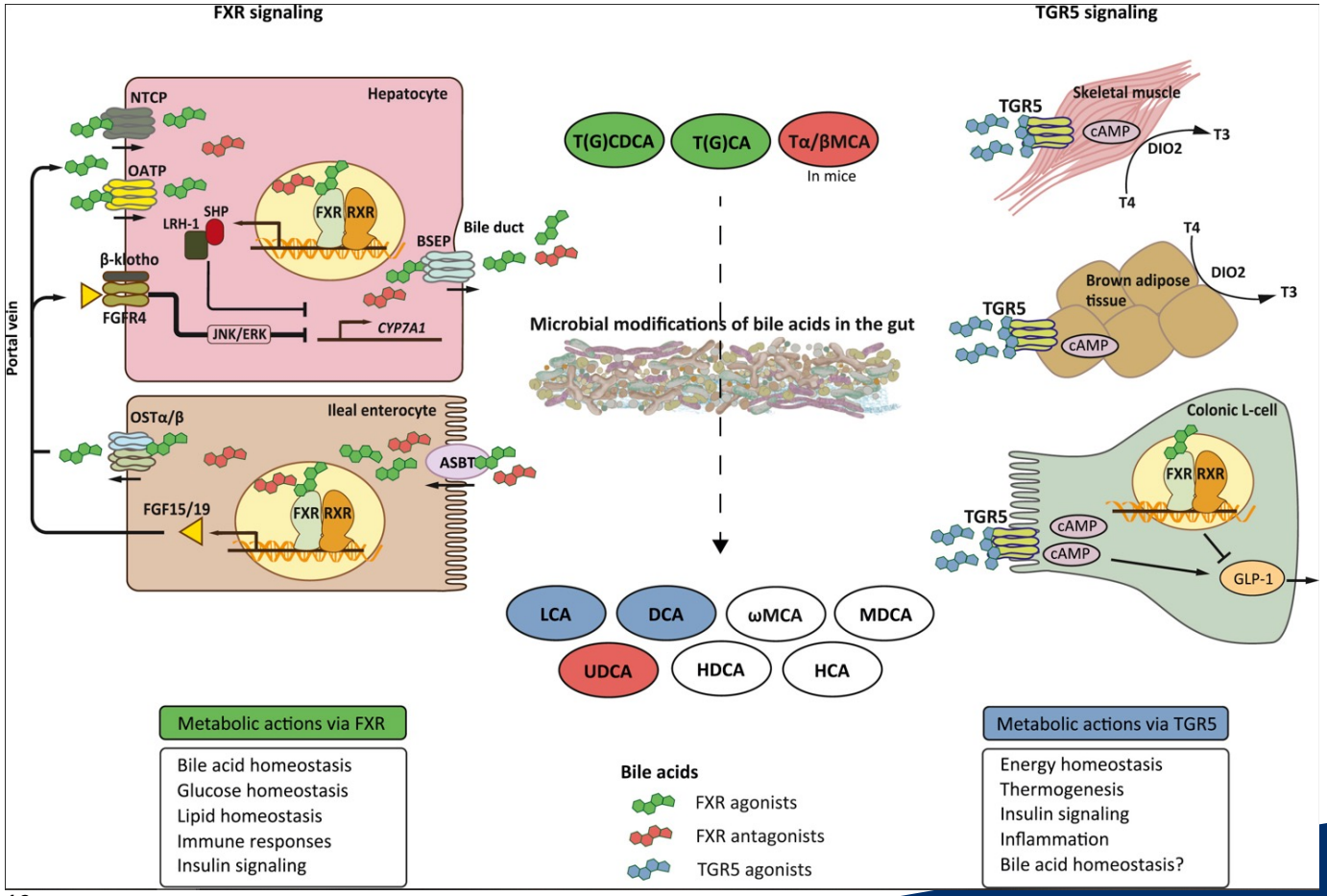
Resistance genes of biome in NASH

- NASH vs NAFL: Endonuclease 4 (nfo); Penicillin-binding protein 1a (mrcA)
- NAS ≥ 4: DNA adenine methylase (dam); outer membrane protein TolC (tolC)
- Clinically significant fibrosis: outer membrane protein TolC (tolC)
- Conferring resistance to antibiotic effects of bile acids

