XXV International Bile Acid Meeting: Bile Acids in Health and Disease 2018

July 6–7, 2018
Clayton Hotel
Burlington Road
Dublin, Ireland

Abstracts/Poster Abstracts Symposium 211

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Symposium 211

XXV INTERNATIONAL BILE ACID MEETING:
BILE ACIDS IN HEALTH AND DISEASE 2018

Dublin, Ireland
July 6 – 7, 2018

Scientific Organization:
D. Häussinger, Düsseldorf (Germany)

Scientific Co-Organization:
U. Beuers, Amsterdam (The Netherlands)
V. Keitel, Düsseldorf (Germany)
M. Trauner, Vienna (Austria)

Local Organizer:
S.J. Keely, Dublin (Ireland)
Session I

Bile acid signaling in health and disease

Chair:
D. Häussinger, Düsseldorf
S.J. Keely, Dublin

Targeting the glucocorticoid receptor for intrahepatic cholestasis
D.D. Moore, Houston

Post-transcriptional regulation of hepatic bile acid and lipid metabolism
T.A. Vallim, Los Angeles

Phospholipid transport by ABCB4: Novel insights into the floppase activity
L. Schmitt, Düsseldorf

Oral Poster Presentation
Taurocholate upregulates IncRNA H19 in cholangiocytes and activates hepatic stellate cells via exosome release under cholestatic conditions
H. Zhou, R. Liu, X. Li, W.M. Pandak, P.B. Hylemon, Richmond

Session II

Interaction of bile acids and the intestinal microbiome

Chair:
U. Beuers, Amsterdam
A.F. Hofmann, La Jolla

Microbiome and bile acid interactions
A. Wahlström, Gothenburg

Modification of the human intestinal microbiome by obeticholic acid
Bile acid microbiome interaction in thermogenesis
J. Heeren, Hamburg

Antibiotics secreted by gut bacteria regulate *Clostridium difficile* growth and the structure of the gut microbiome: Role of secondary bile acids
P.B. Hylemon, J.D. Kang, C.J. Myers, S.C. Harris, G. Kakiyama, J.S. Bajaj, H. Zhou, Richmond

**Oral Poster Presentation**
Enhanced microbial deconjugation of bile acids in pregnancy represses intestinal FXR-mediated regulation of hepatic bile acid synthesis

Presentation of Adolf Windaus Prize
D. Häussinger, Düsseldorf

Adolf Windaus Prize Lecture
B. Stieger, Zürich

**Session III**

**Bile acid transport in health and disease**

Chair:
V. Keitel, Düsseldorf
D.D. Moore, Houston

Visualization of bile flux in liver by 2-photon microscopy: The principle of diffusion-limited bile canalicular transport
J.G. Hengstler, N. Vartak, Dortmund

Inhibition of bile acid reabsorption to ameliorate cholestatic liver injury
S.F.J. van de Graaf, Amsterdam

IgG4-associated cholangitis – Another model cholangiopathy with an underlying defect of the biliary bicarbonate umbrella?
T. Herta, L. Hubers, S. van de Graaf, U. Beuers, Amsterdam

Bile acid transport in the intestine: From genetic variants to therapeutic targets
P.A. Dawson, Atlanta
**Oral Poster Presentation**
Inactivation of the intestinal apical sodium bile acid transporter profoundly inhibits cholesterol absorption  

**Session IV**

**Bile acid transport in health and disease**

Chair:
P.B. Hylemon, Richmond  
R.P.J. Oude Elferink, Amsterdam

Defect in hepatic tight junction proteins (TJP2, DCDC2, Claudin-1) as cause of cholestasis and cholangitis in children (no abstract)  
R.J. Thompson, London

Analysis of transporter defects in children and adults with cholestatic liver disease  
C. Dröge, M. Bonus, H. Gohlke, V. Keitel, D. Häussinger, Düsseldorf 45

Genetic determinants of cholangiopathies: Molecular and systems genetics  
F. Lammert, Homburg 46

**Oral Poster Presentation**
Loss of BSEP/ABCB11 protects MDR2/ABCB4 KO mice from cholestatic liver injury by altering bile acid profile and signaling  

**Session V**

**Extrahepatic effects of bile acids and bile acid receptors**

Chair:
J.M. Banales, San Sebastian  
P. Fickert, Graz

Targeting bile acids in intestinal disease  
S.J. Keely, Dublin 51

Interaction of gut microbiota and bile acids in NAFLD  
S. Kersten, Wageningen 52
Altered bile acid signaling in NAFLD in humans

Bile acids increase serum corticosterone levels in cholemic mice and induce cortisol secretion in adrenocortical H295R cells in an S1PR2-ERK-SF-1-dependent manner

Oral Poster Presentation
Targeting host and microbial choline metabolism by a semi-synthetic bile acid suppresses TMA/TMAO formation and ameliorates atherosclerosis and NASH in mice

Session VI

Bile acid receptors and bile acid signaling as therapeutic targets

Chair:
M.A. Ávila, Pamplona
M. Trauner, Vienna

Targeting TGR5 to treat polycystic liver disease
N.F. LaRusso, T.V. Masyuk, A.I. Masyuk, J. Ding, N.E. Pirius, C.E. Trussoni, Rochester 59

TGR5 (Gpbar1) in inflammatory liver disease
M. Reich, K. Deutschmann, P. Lang, D. Häussinger, V. Keitel, Düsseldorf 60 – 61

FXR agonists in portal hypertension
T. Reiberger, Vienna 62

Intestinal FXR agonism and fibroblast growth factor 19 protect against colitis and intestinal tumorigenesis
A. Moschetta, Bari 63

Oral Poster Presentation
Treatment response of murine sclerosing cholangitis to systemic versus intestinal FXR agonists segregates with their effects on hepatic pro-inflammatory cytokine production
T. Shi, C.S. Lages, R. Kudira, L. Matuschek, M. Mullen, A. Ortiz, K.-J. Lee, D. Zook, B. Wagner, A. Miethke, Cincinnati 64
Session VII

Bile acid receptors and bile acid signaling as therapeutic targets

Chair:
N.F. LaRusso, Rochester
C. Williamson, London

Amphiregulin/EGFR as therapeutic target in liver disease
M.A. Avila, Pamplona

Stimulation of ammonium detoxification via FXR; where isoforms matter
S.W.C. van Mil, Utrecht

Role of miR-506 in primary biliary cholangitis
J.M. Banales, San Sebastian

Clinical and mechanistic aspects of nor-ursodeoxycholic acid (norUDCA)
M. Trauner, Vienna

Oral Poster Presentation
Serum biomarker development demonstrating the transformation of fatty liver to steatohepatitis in association with diabetes mellitus
M. Fuchs, D. Marques, H. Takei, H. Nittono, S. Erickson,
D. Rodriguez-Agudo, G. Gil, P.B. Hylemon, H. Zhou, J.S. Bajaj,
W.M. Pandak, G. Kakiyama, Richmond

Presentation of Poster Awards
D. Häussinger, Düsseldorf

List of Chairpersons, Speakers and Scientific Organizers
1. Obeticholic acid increases cholesterol saturation and FGF19 in human gallbladder bile
(Graz, Vienna, AT; Stockholm, Gothenburg, SE)

2. Rapid regulation of hepatic lipid metabolism by modulation of bile acid fluxes in humans
A. Al-Khaifi, S. Straniero, M. Ghosh Laskar, M. Rudling, B. Angelin
(Stockholm, SE)

3. In vitro rescue of ABCB11 non-sense mutations: Induction of a readthrough of premature stop codons

4. Ubiquitination of Lys-340 reduces NTCP-mediated bile acid uptake and NTCP plasma membrane expression
M.D. Appelman, C. Sachetto, M.J.D. Robin, S.F.J. van de Graaf
(Amsterdam, NL)

5. Sex hormone-dependent intestinal expression of ASBT determines the sclerosing cholangitis phenotype and the response to pharmacological disruption of enterohepatic circulation of bile acids in Mdr2-/- mice

6. The role of microbiota in sex-specific regulation of lipid metabolism

7. Complex treatment of uncomplicated cholelithiasis at the stage of cholecystolithiasis
O. Babii, B. Shevchenko, I. Konenko (Dnipro, UA)

8. The use of SeHCAT scans in patients with undiagnosed chronic diarrhea
A.S. Bancil, J. Cooney, S. Gupta (London, GB)

9. The role of nuclear receptors and histone deacetylases in the regulation of bile acid synthesis in humans: Effects of drug treatment
M. Bertolotti, C. Anzivino, E. Baldelli, L. Carulli, C. Mussi, C. Gabbi (Modena, IT)
10. Hepatoprotective impact of TGR5: central role of gallbladder function and bile acid pool modulation
(Orsay, FR; Basel, CH; Paris, FR)

11. Impact of miR-24 on a MEN1 and SMAD3 gene expression in primary biliary cholangitis
M. Blatkiewicz, P. Milkiewicz, M. Milkiewicz (Szczecin, Warsaw, PL)

12. Early onset of increased hypercholanemia during pregnancy correlates with higher risk of meconium-stained fluid
(Salamanca, ES; Buenos Aires, AR; London, GB)

13. sGC stimulation and PDE5 inhibition decrease sinusoidal resistance and reduce fibrosis in rats with biliary cirrhosis

14. Bile acids induce hepatic chemokine expression by activating Ca^{2+}/NFAT signaling
S.-Y. Cai, A. Mennone, M.T. Guerra, M. Nathanson, J.L. Boyer
(New Haven, US)

15. Modification of the intestinal intraluminal bile acid pool composition upon bariatric surgery in a preclinical minipig model

16. Both intestine-specific and renal/hepatic inhibition of the bile salt transporter ASBT ameliorates cholestatic liver injury
H.-W. Chen, J.B. van Niekerk, D. Slijepcevic, R. Roscam Abbing, S.F.J. van de Graaf (Amsterdam, NL)

17. FXR and TGR5 signaling crosstalk and the gut microbiota in liver metabolism and diseases
J. Chiang, P. Pathak, J.M. Ferrell, S. Boehme (Rootstown, US)

18. Role of a high-fructose diet in early stages of cholelithiasis
R. del Pozo, L. Mardones, M. Villagrán, K. Muñoz, C. Cabezas, L. Troncoso, M. Mellado, M. Muñoz (Concepcion, CL)

19. Role of the bile acid receptor TGR5 (GPBAR1) in cholangiocarcinoma (CCA)
K. Deutschmann, M. Reich, A. Lang, R. Piekorz, C. Gertzen, H. Gohlke, D. Häussinger, V. Keitel (Düsseldorf, DE)
20. Whole genome sequencing of 278 patients with intrahepatic cholestasis of pregnancy: Initial findings

21. Inhibition of hepatic bile acid uptake improves obesity-related metabolic dysfunctions in mice

22. The influence of gut microbiota-mediated bile acid metabolism on the transport of P-glycoprotein substrates across the intestinal epithelium

23. Neutraceutical targeting of the bile acid receptor, farnesoid X receptor, for intestinal disease

24. What is the cost of delayed diagnosis of bile acid malabsorption?
D. Fernandes, D. Poon, L. White, J. Andreyev (Lincoln, GB)

25. Chronic ursodeoxycholic acid treatment protects against acute ischaemia-induced arrhythmias and improves conduction velocity in adult hearts
E. Ferraro, C. Mansfield, J. Gorelik, F.S. Ng (London, GB)