Urdaneta et al, Front Med 2017
Smirnova et al, Hepatology 2022
Begley et al, FEMS Microbiol Rev 2005

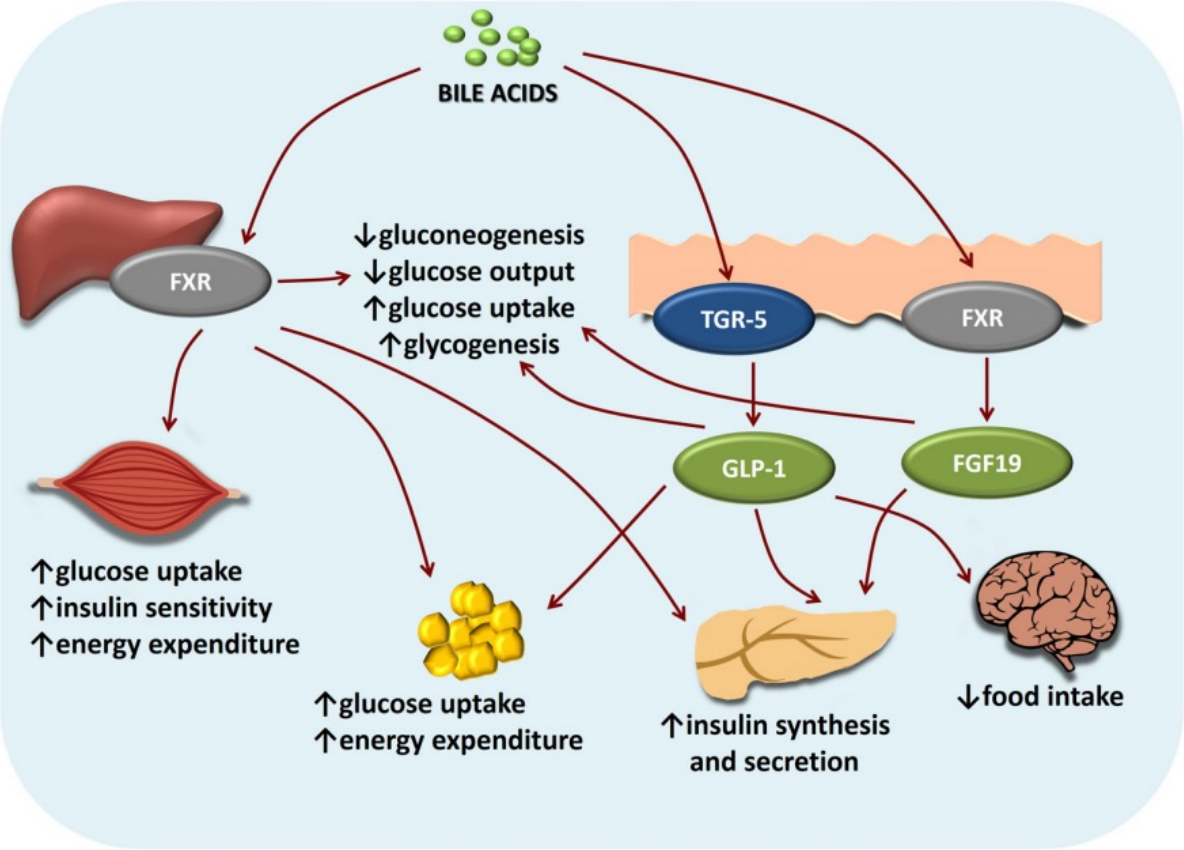
Considerations in bile acid profiles in metabolic disease