B. Flinders, L.R.S. Huizing, M. van Heerden, F. Cuyckens, S.W.M. Olde Damink, R.M.A. Heerens, F.G. Schaap, R.J. Vreeken (Maastricht, NL; Beerse, BE; Aachen, DE)

27. N-(4-[18F]fluorobenzyl)cholylglycine, a novel tracer for positron emission tomography of enterohepatic circulation of bile acids: Proof-of-concept study in rats
K. Frisch, D.H.R. Stimson, T. Venkatachalam, G.K. Pierens, S. Keiding, D. Reutens, R. Bhalla (Aarhus, DK; Brisbane, AU)

28.* Loss of BSEP/ABCB11 protects MDR2/ABCB4 KO mice from cholestatic liver injury by altering bile acid profile and signaling
29.* Serum biomarker development demonstrating the transformation of fatty liver to steatohepatitis in association with diabetes mellitus
(Richmond, San Francisco, US; Tokyo, JP)

30. Circulating fibroblast growth factor 21 is increased during cholestasis and correlates with hepatic expression of genes involved in regulating bile acid homeostasis
C. Gabbi, C. Anzivino, E. Baldelli, L. Carulli, M. Bertolotti (Modena, IT)

31. Low-dose ursodeoxycholic acid in association with low caloric diet in the long term treatment of non-alcoholic steatohepatitis in obese patients
A. Genunche-Dumitrescu, D. Badea, M. Badea, P. Mitrut, C. Deliu, A. Badea
(Craiova, RO)

32. Combined budesonide-UDCA therapy versus UDCA monotherapy in the treatment of the primary biliary cholangitis
A. Genunche-Dumitrescu, D. Badea, M. Badea, P. Mitrut, C. Deliu, A. Badea
(Craiova, RO)

33. Testosterone reduces circulating PCSK9 but does not influence cholesterol or bile acid synthesis in healthy males
M. Ghosh Laskar, L. Beckman, A. Laskar, N. Gårevik, L. Ekström, M. Rudling, B. Angelin (Stockholm, SE)

34. T cell-mediated cholangitis alters bile acid metabolism

35. Intestinal and liver crosstalk in control of cholesterol homeostasis by FXR

36. A novel fibroblast growth factor 15-dependent and bile acid-independent promotion of liver regeneration in mice
G. Guo, B. Kong (Piscataway, US)

37. Increased risk of adverse pregnancy outcomes in gestational diabetes mellitus complicated by intrahepatic cholestasis of pregnancy
(Adelaide, Elizabeth Vale, AU)
38. The number needed to treat with ursodeoxycholic acid to prevent one liver transplantation or death in patients with primary biliary cholangitis

39. Differences in contractile and signalling responses to bile acids and their respective conjugates in neonatal cardiomyocytes: role of Gi protein, muscarinic receptors and TGR5

40. Transcriptional regulation of FGF19 in human intestinal cells by nuclear receptor agonists
D. Jahn, D. Dorbath, H.M. Hermanns, A. Geier (Würzburg, DE)

41. Gut bacteria of the family Coriobacteriaceae influence lipid metabolism in mice

42. Mitochondrial oxysterol biosynthetic pathway gives evidence for CYP7B1 as controller of regulatory oxysterols

43. Oncomir microRNA-346 is upregulated in ascending but not sigmoid colon in patients with primary sclerosing cholangitis (PSC) and ulcerative colitis (UC)
A. Kempinska-Podhorodecka, P. Milkiewicz, E. Wunsch, L. Krupa, K. Gutowski, M. Milkiewicz (Szczecin, Warsaw, Rzeszow, PL)

44. Effect of ursodeoxycholic acid on biochemical markers of cholestasis in children with Alagille syndrome
A.I. Khavkin, G.V. Volynets, A.V. Nikitin, T.A. Skvortsova, E.L. Nikonov (Moscow, RU)

45. Treatment with S-adenosyl-L-methionine (SAMe) may affect immune responses in primary biliary cholangitis (PBC) via its antioxidant properties
E. Kilanczyk, M. Milkiewicz, E. Wunsch, J.M. Banales, P. Milkiewicz (Szczecin, Warsaw, PL; San Sebastian, ES)

46. Isolation and characterization of infant BSH active bacterial isolates
C. Killian, P. Cronin, S.L. Long, C.G.M. Gahan, F. Shanahan, S.A. Joyce (Cork, IE)
47. Difference between two mice strains changes their bile acid composition, gut microbiota, and metabolic regulation system

48. Dynamic determinants of portal hypertension are identified by histological collagen proportionate area estimations

49. Investigation of the modulation of the ATPase activity of human multidrug resistance protein 3 (MDR3/ABCB4) by bile acids
T. Kroll, M. Prescher, S. Smits, L. Schmitt (Düsseldorf, DE)

50. Anti-apoptotic actions of lithocholic acid on colonic epithelial cells: Implications for treatment of inflammatory bowel disease
N.K. Lajczak, A.M. O'Dwyer, S.J. Keely (Dublin, IE)

51. Multiple cholephilic compounds involved in cholestatic itch inhibit autotaxin activity

52. Assessment of drugs that inhibit bile salt export pump (Bsep) in a siRNA Bsep knockdown rat model

53. Changes in plasma bile acid profiles after partial internal biliary diversion in three ABCB11-mutated (PFIC2) patients
T. Liu, R.-X. Wang, J. Han, Y.-Y. Yan, Y.-L. Qiu, L.-L. Liu, C.H. Borchers, V. Ling, J.-S. Wang (Shanghai, CN; Vancouver, Montreal, Victoria, CA)

54. Probiotic potential of new Lactobacillus salivarius isolate with regard to BSH activity
S.-L. Long, F. Shanahan, C.G.M. Gahan, S.A. Joyce (Cork, IE)

55. Histomorphological assessment of hepatic fibrosis progression with accompanying pronounced ductular proliferation in chronic thiacetamide-induced experimental liver fibrosis/cirrhosis in young rats
J.M. Lotowska, M.E. Sobaniec-Lotowska, B. Szukiel, S.B. Lotowska, D.M. Lebensztejn, W. Debek (Bialystok, PL)
56. Evaluation of serum fibroblast growth factor 19 (FGF19) and total free fecal bile acids in stool as markers of bile acid malabsorption in patients with chronic diarrhea: A pilot study
I. Lyutakov, P. Penchev, R. Nakov, B. Asenova, M. Chetirska, R. Vatcheva-Dobrevska, B. Vladimirov (Sofia, BG)

57. Does the placenta contribute to the enhanced risk of pruritus during maternal hypercholanemia?

58. A novel non-immunosuppressive cyclosporine analog inhibits NTCP and shows potential for treatment of metabolic diseases in mouse models
F. Mao, Z. Zhou, Y. Liu, Y. Li, H. Ruan, Z. Zhang, W. Li (Beijing, CN)

59. Bile acid 7-dehydroxylation by Clostridium scindens in vitro and in vivo
S. Marion, N. Studer, L. Desharnais, S. Escrig, A. Meibom, S. Hapfelmeier, R. Bernier-Latmani (Lausanne, Bern, CH)

60. OCA ameliorates dyslipidemia but not insulin resistance in a mouse model of gestational diabetes mellitus

61. TGR5-dependent hepatoprotection through the regulation of biliary epithelium permeability

62. Deviations in peripheral blood subpopulations are connected with the presence of pruritus in primary biliary cholangitis patients

63. Bile acid traffic across the mammary gland: Implications on lactation during maternal cholestasis

64.* Targeting host and microbial choline metabolism by a semi-synthetic bile acid suppresses TMA/TMAO formation and ameliorates atherosclerosis and NASH in mice
T. Moustafa, T. Madl, D. Kratky, S. Stryeck, T. Eichmann, J. Gumhold, S. Racedo, D. Silbert, T. Hitch, C. Kern, T. Clavel, K. Schoonjans, P. Fickert, M. Trauner (Graz, AT; Aachen, DE; Lausanne, CH; Vienna, AT)
65. Asperuloside, the extraction of Tochu-tea improves metabolic syndrome through the induction of bile acid signaling
A. Nakamura, T. Hirata, T. Ueda, A. Honda, N. Kitamura, Y. Yokoyama, M. Watanabe (Fujisawa, Tokyo, Osaka, Ibaraki, JP)

66.* Enhanced microbial deconjugation of bile acids in pregnancy represses intestinal FXR-mediated regulation of hepatic bile acid synthesis

67. Increased endogenously synthesized oxysterol accumulation represents an initiating step in fatty liver’s progression toward inflammation

68. Pharmacological inhibition of the apical sodium-dependent bile acid transporter (ASBT) protects ileal enterocytes from bile acid-induced injury in adult mice
A. Rao, C. Ferrebee, J. Li, S.J. Karpen, P.A. Dawson (Atlanta, US)

69. Upregulation of the membrane-bound bile acid receptor (TGR5) in response to Listeria monocytogenes infection involves Krüppel-like factor 5 (KLF5)
M. Reich, K. Deutschmann, J. Stindt, H.C. Xu, P. Lang, D. Herebian, E. Mayatepek, D. Häussinger, V. Keitel (Düsseldorf, DE)

70. Oxysterol sulfates alleviate injured liver function and decrease mortality in mouse models
S. Ren, J.K. Kim, Y. Ning (Richmond, US)

71. Calnexin depletion by ER-stress during cholestasis inhibits the Na+-taurocholate cotransporting polypeptide (NTCP)
M.J.D. Robin, M.D. Appelman, H.R. Vos, R.M. van Es, J.C. Paton, A.W. Paton, B. Burgering, J. Heijmans, S.F.J. van de Graaf (Amsterdam, Utrecht, NL; Adelaide, AU)

72. Inhibiting NTCP-mediated hepatic bile salt uptake stimulates biliary lipid excretion, independent of changes in bile salt output and hydrophobicity

73. Gallbladder bile supersaturated with cholesterol in gallstone patients develops chiefly from bile acid shortage worldwide
M. Rudling, A. Laskar, S. Straniero (Stockholm, SE)
74. The FXR agonist GS-9674 reduces fibrosis and portal hypertension in a rat model of NASH

75. \( \Delta^4 \)-3-oxosteroid-5\( \beta \)-reductase (AKR1D1) deficiency: Responses and long-term outcomes from oral bile acid therapy
K.D.R. Setchell, M. Zhang, J. Zhao, J. Gong, Y. Lu, J.-S. Wang (Cincinnati, US; Shanghai, CN)

76.* Treatment response of murine sclerosing cholangitis to systemic versus intestinal FXR agonists segregates with their effects on hepatic pro-inflammatory cytokine production

77. Blood-circulating bile acids support hematopoietic recovery after chemotherapy

78. Roux-en-Y gastric bypass induces elevation of plasma bile acids through disturbed intestinal transit and long-term change of synthesis control

79. The role of the population of hepatic progenitor/oval cells in the process of fibrogenesis in the model of biliary fibrosis induced by bile duct ligation in young Wistar Crl: Wi(Han) rats: The transmission electron-microscopic analysis
M.E. Sobaniec-Lotowska, J.M. Lotowska, P. Sobaniec, D.M. Lebensztejn, J. Reszec, W. Debek (Bialystok, PL)

80. The results of ursodeoxycholic acid use in the diagnosis of gallbladder polyps
A. Soylu, S. Cakmak, I. Sevindir, I. Soylu (Istanbul, TR)