Pleotropic effects of bile acids in metabolic disease



Wahlstrom et al, Cell Metab 2016

Pleotropic effects of bile acids in metabolic disease



Danic et al, Front Pharmacol 2018

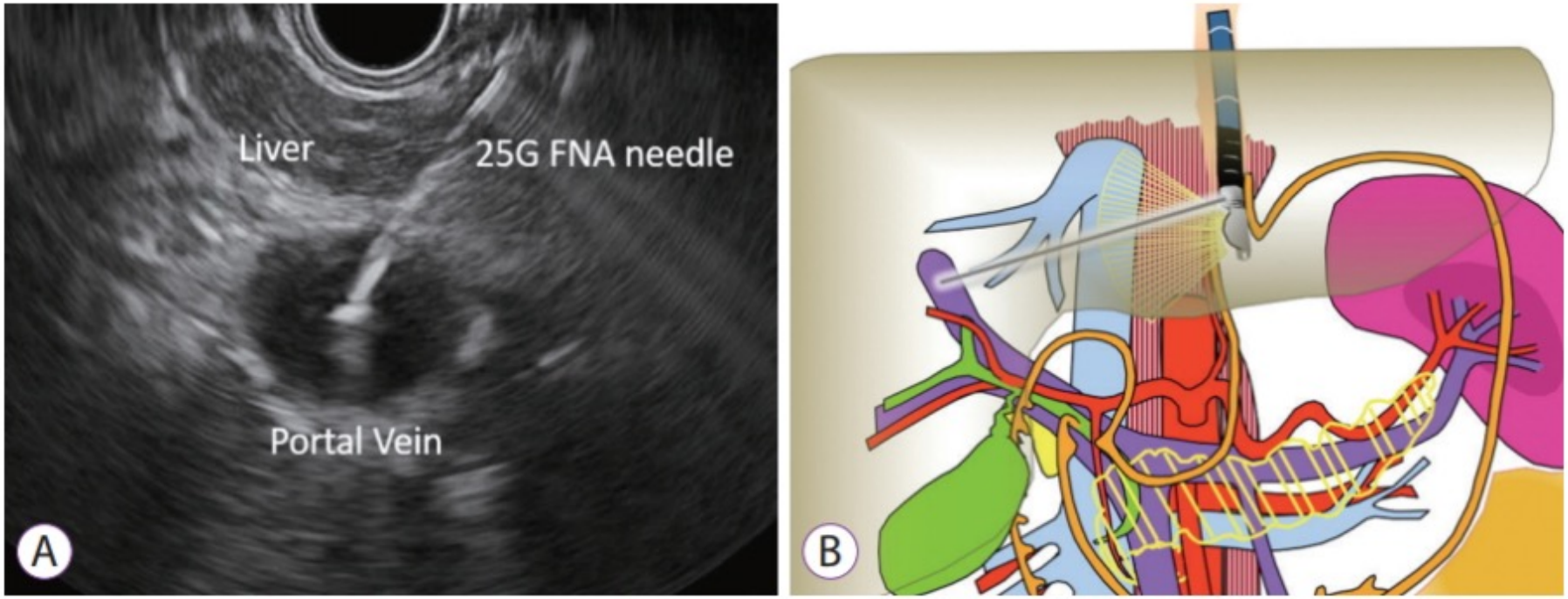
Bile acid receptor affinity

- FXR: CDCA > LCA = DCA > CA
- TGR5: tLCA > LCA > DCA > CDCA > CA

Bile acid	FXR EC_{50}	FXR IC_{50}	TGR5 EC_{50}	VDR EC_{50}	PXR EC_{50}
Cholic acid	100–200 μM[197]	NA	7.72 μM[85], >10 μM[84], 13.6 μM[213]	No effect[211]	No effect[211]
Deoxycholic acid	50 μM[42], 50–75 μM[212]	NA	1.01–1.25 μM[85,213]	No effect[211]	50.2 μM[211]
Chenodeoxycholic acid	1–2 μM[212], 4.5 μM[40], 5.2 μM[185], 7 μM[75], 10 μM[41,212], (T, G) 10 μM[40], 10–30 μM[214], 20 μM[41,212], 25–50 μM[212], 50 μM[42]	NA	4–4.43 μM[84,85], (T) 1.92 μM[213], (G) 3.88 μM[199]	No effect[211]	(T) 104 μM[211]
Lithocholic acid	50 μM[42]	NA	35 nM[84], (T) 0.33 μM[85], 0.53 μM[85], 3 μM[84]	8 μM[41], 12.1 μM[215], 21.6 μM[211]	10.2 μM[211]
3-Keto-lithocholic acid	NA	NA	NA	3 μM[75], 6.8 μM[215]	8.3 μM[211]
Ursodeoxycholic acid	No effect[40]	NA	36.4 μM[213], No effect[85]	No effect[75]	NA

Ahmad et al, Nat Rev Endocrinol 2019

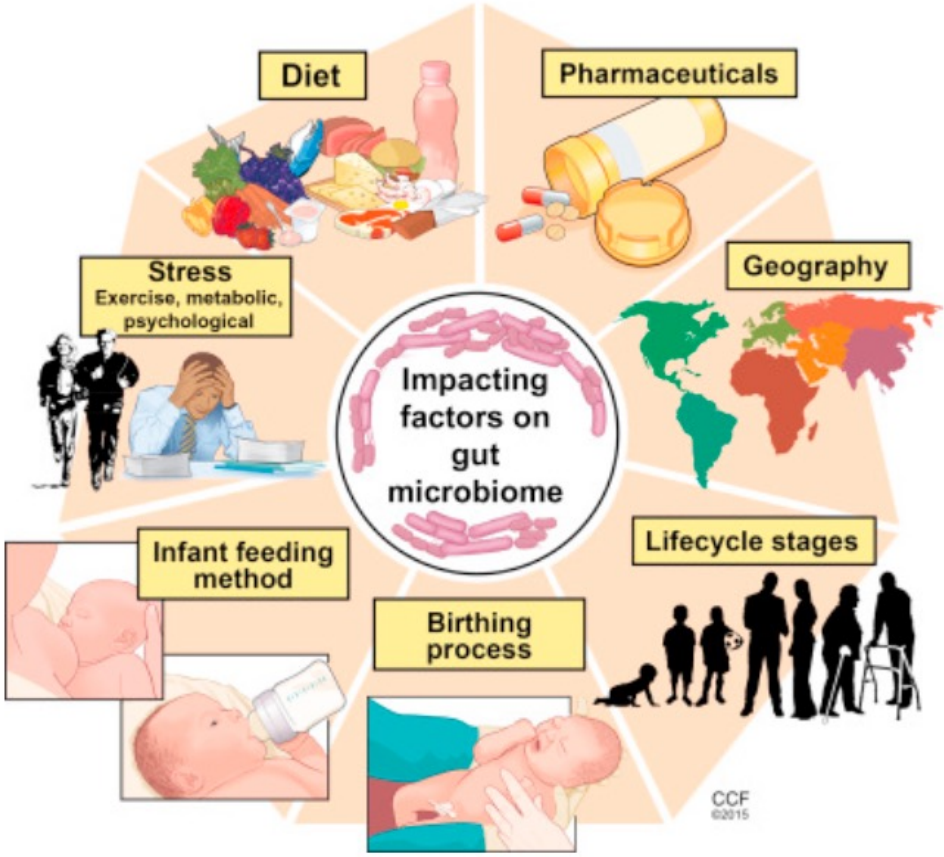
Interrogation of secondary bile acid profiles