81. Interleukin-8 mediates downregulation of TGR5 in biliary epithelial cells, which may contribute to progression of sclerosing cholangitis
L. Spomer, M. Reich, J. Hönhe, J.R. Hov, T.H. Karlsen, D. Nierhoff, D. Häussinger, V. Keitel (Düsseldorf, Cologne, DE; Oslo, NO)

82. A novel, cell-based assay for measuring bile salt transport inhibition by BSEP antibodies in sera from antibody-induced BSEP deficiency (AIBD) patients
J. Stindt, C. Dröge, M. Wammers, P. Philippki, C. Wiek, H. Hanenberg, D. Häussinger, V. Keitel (Düsseldorf, DE)
83. Effects of bile acid signaling and microbiota during HCC progression in NASH
S. Sydor, J. Best, P. Manka, I. Messerschmidt, K.N. Faber, H. Moshage,
R. Vilchez Vargas, G. Gerken, A. Canbay, L.P. Bechmann
(Magdeburg, Essen, DE; Groningen, NL)

84. Bile acid alterations are associated with insulin resistance but not NASH in
obese patients
A. Tailléux, V. Legry, S. Francque, J. Haas, A. Verrijken, S. Caron, P. Lefebvre,
J.-F. Goossens, M. Kouach, A. Descat, E. Vallez, O. Chávez-Talavera,
S. Lestavel, L. Van Gaal, R. Paumelle, B. Staels (Lille, FR; Antwerp, BE)

85. Topical intestinal TGR5 agonists promote glucagon-like peptide-1 secretion and
improve glucose tolerance
A. Tailléux, O. Chávez-Talavera, S. Lestavel, N. Hennuyer, F. Leroux,
C. Piveteau, E. Vallez, E. Dorchies, I. Duplan, B. Staels, B. Deprez, J. Charton
(Lille, FR)

86. Evidence-based clinical guideline for primary sclerosing cholangitis in Japan
2017
S. Tazuma, H. Isayama, T. Nakazawa, K. Notohara, T. Tsuyuguchi,
M. Serikawa, T. Mori, A. Tanaka, H. Takikawa (Hiroshima, JP)

87. A real-time bioluminescent method for assessing bile acid transporter activity
(Chicago, US)

88. Targeting organic solute transporter alpha-beta to attenuate liver damage
induced by bile duct ligation
S.M.W. van de Wiel, S.F.J. van de Graaf (Amsterdam, NL)

89. Ursodeoxycholic acid is associated with an improved liver transplant-free
survival in all patients with primary biliary cholangitis
A. van der Meer, M.H. Harms, H.R. van Buuren, C. Corpechot, D. Thorburn,
H.L.A. Janssen, K.D. Lindor, G.M. Hirschfield, A. Parés, A. Floreani, M.J. Mayo,
P. Invernizzi, P.M. Battezzati, F. Nevens, C.Y. Ponsioen, A.L. Mason,
K.V. Kowdley, W.J. Lammers, B.E. Hansen
(Rotterdam, Amsterdam, NL; Paris, FR; London, Birmingham, GB; Toronto,
Edmonton, CA; Rochester, Phoenix, Seattle, Dallas, US; Barcelona, ES;
Padova, Milan, IT; Leuven, BE)

90. The postoperative serum course of liver regeneration-associated signaling
factors FGF19 and bile salts, in non- and post-cholestatic patients undergoing
liver resection
K.M. van Mierlo, K.V. Koelfat, M. Schmeding, T. Cramer, I. Sauer, C.H. Dejong,
F.G. Schaap, U.P. Neumann, S.W. Olde Damink (Maastricht, NL; Aachen, DE)
91. Elongation of the fetal PR interval associated with intrahepatic cholestasis of pregnancy is normalised by UDCA therapy
(London, Nottingham, GB; New York, US)

92.* Inactivation of the intestinal apical sodium bile acid transporter profoundly inhibits cholesterol absorption
H.J. Verkade, I. van de Peppel, A. Bertolini, T.H. van Dijk, A.K. Groen, J.W. Jonker (Groningen, NL)

93. Effect of ursodeoxycholic acid on biochemical markers of cholestasis in children with progressive intrahepatic cholestasis of type 1 and type 2
G.V. Volynets, A.I. Khavkin, A.V. Nikitin, T.A. Skvortsova, E.L. Nikonov (Moscow, RU)

94. A physiology-based model of the distribution of individual bile acids within the enterohepatic circulation under normal and pathological conditions in humans
V. Voronova, V. Sokolov, D. Chenikova, A. Al-Khaifi, S. Straniero, C. Kumar, K. Peskov, G. Helmlinger, M. Rudling, B. Angelin (Moscow, RU; Stockholm, Mölndal, SE; Waltham, US)

95. Obeticholic acid compassionate use therapy for severe primary bile acid diarrhoea
J.R.F. Walters (London, GB)

96. Expression of mir-21 and mir-150 in patients with primary biliary cholangitis (PBC)
U. Wasik, E. Wunsch, P. Milkiewicz, M. Milkiewicz (Szczecin, Warsaw, PL)

97. Porphyran, a functional ingredient of Japanese "Nori", improves visceral obesity and non-alcoholic fatty liver disease via alteration of bile acids and intestine interactions in mice and humans
M. Watanabe, Y. Takahina, K. Tanaka, S. Fukuda, K. Tsubota, K. Ishihara (Fujisawa, Tokyo, Tsuruoka, Yokohama, JP)

98. Humanized bile acids lead to increased fibrosis in a toxin-induced mouse model of extrahepatic bile duct injury
A. Wehrman, A. Kriegermeier, O. Waisbourd-Zinman, R. Wells (Philadelphia, US; Tel-Aviv, IL)

99. Dietary protein quality and quantity changed energy metabolism via liver and intestine interactions signals
100. The mechanistic target of rapamycin complex 1 (mTORC1) regulates bile acid biosynthetic and transporter gene expression via activity of the farnesoid X receptor (FXR)
   (Graz, Innsbruck, AT; Utrecht, NL)

101. Renal lesions in HSD3B7 deficiency resolved with primary bile acid replacement therapy
   J. Zhao, L.-J. Fang, K.D.R. Setchell, J.-X. Wang, Y. Gong, Y. Sun, J.-S. Wang
   (Shanghai, CN; Cincinnati, US)

102.* Taurocholate upregulates lncRNA H19 in cholangiocytes and activates hepatic stellate cells via exosome release under cholestatic conditions
    H. Zhou, R. Liu, X. Li, W.M. Pandak, P.B. Hylemon (Richmond, US)

103. Immunomodulatory mechanisms of the novel therapeutic bile acid 24-norursodeoxycholic acid
    (Vienna, Graz, AT)

* = Posters of Distinction
Session I

Bile acid signaling in health and disease
Targeting the glucocorticoid receptor for intrahepatic cholestasis

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Cholestasis is a hereditary or acquired disruption of bile flow that interrupts the normal circulation of bile acids from the liver to the intestine and back. Progressive familial intrahepatic cholestasis (PFIC) is a heterogeneous group of autosomal recessive disorders of childhood caused by mutations in genes of bile acid circulation and homeostasis, including the bile acid/phospholipid transporters \( ABCB11 \) (\( BSEP \), PFIC2), \( ABCB4 \) (human \( MDR3 \), PFIC3) and the bile acid-activated nuclear receptor \( NR1H4 \) (\( FXR \), PFIC5). In PFIC toxic bile acids accumulate in the liver and, in severe forms, cause liver failure and mortality. We have characterized differential pathogenesis in two mouse PFIC models: \( Fxr/Shp \) double knockouts (\( Fxr^{--} \); \( Shp^{--} \), DKO) and \( Bsep \) knockout (\( Abcb11^{--} \), BKO), representative of PFIC5 and PFIC2, respectively. Despite gross phenotypic similarities, DKO and BKO mice exhibit quite distinct molecular profiles of accumulated BA species and BA-related gene expression that are a direct consequence of DKO-specific xenobiotic nuclear receptor signaling. This result emphasizes the heterogeneity of PFIC syndromes and the importance of understanding molecular pathogenesis as well as developing targeted therapeutics.

We previously demonstrated that glucocorticoids have both beneficial and deleterious effects in the mouse \( CCl_4 \) model of hepatic fibrosis due to differential effects in distinct target cells. Both standard glucocorticoid receptor (GR) agonists and the selective GR modulator Compound A (CpdA) act in stellate cells to repress profibrotic gene expression, but also decrease beneficial inflammatory cell infiltration and increase liver injury through immune cell modulation. We have extended these divergent results to the DKO and BKO PFIC models. In DKO mice, the GR ligands decrease both liver and serum BAs with particularly striking effects in CpdA-treated mice. They also show anti-fibrotic effects. These effects are absent in BKO mice. These results show that GR differentially modulates BA homeostasis in PFIC models, dependent on the disease context, and predict that GR ligands may be effective treatments for specific forms of PFIC diseases.
Post-transcriptional regulation of hepatic bile acid and lipid metabolism

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Bile acids are detergents and important signaling molecules that activate the nuclear receptor FXR to control key metabolic processes, including feedback mechanisms to maintain bile acid homeostasis. FXR is the central rheostat of bile acid metabolism, and activation of FXR decreases the mRNA levels of bile acid synthetic genes, including Cyp7a1, the gene encoding the rate-limiting enzyme of bile acid synthesis. We have recently discovered that Cyp7a1 mRNA levels are rapidly reduced after pharmacologic FXR activation in wild-type, but not Fxr−/− or liver-specific Fxr knockout mice (FxrL-KO). The rapid decrease in Cyp7a1 mRNA suggested a previously unidentified post-transcriptional mechanism, and we hypothesized that this mode of regulation would be mediated by an RNA binding protein. Using synthetic and endogenous FXR agonists we identified the RNA binding protein ZFP36L1 as a novel FXR target gene. ZFP36L1 mRNA and protein levels were increased as early as 30 minutes after FXR activation in mice. ZFP36L1 is a bona-fide RNA binding protein that promotes degradation of mRNA targets by binding to AU-rich elements (AREs) in the 3’ UTR. The Cyp7a1 UTR contains multiple AU-rich elements, and we used reporter assays to show that ZFP36L1 targets the human and mouse Cyp7a1 UTR. We generated in vivo and in vitro ZFP36L1 gain-of-function models and show that hepatic overexpression of ZFP36L1 decreased Cyp7a1 mRNA and protein and decreased bile acid levels. In contrast, liver-specific Zfp36l1 knockout mice (Zfp36l1L-KO) have elevated Cyp7a1 mRNA and protein, and increased biliary bile acid levels as well as an altered bile acid pool composition. Given that bile acids are important metabolites that control lipid absorption and signaling, we investigated whether loss of hepatic Zfp36l1 resulted in broader metabolic dysfunction. Western diet fed Zfp36l1L-KO mice had reduced body weight gain, specifically in adipose tissue depots, as well as reduced steatosis compared to littermate Zfp36l1flx-flx mice. The differences in adiposity and steatosis were attributed to reduced lipid absorption, as Zfp36l1L-KO mice have increased fecal caloric content and reduced triglyceride absorption as determined by an intragastric fat tolerance test. The decreased lipid absorption is consistent with an altered bile acid metabolism. Thus, we have identified a novel pathway that controls Cyp7a1 and bile acid metabolism that has wider implications in obesity and hepatosteatosis.
Phospholipid transport by ABCB4: Novel insights into the floppase activity

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Bile acids are harsh detergents that are capable of solubilizing biological membranes. Synthesized in the cytosol of hepatocytes, they are translocated across the canalicular membrane into the bile duct by the ABC transporter BSEP (bile salt export pump or ABCB11). Here, the harsh detergent of bile acids is counteracted by the formation of so-called mixed micelles composed of bile acids, lipids of the phosphatidylcholine (PC) family and cholesterol. Cholesterol is secreted into the bile duct by the ABC transporter ABC G5/G8 and PC lipids are flopped from the inner leaflet of the membrane to the outer leaflet by the ABC transporter MDR3 (multidrug resistance protein 3 or ABCB4). Subsequently, the lipids are incorporated into mixed micelles by an unknown mechanism. Despite the high sequence identity of MDR3 and MDR1 (P-gp or ABCB1) of 76%, MDR3 is highly specific for phospholipids of the PC family, while MDR1 possesses an extremely promiscuous substrate spectrum, which often provide resistance of cancer cells against chemotherapeutics. To understand BSEP and MDR3 on a molecular level, we have established an efficient heterologous expression system and set-up an in vitro system to study the function of these two transporters.

While we could establish a structure-function relation of BSEP based on an analysis of clinical relevant BSEP mutations, we also observed an unexpected modulation of the activity of ABCB4/MDR3 by bile acids. Here, this modulation will be described in detail and an underlying mechanism will be postulated that puts a MDR3 bile acid interaction into its biological context.
Taurocholate upregulates IncRNA H19 in cholangiocytes and activates hepatic stellate cells via exosome release under cholestatic conditions

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Introduction: Accumulation of bile acids, especially conjugated bile acids, plays important roles in the pathogenesis of cholangiopathies. Long noncoding RNA (lncRNA) is a novel class of master regulators of gene expression and involved in regulating many physiological and pathological processes. Our recent studies showed that cholangiocyte-derived exosomal IncRNAH19 is an important player in cholestasis. However, the effect of bile acids on IncRNAH19 expression and hepatic stellate cell (HSC) activation remains unclear and is the focus of the current study.

Methods: Cultured mouse large cholangiocytes (MLE), human H69 and LX2 cells, primary cholangiocytes and HSCs from wild type (WT), Mdr2−/− and H19−/− mice were used this study. Exosomes were isolated from culture media and mouse serum by ultracentrifugation and characterized. The MLE-derived exosomes were labelled with PHK67 to track the uptake by different hepatic cells. The collagen gel contraction assay was used to determine HSC contraction. Both Mdr2−/− and bile duct ligation (BDL) mouse models of cholestatic liver injury were used.

Results: Taurocholate (TCA) dose- and time-dependently induced the expression of IncRNAH19 in MLE cells. Hepatic IncRNAH19 level was correlated to the activation of HSCs and cholestatic liver injury in both Mdr2−/− and BDL mouse models. Cholangiocyte-derived exosomes were taken up by all hepatic cells in the following order: Kupffer cells>HSCs>Hepatocytes. Cholangiocyte-exosomal H19 significantly induced activation and contraction of HSCs. Knocking-down H19 in MLE cells had no effect on exosome release, but blocked TCA-induced activation of HSCs. In contrast, H19 overexpression-derived MLE exosomes significantly induced activation of HSCs. Furthermore, BDL-induced cholestatic liver injury was significantly reduced in H19−/− mice.

Discussion/Conclusion: The current study shows that bile acid-induced H19-containing exosomes from cholangiocytes play a critical role in cholestatic liver injury by activating HSCs. This study suggests that serum cholangiocyte-derived H19-containing exosomes can be used as a potential biomarker for cholangiopathies.
Session II

Interaction of bile acids and the intestinal microbiome
Microbiome and bile acid interactions

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The gut microbiota is considered a metabolic “organ” that not only facilitates harvesting of nutrients and energy from the ingested food but also produces numerous metabolites that signal through their cognate receptors to regulate host metabolism. One such class of metabolites is bile acids, which are produced in the liver from cholesterol and metabolized in the intestine by the gut microbiota. These biotransformations modulate the signaling properties of bile acids via the nuclear farnesoid X receptor (FXR) and the G-protein coupled membrane receptor 5 (TGR5), which regulate numerous metabolic pathways in the host. The gut bacteria convert primary bile acids into secondary bile acids and thereby change their affinity for their receptors. FXR and TGR5 have become major targets for studies of metabolic diseases and it is clear that the microbiota can modulate signaling through both FXR and TGR5 via modifications of bile acids. Conversely, bile acids can modulate gut microbial composition both directly and indirectly through activation of their receptors.

To study the influence of microbiota and bile acid interactions on host metabolism we use germ-free mice that can be colonized with specific communities of bacteria. These mice are important tools but interpretation and translation of results from mouse models must be done carefully since mice and humans have substantial differences in bile acid composition.

Targeting the interplay between microbiota, bile acids and FXR and/or TGR5 signaling seems to evolve as a promising avenue for the treatment of metabolic diseases but much more research is needed especially in humans.
Modification of the human intestinal microbiome by obeticholic acid

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Background and aims: There is only limited information about the composition of the human small intestinal microbiome and no data on its dynamic response to perturbation. Based on the high concentration of bile acids in the mammalian small intestine and their effects on bacterial growth, we examined the effect of farnesoid X receptor (FXR)-dependent bile acid synthesis on the human gut microbiome.

Methods: The effect of FXR-dependent inhibition of bile acid synthesis via the administration of obeticholic acid (OCA) on the human and murine gut microbiome was determined by shotgun metagenomic and 16S tagged sequencing, respectively. In vitro bacterial culture experiments were performed to determine taxonomic-specific effects on bacterial growth.

Results: Suppression of endogenous bile acid synthesis by OCA in healthy human subjects led to a reversible induction of Gram-positive bacteria that are found in the small intestine, are components of diet and the oral microbiota, and are sensitive to growth inhibition by bile acids in vitro. OCA treatment enhanced the representation of genomic pathways involved in DNA synthesis and amino acid metabolism, suggesting greater growth of these bacterial taxa. Indeed, mice fed OCA had reduced endogenous bile acid levels and an increased proportion of Firmicutes, specifically in the small intestine.

Conclusion: These results demonstrate the dynamic interplay between bile acids and the human small intestinal microbiome, suggesting opportunities for microbiome biomarker discovery as well as novel modalities to engineer the human microbiome via FXR activation.
Bile acid microbiome interaction in thermogenesis

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Adaptive thermogenesis is an energy-demanding process mediated by cold-activated beige and brown adipocytes, which requires increased uptake of dietary carbohydrates and lipids for maintaining caloric balance. The canonical lipid uptake pathway involves the hydrolysis of triglyceride-rich lipoproteins (TRL) by active lipoprotein lipase and the subsequent fatty acid uptake by active adipocytes. In addition to fatty acid uptake, we have shown whole TRL particle internalization into active brown adipose tissue (BAT).

Recently, we investigated in more detail the regulation as well as the molecular processes of lipid disposal into activated BAT, using pharmacological and genetic interventions in mice. We found that short-term BAT activation by cold exposure or beta-3-adrenergic receptor agonism triggers insulin secretion, a process depending on fatty acid release by white adipose tissue. Furthermore, we showed that both insulin release and brown adipocytes insulin sensitivity is essential for the replenishment of endogenous energy stores and efficient adaptive thermogenesis. Our data demonstrate that both catabolic and anabolic processes are important for energy balance and function of BAT.

In addition to increased fatty acid disposal, we found enhanced uptake of dietary cholesterol into activated BAT as consequence of lipoprotein internalization. Following the fate of cholesterol, we observed the induction of hepatic bile acid synthesis, interestingly via the alternative but not the classical pathway. This process, depending on hepatic CYP7B1 induction, results in elevated plasma levels and pronounced fecal excretion of conjugated bile acids, accompanied by distinct changes in gut microbiota. Pharmacological intervention using ezetimibe, a drug blocking dietary cholesterol uptake, prevented both the rise in bile acid excretion and compositional changes in gut bacteria in response to cold. These results identify bile acids generated in the liver as the determinant of cold-induced gut microbiota, highlighting the relevance of cholesterol metabolism by the host for diet-induced changes on gut microbiota.
Antibiotics secreted by gut bacteria regulate *Clostridium difficile* growth and the structure of the gut microbiome: Role of secondary bile acids

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**Introduction:** *Clostridium difficile* is the cause of antibiotic associated diarrhea and colitis. *C. scindens*, which biotransforms primary bile acids (PBA) into secondary bile acids (SBA), is highly correlated with inhibition of *C. difficile* growth *in vivo*. The aim of this study was to determine how *C. scindens* and bile acids regulate *C. difficile* growth *in vitro* and the structure of the gut microbiome.

**Methods:** *C. difficile* and *C. scindens* were grown in co-culture with and without added CA (100 \(\mu\)M). Gene specific PCR assays to quantify *C. scindens* (bai CD gene) and *C. difficile* (Toxin A gene) were developed. Antibacterial compounds secreted by *C. difficile*, *C. scindens*, and *C. sordellii* were purified and their structures determined by liquid chromatography-electrospray ionization mass spectrometry (LC-ESI/MS) and \(^1\)H and \(^13\)C NMR chemical shifts.

**Results:** *C. difficile* strongly inhibited *C. scindens* growth under co-culture conditions in the absence but not presence of cholic acid (CA). *C. difficile* was discovered to secrete two cyclic dipeptides, cyclo(Phe-Pro) and cyclo(Leu-Pro), which are known quorum-sensing molecules with antibacterial activity. Bile acid 7\(\alpha\)-dehydroxylating (7\(\alpha\)-DeOH) gut bacteria, *C. scindens* and *C. sordellii*, were found to secrete 1-acetyl-\(\beta\)-carboline and turbomycin A, respectively. Both antibiotics inhibited *C. difficile* growth *in vitro*. Deoxycholic acid and lithocholic acid, but not CA, strongly enhanced the inhibitory activity of these antibiotics. The addition of these antibiotics to culture media resulted in the formation of highly elongated *C. difficile* cells indicating inhibition of a division septum.

**Discussion:** These results helps to explain how *C. difficile* is able to colonize the colon in the absence of 7\(\alpha\)-DeOH bacteria and how SBA, and endogenously synthesized antibiotics, regulate the structure of the gut microbiome.

**Conclusion:** The concentration of bile acids in the intestines appears to be a major regulator of the structure of the gut microbiome.
Enhanced microbial deconjugation of bile acids in pregnancy represses intestinal FXR-mediated regulation of hepatic bile acid synthesis

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Introduction: Pregnancy is associated with maternal metabolic adaptations, including progressive hypercholanaemia, hypercholesterolaemia and hypertriglyceridaemia, which can develop into maternal metabolic disease. Gut signals modify hepatic homeostatic pathways, linking intestinal content to metabolic activity. We sought to identify whether enteric signals originating in response to intestinal contents contribute to raised serum bile acids observed in human and murine pregnancies.

Methods: Following a standardised diet, we measured FGF19 and 7α-hydroxy-4-cholesten-3-one serum levels in 26 women, and measured distal ileal mRNA expression of FGF15 in C57BL/6 mice (n = 6–10), in non-pregnant and pregnant individuals. To determine the effects on FXR signalling, we exposed human terminal ileal explants and murine intestines in vivo to the hepatic FXR antagonist epiallopregnanolone sulfate. We used shotgun whole genome sequencing and UPLC-MS to determine the caecal microbiome and metabolome, performing targeted and untargeted pathway analyses to predict the effects of the altered metagenome and metabolite profiles. Finally, we supplemented a murine diet with cholic acid to determine whether the observed alterations could be overcome by the presence of an intestinal FXR agonist.