Samarasena et al, Clin Endoscopy 2018

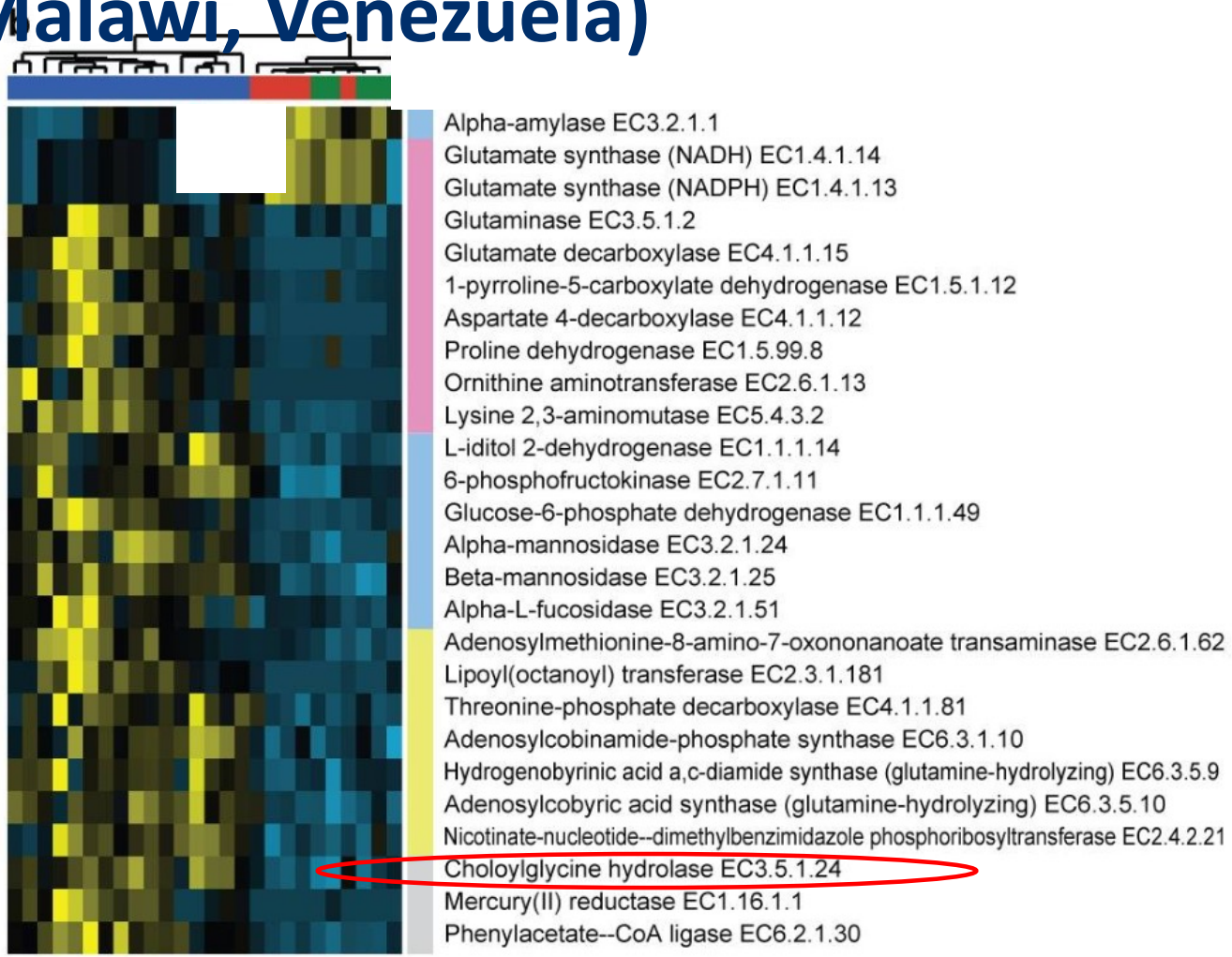
Heterogeneity of the biome

Heterogeneity of gut microbiome



Gail et al, Adult Short Bowel Syndrome 2019

Bile acid metabolizing enzyme heterogeneity (USA, Malawi, Venezuela)



Yatsunenko et al, Nature 2012

Study	Genus changes
Wong 2013	Parabacteroides, Allisonella (i); Faecalibacterium, Anaerosporobacter (d)
Zhu 2013	Prevotella, Peptoniphilus (i); Bifidobacterium, Oscillospira, Roseburia, Alistipes, Blautia, Coprococcus, Ruminococcus (d)
Raman 2013	Lactobacillus, Dorea, Robinsoniella, Roseburia (i); Oscillibacter (d)
Michail 2015	Prevotella (i)
Jiang 2015	Escherichia, Anaerobacter, Lactobacillus, Streptococcus, Clostridium, Alistipes, Prevotella, Oscillibacter, Odoribacter, Flaconifractor (d)
Boursier 2016	Ruminococcus, Bacteroides (i); Prevotella (d)
Wang 2016	Coprococcus, Pseudobutyrivibrio, Moryella, Roseburia, Anaerosporobacter, Anaerotruncus, Ruminococcus, Lactobacillus (d)
Del Chierico 2017	Bradyrhizobium, Anaerococcus, Peptoniphilus, Propionibacterium, Dorea, Ruminococcus, Blautia (i); Oscillospira (d)
Shen 2017	Escherichia, Shigella, Lachnospiraceae, Blautia (i); Prevotella (d)
Caussy 2019	Streptococcus, Bacillus, Lactococcus, Megasphaera, Gallibacterium (i); Catenibacterium, Pseudomonas (d)

Summary

- Changes in bile acids closely associated with NASH and disease progression
- Potential role of gut modified secondary bile acids and bile acid epimers
- Distinct changes in fecal microbiome can account for above changes
- Bidirectional relationship between biome and bile acid profile

Thank you.

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