Results: Human and murine pregnancy were associated with reduced intestinal FXR signalling, with lower FGF19/15, and resultant increased hepatic bile acid synthesis. Ileal FXR signalling was not affected by epiallopregnanolone sulfate exposure. Caecal conjugated bile acids were lower in pregnancy due to elevated bile salt hydrolase-encoding Bacteroidetes. Cholic acid supplementation induced intestinal FXR signalling, which was not abrogated by pregnancy. Despite the intestine being loaded with cholic acid, the microbiota of cholic acid feeding was strikingly similar to that of pregnancy, with similar metabolite changes.

Discussion/Conclusion: The altered intestinal microbiota of pregnancy enhances bile acid deconjugation, reducing ileal bile acid uptake and FXR induction. There is, therefore, reduced FGF19/15-mediated hepatic repression of hepatic bile acid synthesis, a proportion of which enters the blood, causing hypercholanaemia.
Session III

Bile acid transport in health and disease
Visualization of bile flux in liver by 2-photon microscopy: The principle of diffusion-limited bile canalicular transport

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A well-accepted concept in hepatology is that bile flows inside the bile canaliculi from the centre of the liver lobule to the portal vein area, where it drains into bile ducts. Recent work has investigated this idea as an in silico model, predicting that such a mechanism would be critically depending on osmotic water influx, and impose a 10-fold acceleration in bile velocity from the central vein to portal vein neighbourhood. However, we now present empirical evidence for a fundamentally different mechanism. We used photoactivation of CMNB fluorescein in canaliculi, which allows the spatially controlled fluorescent tracking in the canalicular network to measure directly the spatial flux profiles of bile constituents. In canaliculi, photoactivation was followed by a symmetric dispersion of fluorescence in the canalicular network, without directional preference for movement towards the portal region. Moreover, photoactivation within interlobular bile ducts was followed by retrograde dispersion into the canaliculi, which is against the direction of hitherto postulated convective flow. Second, in vivo raster image correlation spectroscopy (RICS) was established to quantify convective flow and diffusion coefficients. In liver sinusoids, convective flow was 65 µm/s and the diffusion coefficient approx. 2 µm²/s. In bile canaliculi, convective flow was extremely low with less than 0.02 µm/s, while the diffusion coefficient was approx. 2 µm²/s, similar to that in sinusoids. Only in interlobular bile ducts that were visualized by HNF1beta reporter expression convective flow was measurable. Therefore, bile canaliculi can be considered as pipes with stagnant water from where bile acids but also biliary excreted drugs reach the bile ducts by diffusion; convective flow only sets in from interlobular bile ducts and downstream. This novel concept of canalicular diffusion explains several physiological and pathological observations better than the conventional model of osmosis and contractility driven canalicular convective flow: first, clearance of compounds from bile canaliculi depends on their molecular weight; second, rupture events of the apical hepatocyte membrane as observed in cholestasis are followed by a gradient of increased hepatocellular bile salt concentrations that correspond to the here determined diffusion coefficient. Finally, analysis of the liver lobules of 22 mammalian species (smallest mice, largest elephants) demonstrates that the lobular radius (in contrast to liver weight) does not increase proportionally to body weight but always remains below approximately 300 µm, which suggests that limits imposed by diffusion for bile canalicular transport have shaped liver architecture in evolution.
Inhibition of bile acid reabsorption to ameliorate cholestatic liver injury

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Accumulation of bile salts during cholestasis leads to hepatic and biliary injury, driving inflammatory and fibrotic processes. Currently, several trials suggest that specific inhibition of intestinal reabsorption ameliorates liver injury and pruritus\(^1\), but induces intestinal discomfort due to the increased presence of bile salts in the colon.\(^2\) In this presentation, we will discuss alternative possibilities to dampen cholestatic liver damage by targeting more downstream transporters, such as the \(\text{Na}^+\)-Taurocholate Cotransporting Polypeptide (NTCP).\(^3\) The latter is the major hepatic uptake transporter of conjugated bile salts, and can be specifically inhibited by myrcludex B.\(^4\) Acute cholestasis was induced in mice by a 3.5-diethoxycarbonyl-1.4-dihydrocollidine (DDC) diet or by bile duct ligation (BDL). Chronic cholestasis was investigated in \(\text{Atp}8\text{b}1\)-G308V and \(\text{Abcb}4\)/\(\text{Mdr}2\) deficient mice. Mice were injected daily with myrcludex B or vehicle. Myrcludex B reduced plasma alkaline phosphatase (ALP) levels in DDC-fed, \(\text{Atp}8\text{b}1\)-G308V and BDL mice by 39%, 27% and 48% respectively. NTCP-inhibition reduced biliary bile salt output in DDC-fed and \(\text{Atp}8\text{b}1\)-G308V mice by ~50% whilst phospholipid (PL) output was maintained, resulting in a higher PL/bile salt ratio. Conversely, in \(\text{Abcb}4\) deficient mice, lacking biliary phospholipid output, liver injury was aggravated after myrcludex B treatment. In all models except \(\text{Abcb}4\) knockout mice, NTCP inhibition increased bile salt levels in urine and plasma.

**Conclusion:** NTCP-inhibition by myrcludex B has hepatoprotective effects\(^5\), by reducing bile salt load in hepatocytes and increasing the biliary PL/bile salt ratio. Elevated plasma bile salt levels after NTCP-inhibition were well tolerated up to high micromolar levels. In conclusion, NTCP-inhibition may be a new strategy to protect the liver in certain forms of cholestasis.

**References:**


**IgG4-associated cholangitis – Another model cholangiopathy with an underlying defect of the biliary bicarbonate umbrella?**

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**Introduction:** We have introduced the ‘biliary bicarbonate umbrella hypothesis’ as a protective mechanism of human cholangiocytes and hepatocytes against toxic effects of human hydrophobic bile acids.1-3 Primary biliary cholangitis (PBC) shows features of defective biliary bicarbonate secretion.4 IgG4-associated cholangitis (IAC) is the biliary manifestation of IgG4-related disease, a B-cell driven autoimmune multiorgan disease targeting among others the biliary epithelium. IAC is characterized by oligoclonal B-cell receptor clones in blood and affected tissues5, frequently affects ‘blue collar workers’ with long-term exposure to solvents, oil products and other organic agents6, and is diagnosed with high accuracy by determination of the blood IgG4/IgG RNA ratio using qPCR.7 We have recently identified the first IgG4/IgG1 autoantigen in IAC, Annexin A11.8 The Ca2+-regulated protein Annexin A11 is known to participate in Ca2+-dependent exocytosis in pancreatic β-cells. Here, we studied the potential role of Annexin A11 in mediating bicarbonate secretion in human cholangiocytes.

**Methods:** Expression pattern of Annexin A11 in human liver tissue sections was visualized by immunohistochemistry. Human SV40-transformed cholangiocytes (H69) were transduced with short hairpin RNA (shRNA) against Annexin A11 or nontargeting shRNA as negative control. AE2 surface expression was quantified using biotinylation assays. Intracellular pH was determined by a ratiometric approach. Apoptosis and cell viability after exposure to hydrophobic bile acids were measured by established assays.2,3

**Results:** Annexin A11 is highly expressed in human cholangiocytes. Preliminary data suggest that Annexin A11 is involved in biliary bicarbonate secretion in the biliary epithelium.

**Discussion/Conclusion:** Our preliminary data are in line with our hypothesis that IAC might be another cholangiopathy related to an underlying secretory defect of biliary bicarbonate secretion.
References:


Bile acid transport in the intestine: From genetic variants to therapeutic targets

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The list of transporter genes with inherited defects that impact hepatic secretion of biliary constituents or the enterohepatic cycling of bile acids include: \textit{ATP8B1}/FIC1 (Progressive familial intrahepatic cholestasis type 1; PFIC1), \textit{ABCB11}/BSEP (PFIC2), \textit{ABCB4}/MDR3 (PFIC3), \textit{SLCO1B1}/OATP1B1 and \textit{SLCO1B3}/OATP1B3 (Rotor Syndrome), \textit{SLC10A1}/NTCP (Conjugated Hypercholanemia), and \textit{SLC10A2}/ASBT (Primary Bile Acid Malabsorption). Notably absent from that list is the \textit{SLC51A}-\textit{SLC51B} (OSTα-OSTβ) heterodimer, a major basolateral membrane bile acid transporter, that is expressed by ileal enterocytes, cholangiocytes, and renal proximal tubule cells.

To determine the pathophysiological consequences associated with loss of OSTα-OSTβ, we examined the phenotype of Ostα-deficient mice and two pediatric patients that were recently identified with OSTβ-deficiency. The Ostα-deficient mice present with a small growth deficit, reduced hepatic bile acid synthesis and bile acid pool size, and reduced intestinal cholesterol and fat-absorption. In the absence of Ostα, the ileal epithelium exhibited evidence of on-going bile acid-induced injury and restitution, which could be rescued by genetic or pharmacological inhibition of ASBT-mediated bile acid uptake. In contrast to the intestinal changes, the livers of Ostα-deficient mice were indistinguishable from matched wild type mice. The pediatric patients are two brothers who presented with congenital chronic diarrhea and severe fat-soluble vitamin-deficiency. The patients are homozygous for a single nucleotide deletion in codon 27 of OSTβ, which truncates the protein and abolishes its ability to support OSTα expression and bile acid transport activity. In addition to intestinal malabsorption and diarrhea, the OSTβ-deficient patients exhibited elevated serum levels of liver enzymes, particularly gamma-glutamyltransferase activity, elevated liver copper, and mild liver histological changes, which had not been observed in patients with ASBT-deficiency. In conclusion, study of the phenotype of OSTα-OSTβ-deficient mice and human subjects further supports a critical role of this unusual transporter in bile acid homeostasis and gut-liver function.
Inactivation of the intestinal apical sodium bile acid transporter profoundly inhibits cholesterol absorption

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Introduction: Cholesterol enters the intestinal lumen via diet, via biliary secretion or via transintestinal cholesterol excretion. Cholesterol disposal from the body is achieved by fecal excretion as neutral sterols and bile acids. Inhibition of the intestinal apical sodium-dependent bile acid transporter (ASBT) increases both fecal bile acid and neutral sterol excretion and inhibits atherosclerosis in animal models. We aimed to determine whether the increase in fecal neutral sterol excretion upon ASBT inactivation is due to decreased cholesterol (re)absorption or enhanced transintestinal excretion.

Methods: To differentiate between these two mechanisms, Asbt⁻/⁻ mice and wildtype (WT) littermates were fed chow with or without 0.005% (w/w) ezetimibe, an intestinal cholesterol absorption inhibitor. We measured and calculated cholesterol fluxes originating from the diet, the bile and from across the intestine, using mass determinations and a dual stable isotope method.

Results: Fractional cholesterol absorption was profoundly lower in Asbt⁻/⁻ mice compared to WT controls (5.7% vs. 46.2%). Ezetimibe treatment virtually abolished fractional cholesterol absorption in Asbt⁻/⁻ and in WT mice (0.8% and 4.0%, respectively). Fecal excretion of neutral sterols (consisting of cholesterol and its intestinal metabolites) was threefold higher in Asbt⁻/⁻ mice, compared with WT mice. Ezetimibe treatment to Asbt⁻/⁻ mice did not further increase or affect fecal neutral sterol secretion. The strong inhibition of cholesterol absorption by ezetimibe in WT mice increased fecal neutral sterol secretion to a similar level as in untreated or in ezetimibe-treated Asbt⁻/⁻ mice. Flux calculations indicated that the predominant fraction of fecal neutral sterols originated from transintestinal excretion of cholesterol that was not reabsorbed upon ezetimibe treatment.

Discussion/Conclusion: Prevention of intestinal bile acid reabsorption by ASBT inactivation is as effective as ezetimibe in inhibiting intestinal cholesterol (re)absorption and, thereby, in increasing total fecal sterol excretion. Combining ASBT inhibition with ezetimibe treatment results in a higher total fecal sterol excretion which could have an additional benefit for the treatment of hypercholesterolemia and atherosclerosis.
Session IV

Bile acid transport in health and disease
Analysis of transporter defects in children and adults with cholestatic liver disease

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The ATPase familial intrahepatic cholestasis 1 (FIC1; ATP8B1), the bile salt export pump (BSEP; ABCB11), and the multidrug resistance protein 3 (MDR3; ABCB4) are essential for bile formation. Genetic variants in these genes are associated with cholestatic liver diseases ranging from milder forms to progressive familial intrahepatic cholestasis (PFIC).

Over the last decades, Sanger sequencing was the method of choice for the identification of genetic variants. Nowadays, next generation sequencing (NGS) techniques are in use to achieve higher throughput. Applying NGS approaches in PFIC patients lacking mutations in ATP8B1, ABCB11 or ABCB4 led to the identification of three novel PFIC-associated genes comprising tight junction protein 2 (TJP2), nuclear receptor FXR (NR1H4) and Myosin5B (MYO5B) by others.

Since 2006, single gene sequencing of ATP8B1, ABCB11, and ABCB4 was a standard method in the Düsseldorfer Cholestasis Lab to elucidate the genetic background of patients with assumed genetically based cholestasis. With upcoming importance of TJP2, FXR and MYO5B for cholestasis, a custom-designed NGS panel was established in the last years. This panel includes not only the known cholestasis-relevant genes but also other genes related to liver diseases as well as specific hot spots.

About 230 variants were detected in these six genes including over 65 novel variants first described in our lab. Since roughly 50% of the mutations represent missense mutations, effects of new variants were evaluated using bioinformatics tools and 3D protein homology modeling regarding local structural stability and function of BSEP and MDR3 by inspecting their location, their occurrence in functionally important sequence motifs, and a potential impact on interactions with the surrounding residue environment after mutation. These structure-based interpretations complemented results from sequence-based prediction tools by exploiting structure-function relationships in terms of assessing the influence of a mutation on its local environment in the context of the overall protein function.
Genetic determinants of cholangiopathies: Molecular and systems genetics

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Familial cholangiopathies are rare but potentially severe diseases. Their spectrum ranges from fairly benign conditions as, for example, benign recurrent intrahepatic cholestasis to low-phospholipid associated cholelithiasis and progressive familial intrahepatic cholestasis (PFIC). Many cholangiopathies such as primary biliary cholangitis (PBC) or primary sclerosing cholangitis (PSC) affect first the bile ducts but others, such as PFIC, start upstream in hepatocytes, causing progressive damage of liver parenchyma and biliary tree. In recent years our understanding of cholestatic diseases has improved, since we have been able to pinpoint numerous disease-causing mutations that cause familial cholangiopathies. Accordingly, six PFIC subtypes (PFIC type 1–6) have now been defined. Given the availability of genotyping resources, these findings can be introduced in the diagnostic work-up of patients with peculiar cholestasis. In addition, functional studies have defined the pathophysiological consequences of some of the detected variants, leading to novel therapies with chaperones and potentiatators. Furthermore, ABCB4 variants do not only cause PFIC type 3 but confer an increased risk for chronic liver disease in general. Here we present the latest data on the genetic background of familial cholangiopathies and discuss their application in clinical practice for the differential diagnosis of cholestasis of unknown aetiology. As look in the future we introduce system genetics as a novel experimental tool for the study of cholangiopathies and disease-modifying genes.
Oral Poster Presentation

Loss of BSEP/ABCB11 protects MDR2/ABCB4 KO mice from cholestatic liver injury by altering bile acid profile and signaling

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Introduction: Cholestasis is characterized as intrahepatic accumulation of potentially cytotoxic bile acids (BAs) subsequently leading to liver injury reflected by disruption of hepatocellular integrity, inflammation, fibrosis, cirrhosis and increased risk for cancer. Bile salt export pump (Bsep/Abcb11) is the main canicular BA transporter and rate limiting step for hepatobiliary BA secretion. Here we aim to investigate the role of Bsep in development of liver injury in a mouse model of sclerosing cholangitis - the Mdr2KO mouse.

Methods: To explore the consequences of Bsep loss in a mouse model of sclerosing cholangitis, Mdr2/Bsep double knockout (DKO) mice were generated. WT mice subjected to bile duct ligation (BDL) as well as Mdr2 KO mice were fed with a tetrahydroxylated bile acid (THBA). Gene expression profile of inflammatory and fibrotic markers, serum biochemistry, liver histology, immunohistochemistry (IHC) and serum BA composition were investigated. Amount of RORgt+CD4+CD3+ T cells as well as FOXP3+CD4+CD3+ T cells in livers of Mdr2 KO and DKO mice were assessed by FACS analysis.

Results: In contrast to Mdr2 KO mice, DKO mice were protected against liver and bile duct injury, reflected by serum biochemistry and H&E staining. Gene expression of inflammatory markers F4/80, Tnfa and Mcp1 remained unchanged (compared to WTCtrls) in DKO mice, while in Mdr2 KO mice these markers were increased (4-fold, 6-fold and 8-fold, respectively; p < 0.05). Fibrosis markers were increased in Mdr2 KO mice (Desmin 9-fold, Col1a1 24-fold; p < 0.05) but remained unchanged in DKO mice. mRNA expression of Cyp3a11 and Cyp2b10, two enzymes involved in BA hydroxylation/detoxification were increased 5-fold and 100-fold in DKO mice, respectively, while Mdr2 KO mice showed unchanged levels. In line, 67% of serum BAs in DKO mice were polyhydroxylated, with tetrahydroxylated BAs being most prominent. In Mdr2 KO mice, polyhydroxylated BAs were completely absent. Within the CD4+CD3+ T cell population about 50% are RORgt+ and 5% FOXP3+ in Mdr2 KO mice versus 10% RORgt+ and 30% FOXP3+ cells in DKO mice. Notably THBA feeding
profoundly reduced expression levels inflammatory markers (IL1b and Cxcl1 by 50\%) and fibrotic marker (Col1a2 by 80\%) in Mdr2 KO. In line, THBA feeding improved inflammation in WT BDL mice, reflected by reduced number of F4/80 positive cells and improved gene-expression profile (F4/80 by 50\%, Cxcl1 by 55\% and Cxcl2 by 75\%; p < 0.05) compared to WT BDL mice.

**Discussion/Conclusion:** Loss of Bsep results in increased expression/activity of enzymes involved in BA hydroxylation/detoxification, thereby modifying CD4+CD3+ T cell population, thus protecting Mdr2 KO mice from development of cholestatic liver disease. Therefore, THBA may be a new potential treatment strategy for cholestatic liver diseases.
Session V

Extrahepatic effects of bile acids and bile acid receptors
Targeting bile acids in intestinal disease

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Bile acids, classically known for their roles in facilitating lipid digestion and absorption, are now also established as a family of enterocrine hormones that regulate many aspects of intestinal physiology. Altered levels of luminal bile acids are associated with changes in epithelial transport and barrier function which underpin the onset of several common intestinal diseases, including inflammatory bowel disease, bile acid diarrhea, and colorectal cancer. With this in mind, the overall goal of our research is to elucidate the roles that bile acids play in regulating colonic epithelial physiology/pathophysiology and to develop their potential as targets for disease treatment. Most recently our work has focused on the secondary bile acid, ursodeoxycholic acid (UDCA). Although UDCA has long been used in treatment of liver inflammation, its potential as a therapeutic for intestinal disease is less clear. Our studies demonstrate UDCA prevents colonic epithelial apoptosis and cytokine release and to promote restitution, actions which serve to enhance barrier function and dampen inflammation in vivo. Interestingly, we have found that a metabolically stable analogue of UDCA, 6-methyl-UDCA, does not exert protective effects in vivo, suggesting that bacterial metabolism of UDCA is necessary for the full expression of its protective actions. Accordingly, we have found that the UDCA metabolite, lithocholic acid (LCA), potently inhibits epithelial cytokine secretion and colonic inflammation in vivo. Our ongoing investigations aim to further understand the relationships between UDCA and LCA in regulation of colonic epithelial barrier function and to elucidate the potential of these bile acids in treatment of intestinal disease.
Interaction of gut microbiota and bile acids in NAFLD

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Growing evidence implicates the bacteria present in our gut in the effect of genetics and lifestyle on metabolic diseases, including NAFLD. Most of the current literature on gut bacteria consists of cross-sectional and correlative studies, rendering it difficult to make any causal inferences on the role of gut bacteria in NAFLD. Interventions with germ-free animals, treatment with antibiotic agents, and bacterial transfer experiments are providing growing evidence that disturbances in gut bacteria may causally contribute to NAFLD. We investigated the role of the gut microbiota in NAFLD in mice by stimulating the gut bacteria via chronic feeding of the fermentable dietary fiber guar gum and suppression of the gut bacteria via chronic oral administration of antibiotics. Feeding mice guar gum profoundly altered gut microbiota composition. Guar gum protected against diet-induced obesity and improved insulin resistance and adipose tissue inflammation. Strikingly, instead of ameliorating NAFLD, guar gum enhanced hepatic inflammation and fibrosis. By contrast, the poorly fermentable dietary fiber resistant starch had minimal impact on liver pathology, metabolic parameters, and microbial composition. Quantification of gut-derived metabolites revealed that guar gum raised plasma levels of bile acids and trimethylamine N-oxide, but did not affect plasma lipopolysaccharide. Chronic treatment of mice with trimethylamine N-oxide had no effect on hepatic inflammation or fibrosis, whereas treatment with the bile acid taurocholic acid stimulated hepatic inflammation and fibrosis. Chronic oral administration of antibiotics effectively suppressed the gut bacteria and markedly attenuated hepatic inflammation and fibrosis, concurrent with a significant decrease in portal secondary bile acid levels.

Conclusions: We find that modulation of the gut microbiota by dietary guar gum or antibiotics markedly influenced hepatic inflammation and fibrosis in a mouse model of NAFLD, likely via alterations in portal bile acid levels. Our data suggest a causal link between disturbances in gut microbial community, bile acids, and NAFLD.
Altered bile acid signaling in NAFLD in humans

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Bile acids regulate human metabolism through multiple pathways such as those mediated by bile acid receptors farnesoid X receptor (FXR) and G-protein coupled bile acid receptor 1 (GPBAR1 or TGR5). Therefore, these pathways are potential intervention targets for non-alcoholic fatty liver disease (NAFLD), which is an obesity associated liver disease and considered the liver component of metabolic syndrome.

In human, liver produced primary bile acids are mainly cholic acid and chenodeoxycholic acid (CDCA), which can be converted by gut microbiota into secondary bile acids such as deoxycholic acid (DCA) and lithocholic acid. We found that both primary and secondary bile acids were elevated in NAFLD patients. Increased hepatic expression of CYP7A1 and other relevant genes indicated that elevated serum bile acids were driven by increased bile acid synthesis, and that the feedback inhibition of FXR signaling by bile acid was suppressed in NAFLD. Further evidence for suppressed FXR signaling in NAFLD include increased expression of Na+-taurocholate cotransporting polypeptide (NTCP) and paraoxonase 1, no change in expression of small heterodimer partner (SHP) and bile salt export pump (BSEP), and reduced serum FGF19.

One explanation for suppressed FXR signaling is the relatively increased level of DCA (FXR antagonistic), while the agonistic CDCA was relatively decreased in NAFLD. These observations were consistent with the microbiome data showing taurine and glycine metabolizing bacteria increased in the gut of NAFLD patients, an indication for increased secondary bile acid production.

Conclusions: NAFLD patients exhibit elevated bile acid production. The altered microbiome composition was consistent with increased proportion of FXR antagonistic DCA, which in turn may suppress the FXR signaling in liver and other organs, contributing to NAFLD pathogenesis. Our observations suggest that the FXR signaling pathway and the bile acid converting microbiome are potential intervention targets for NAFLD.
Bile acids increase serum corticosterone levels in cholemic mice and induce cortisol secretion in adrenocortical H295R cells in an S1PR2-ERK-SF-1-dependent manner

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Bile acids are now accepted as important signaling molecules for the regulation of glucose, amino acid, and lipid metabolism. Adrenal gland cortex cells express bile acid receptors such as FXR, TGR5, and S1PR2. In this study, we aimed to determine the effects of cholestasis and more specifically of bile acids on adrenal cortisol production. To accomplish the objectives, FXR and TGR5 knock-out mice and respective wild type controls were subjected to common bile duct ligation (CBDL) or chenodeoxycholic acid (CDCA) feeding. Human adrenocortical H295R cells were challenged with bile acids for mechanistic studies. We found that CBDL and CDCA feeding in mice increased the levels of corticosterone, the rodent equivalent to human cortisol, and mRNA and protein levels of steroidogenesis-related enzymes in adrenals independent of FXR and TGR5. Tauro-conjugated CDCA (TCDCA) significantly stimulated cortisol secretion, phosphorylation of ERK, and expression of steroidogenesis-related genes in H295R cells. FXR and TGR5 agonists failed to induce cortisol secretion in H295R cells. Pharmacological inhibition of S1PR2 expressed in adrenals using JTE-013 significantly abolished CDCA-induced cortisol secretion, lowered phosphorylation of ERK, and abrogated enhanced transcription abundance of steroidogenesis-related genes in H295R cells. Likewise, S1PR2-siRNA treatment reduced phosphorylation of ERK and subsequently cortisol secretion. In addition, steroidogenic factor-1 (SF-1) transcription activity was increased upon TCDCA treatment in H295R cells. Addition of a SF-1 inverse agonist also reduced TCDCA-induced steroidogenesis. Pharmacological inhibition of S1PR2 and ERK phosphorylation caused excessive loss of SF-1 protein. Our combined in vivo and in vitro experimental results therefore indicate that bile acids directly stimulate steroidogenesis in adrenals via an S1PR2-ERK-SF-1 signaling pathway.
Oral Poster Presentation

Targeting host and microbial choline metabolism by a semi-synthetic bile acid suppresses TMA/TMAO formation and ameliorates atherosclerosis and NASH in mice

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Introduction: Microbial conversion of dietary phospholipids (PLs) produces in a sequential reaction trimethylamine (TMA) in the gut and trimethylamine-N-oxide (TMAO), a pro-atherogenic metabolite in the liver. Herein we investigated the role of biliary PLs to TMA/TMAO formation, and explored whether the biliary PL lowering effect of norursodeoxycholate (norUDCA) impacts on TMA/TMAO formation and concomitant cardiac/hepatic disease progression in ApoE−/− mice.

Methods: Wildtype (WT) and Mdr2−/− (lacking biliary PLs) mice on chow and 5%PL-enriched diet; ApoE−/− mice on (High-Fat/High-Cholesterol) diet ± 0.5% (w/w) of norUDCA. Histology, immunohistochemistry, serum/hepatic lipids, western-blot and qPCR from aortic root and liver tissue; 16S rRNA gene amplicon sequencing of intestinal microbiota; LC/MS and NMR spectroscopy to quantify biliary lipid composition, and TMA/TMAO from serum, urine and faeces of mice and in vitro cultures of gut-derived microbiota.

Results: Colonic TMA levels were significantly reduced in Mdr2−/− compared to WT littermates, associated with a distinct shift in gut microbiota. Similarly, treatment of ApoE−/− mice with norUDCA resulted in significant reduction of biliary choline, glycerophosphocholine, and phosphatidylcholine, accompanied by reduced faecal- and urinary TMA, as well as urinary/serum TMAO levels. In anaerobic polymicrobial cultures obtained from norUDCA fed animals, or after exogenous addition of norUDCA, TMA formation from choline was inhibited. Notably, ApoE−/− mice fed norUDCA showed a substantial reduction in hepatic steatosis and inflammation, reduced aortic plaque surface area and macrophage infiltration. The anti-inflammatory effects of norUDCA could be associated to reduce TMA/TMAO levels as we demonstrate that both can antagonize LCA-induced TGR5 activity in vitro.

Discussion/Conclusion: We provide evidence that biliary PLs substantially contribute to microbial TMA formation. norUDCA by lowering PL excretion, and by interfering with microbial choline catabolism can block TMA formation in vivo and in vitro. In an animal model of atherosclerosis and NASH, norUDCA is able to ameliorate hepatic- and cardiac disease progression.
Session VI

Bile acid receptors and bile acid signaling as therapeutic targets
Targeting TGR5 to treat polycystic liver disease

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The polycystic liver diseases (PLD) are genetically heterogeneous conditions that occur alone or with kidney cysts (1). To date, no curative or preventative therapies for PLD exist. Hepatic cystogenesis in PLD is associated with increased levels of cAMP in the cholangiocytes lining liver cysts. Recently, we reported that TGR5, a membrane receptor for bile acids normally expressed in cholangiocytes, is overexpressed in PLD and that interventions that modify TGR5 expression influence hepatic cystogenesis (2). More specifically, compared to control, expression of TGR5 and Gαs (but not Gαi and Gαq) proteins was increased 2-fold to 3-fold in cystic cholangiocytes in vitro and in vivo. In vitro, TGR5 stimulation enhanced cAMP production, cell proliferation, and cyst growth by ~40%; these effects were abolished after TGR5 reduction by short hairpin RNA. Oleanolic acid, a TGR5 agonist, increased cystogenesis in polycystic kidney rats by 35%; in contrast, hepatic cystic areas were decreased by ~45% in TGR5-deficient TGR5−/−/Pkhd1del2/del2 crossbred mice. Levels of cAMP, cell proliferation, and cyst growth in vitro were decreased by ~30% in cystic cholangiocytes after treatment with a novel TGR5 antagonist (SBI-115) alone and by ~50% when SBI-115 was combined with pasireotide, a somatostatin analogue. While targeting cAMP via TGR5 seems a promising approach given these preclinical data, it is likely that simultaneously addressing the several different pathways that control the wide range of cellular processes involved in hepatic cystogenesis, (e.g. benign hyperproliferation, disturbed morphogenesis, autophagy, altered ciliary assembly/disassembly) will be required (3).

References:


TGR5 (Gpbar1) in inflammatory liver disease

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Introduction: TGR5 (Gpbar1) is a G protein coupled receptor responsive to bile acids (BA), which has been detected in different non-parenchymal cells of the liver, including Kupffer cells [1, 2]. Furthermore, TGR5 is highly expressed in monocytes and macrophages of the peripheral blood [3]. Stimulation of TGR5 in macrophages reduces both cytokine and chemokine expression and secretion as well as suppresses the NLRP3 inflammasome [4–7]. Through TGR5 BAs exert a broad range of anti-inflammatory effects in monocytes and macrophages. Aim of our study was to investigate the in vivo relevance of TGR5 in inflammatory liver disease using different mouse models.

Methods: Male, 8–12 week old TGR5 knockout and wildtype mice were injected intraperitoneally (i.p.) with lipopolysaccharide and monitored for up to 3 days. For the second model male mice were intravenously (i.v.) infected with 8 x 10⁴ CFU/ml Listeria monocytogenes (L.m.) and observed for up to 7 days. Serum markers of liver disease were determined using Spotchem-biochemical analyzer. Flow cytometry was used to quantify and differentiate immune cells within the liver. Immunohistochemistry was performed on liver tissue.

Results: TGR5 mRNA and protein expression was significantly up-regulated in livers from WT mice after LPS injection and L.m. infection as demonstrated by realtime PCR and immunofluorescence staining. Similar to TGR5, mRNA expression of the transcription factor Krüppel-like factor 5 (KLF5) was significantly increased in wildtype animals after LPS or Listeria injection. Chromatin immunoprecipitation confirmed binding of KLF5 to the TGR5 promotor, which was further supported by cloning and functional analysis of the putative TGR5 promotor region. LPS and L.m. injection resulted in aggravated liver injury in TGR5 deficient mice as determined by AST and ALT elevation, by the amount of inflammatory infiltrates within the livers as well as by increased levels of cytokines and chemokines in serum. Moreover, TGR5 knockout mice had a significantly increased mortality as compared to their wildtype littermates. This phenotype was mirrored by immune-cell specific TGR5 knockout mice.

Conclusion: TGR5 knockout mice suffer from increased mortality following LPS or L.m. injection. Wildtype animals show a KLF5 dependent upregulation of TGR5 in immune cells after LPS or L.m. injection, thereby increasing the anti-inflammatory effects mediated through TGR5. In contrast this adaptive mechanism is absent in TGR5 deficient mice resulting in increased mortality following either LPS or L.m. injection further underscoring the important role of TGR5 in inflammatory liver disease.
References:


FXR agonists in portal hypertension

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Portal hypertension (PHT) may cause ascites formation and variceal bleeding in patients with cirrhosis. While increased intrahepatic resistance is the main initial trigger for PHT, the current medical therapy with betablockers only targets the increase in splanchnic blood inflow and collateral blood flow to reduce portal pressure. More recently FXR agonists, such as the steroidal FXR agonist obeticholic acid (OCA) has been shown to decrease portal pressure in rats with cholestatic (BDL) and toxic (TAA) cirrhosis by enhancing sinusoidal endothelial NO signaling. Additionally, OCA has been shown to reduce fibrosis in TAA rats and in murine NASH models. First promising data in human on beneficial effects of OCA on portal pressure in patients were presented. The non-steroidal FXR agonist PX20606 and decreased both fibrosis and portal pressure in rats with toxic and biliary fibrosis by ameliorating structural and functional abnormalities in the sinusoidal microcirculation. The mechanisms how PX20606 and other FXR agonist enhance sinusoidal endothelial function, include effects on gaseous vasodilators such as NO, H2S and a reduction in vasoconstrictors, such as endothelin-1. GS9674 another non-steroidal FXR agonist has also inhibited fibrogenesis and decreased portal pressure, both by acute and chronic administration in a rat NASH model.

Furthermore, OCA has also been shown to improve intestinal barrier function by enhancing microbial defense mechanisms in the ileum and by promoting mucosal integrity. The intestinal microbiome was also beneficially influenced by chronic OCA administration in rats with advanced CCl4-induced cirrhosis with ascites.

In summary, current experimental evidence suggests that FXR ligands can effectively decrease portal hypertension by targeting functional (i.e. sinusoidal vasoconstriction) and structural (i.e. liver fibrosis) components and additional, extrahepatic effects on the microbiome, antimicrobial defense mechanisms and intestinal mucosal integrity.
Intestinal FXR agonism and fibroblast growth factor 19 protect against colitis and intestinal tumorigenesis

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Disruption of bile acid (BA) homeostasis plays a key role in intestinal inflammation. BA pool size and composition is mainly regulated via the dual action of nuclear receptor FXR and the enterokine fibroblast growth factor 19 (FGF19). It has been previously shown that FXR ablation in mice predispose to colitis and intestinal tumorigenesis. We will present here data on the role of intestinal FXR agonism in the gut. First, using enterocyte specific transgenic mice with constitutive FXR activation we propose a novel scenario in which selective intestinal FXR activation protects against colitis and cancer. Since it has been postulated that the intestinal FXR driven protection is mainly due to reduction of bile acid pool via CYP7A1 inhibition, we also tested if a novel generated non-tumorigenic FGF19 analogue that reduces BA synthesis and pool size, could protect mice from experimental colitis and intestinal tumorigenesis. Indeed, mice treated with FGF19 presented inhibition of inflammatory immune response and preservation of the intestinal epithelial barrier integrity via direct promotion of enterocyte renewal in the crypt-to-villus axis. These events lead to protection against colitis and tumorigenesis. From a mechanistic point of view, BA administration reverted the FGF19 protective action, while the presence of FXR in the enterocyte was necessary to ensure FGF19 driven anti-inflammatory systemic and local activity. Indeed, FGF19 was not able to protect against colitis in FXR null mice even if hepatic CYP7A1 is repressed and bile acid levels reduced, thus pointing to the central role of FXR in the epithelial barrier integrity and inflammation in the gut. Our data emphasize the therapeutic potential of intestinal FXR agonism and FGF19 in the treatment of enteritis with concomitant derangement of BA homeostasis.
Oral Poster Presentation

Treatment response of murine sclerosing cholangitis to systemic versus intestinal FXR agonists segregates with their effects on hepatic pro-inflammatory cytokine production

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Introduction: FXR agonists are potent therapeutic regulators of liver metabolic function. Their role in the treatment of inflammatory cholestatic liver disease is less defined. Here, we use Mdr2⁻/⁻ mice, a model of defective canalicular excretion of phospholipids which leads to biliary precipitation of bile acids, cholangiocyte injury, sterile inflammation and fibrosis.

Methods: 45-day-old mdr2⁻/⁻ female mice underwent oral gavage for 7 days with 30 mg/kg/day of systemic FXR agonist M345 or 100 mg/kg/day of intestinal FXR agonist M379, both derivatives of fexaramine, or with vehicle (corn oil) in controls. Serum liver biochemistries and bile acid levels were determined by colorimetric assays and liver and intestinal gene expression (8hr after the last oral dose of FXR agonist) was quantitated by Taqman-based qPCR.

Results: Compared to vehicle control mice, M345-treated mice displayed improved weight gain (+4.8 vs. -0.9 g compared to baseline; p = 0.00), lower serum liver biochemistries (ALT 298 vs. 981 IU/l; p = 0.01, ALP 149 vs. 197 IU/l; p = 0.03, TB 1.2 vs. 6.8 mg/dl; p = 0.01) and decreased serum bile acid levels (282 vs. 923 umol/l; p = 0.03). In contrast, M379-treated mice showed continued weight loss and no significant change in serum liver biochemistries or serum bile acid levels when compared to controls. While both M345- and M379 significantly induced intestinal Shp and Fgf15 gene expression and reduced hepatic mRNA expression of Cyp8b1 (down 91% vs. 79%; p = 0.22) and liver bile acid levels (down 38% vs. 29%; p = 0.36), only M345-treatment reduced hepatic TNFα mRNA expression when compared to controls (down-89%; p = 0.00).

Discussion/Conclusion: While both systemic and intestinal FXR agonists down-regulate transcription of key enzymes of de novo bile acid synthesis and subsequently liver bile acid concentration, only the systemic FXR agonist M345 represses hepatic TNFα expression, which is associated with attenuation of the sclerosing cholangitis phenotype.
Session VII

Bile acid receptors and bile acid signaling as therapeutic targets
Amphiregulin/EGFR as therapeutic target in liver disease

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Intrahepatic accumulation of bile acids (BAs) may cause hepatocytes and cholangiocytes death. Upon liver injury, a potent protective and regenerative response ensues. Epidermal growth factor receptor (EGFR) signaling is essential for experimental liver regeneration. EGFR can be activated by several growth factors, among which amphiregulin (Areg) is key for liver regeneration. We studied the role of Areg during cholestatic liver injury and the mutual regulation of Areg expression and BA synthesis. Bile duct ligation (BDL) and oral alpha-naphtyl-isothiocyanate (ANIT) administration were implemented in wild type (Areg-WT) and Areg knockout (Areg-KO) mice. Areg expression was examined in: -livers from patients with primary biliary cholangitis (PBC) and primary sclerosing cholangitis (PSC) -mice and cultured liver cells treated with BAs -farnesoid X receptor knockout mice (FXR-KO) after BDL. Areg mRNA and protein were up-regulated in PBC and PSC patients (hepatocytes and cholangiocytes). BA gavage to mice induced ileal and hepatic Areg expression, and cholestyramine feeding reduced postprandial ileal and liver Areg upregulation. Areg-KO mice display higher Cyp7a1 expression and intrahepatic BA concentrations. Liver Areg expression was induced in BDL and ANIT. Liver damage was exacerbated in Areg-KOs. BAs induced Areg expression in cultured liver cells partially through FXR. Consistently, after BDL FXR-KO mice showed reduced liver Areg expression. Areg protected from ANIT-induced liver injury and from BAs toxicity in hepatocytes.

Conclusions: Areg participates in BA physiological homeostasis. Liver Areg expression is activated during cholestasis partially through FXR. Areg plays an important role in protecting the liver from BA induced toxicity.
Stimulation of ammonium detoxification via FXR; where isoforms matter

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In the last two decades, the Farnesoid X Receptor (FXR) has drawn great attention because of its role as a bile acid-activated transcription factor. Next to bile acid homeostasis, FXR regulates lipid and glucose homeostasis, dampens the immune response and promotes liver regeneration. We have recently shown that FXR also regulates amino acid catabolism and detoxification of ammonium via ureagenesis and glutamine synthesis in livers of mice. Compounds that activate FXR are therefore expected to promote ammonium clearance in patients with hyperammonemia, a common feature of chronic liver disease. Strikingly, Ass1, the rate-limiting enzyme in arginine biosynthesis and urea production, is solely activated by FXRα2/4 isoforms and not by FXRα1/3 in liver organoids expressing single FXR isoforms. FXRα2-transduced organoids showed higher consumption of citrullin, the substrate of the Ass1 enzyme, while increasing arginine concentrations; this was not observed in the FXRα1 organoids. We showed that the previously identified FXR-bound regulatory region in the Ass1 locus is solely bound by FXRα2/4. Subsequent ChIP-sequencing analysis revealed many more FXRα2/4-selective DNA binding locations enriched for a novel discriminant binding motif. Binding and activation by FXR from this DNA binding element does not require RXR heterodimerization, providing potential therapeutic avenues for partial agonism of FXR.

Conclusion: Our data support that Ass1 and many more genes are transcriptionally regulated by FXR α2/4 selectively. The relative expression of the FXR isoforms and RXR strongly impact on which motif is used, and therefore on the transcriptional output. Understanding the regulation of FXR isoform expression may therefore prove instrumental in optimizing the therapeutic efficacy of FXR full agonists.
Role of miR-506 in primary biliary cholangitis

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Primary biliary cholangitis (PBC) is a chronic cholestatic liver disease associated with autoimmune phenomena targeting the intrahepatic bile duct cells (cholangiocytes). Although its etiopathogenesis remains obscure, development of anti-mitochondrial auto-antibodies (AMAs) against the pyruvate dehydrogenase E2 (PDC-E2) complex is a common feature. MicroRNA (miR) dysregulation occurs in liver and immune cells of PBC patients, but its functional relevance is largely unknown. Nevertheless, miR-506 arises as an important actor in the disease. MiR-506 is overexpressed in PBC cholangiocytes and directly targets both Cl-/HCO3- anion exchanger 2 (Banales JM et al. Hepatology. 2012) and type III inositol 1,4,5-trisphosphate receptor (Ananthanarayanan M et al. J Biol Chem. 2015), leading to cholestasis. Likewise, miR-506 regulates cholangiocyte pathobiology and stimulates immune activation (Erice O et al. Hepatology. 2018). Several pro-inflammatory cytokines overexpressed in PBC livers (such as interleukin-8 [IL8], IL12, IL17, IL18, and tumor necrosis factor alpha) stimulate miR-506 promoter activity in human cholangiocytes. MiR-506 dysregulates the proteomic profile of cholangiocytes, affecting proteins involved in different biological processes including mitochondrial metabolism. In cholangiocytes, miR-506 (1) induces dedifferentiation with down-regulation of biliary and epithelial markers together with up-regulation of mesenchymal, pro-inflammatory, and pro-fibrotic markers; (2) impairs cell proliferation and adhesion; (3) increases oxidative and endoplasmic reticulum stress; (4) causes DNA damage; and (5) sensitizes to apoptosis induced by cytotoxic bile acids. These events are also associated with impaired energy metabolism in mitochondria (proton leak and less adenosine triphosphate production) and PDC-E2 overexpression. Co-culture of miR-506 overexpressing cholangiocytes with PBC immunocytes induces activation and proliferation of immune cells. In conclusion, miR-506 promotes PBC-like features in cholangiocytes and immune activation, representing a potential target for therapy.
Clinical and mechanistic aspects of nor-ursodeoxycholic acid (norUDCA)

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24-norursodeoxycholic acid (norUDCA) is a side-chain shortened derivate of ursodeoxycholic acid (UDCA) and lacks a methyl group in its side chain. Despite some similarities in the name, the pharmacological effects and mechanisms of norUDCA profoundly differ from UDCA. The term ‘nor’ is based on older chemical nomenclature and stands for ‘Stickstoff (N) ohne Reste’ (nitrogen without remnants/residues) as also used to distinguish nor-epinephrine from epinephrine. This choice of nomenclature reflects the deep (bio)chemical level of knowledge and understanding of Alan F. Hofmann, who initially synthesized norUDCA among other conjugation resistant nor-bile acids to prove the concept of cholehepatic shunting. Side-chain shortening of orUDCA results in relative resistance to amidation with taurine or glycine compared with UDCA. Consequently, norUDCA undergoes cholehepatic shunting (instead of undergoing a full enterohepatic circulation) resulting in 'ductular targeting' to bile ducts/ductules and hepatic enrichment. Importantly, cholehepatic shunting results in a bicarbonate-rich hypercholeresis which counteracts bile acid toxicity and reinforces the biliary 'bicarbonate umbrella'. As proof of principle, taurin-conjugated norUDCA lacks cholehepatic shunting with loss of its therapeutic efficacy in preclinical models. In addition, norUDCA is more hydrophilic and thereby even less toxic than its ‘mother compound’ UDCA which may further help to counteract (intrinsic) biliary toxicity. As such, norUDCA (but not ‘conventional’ UDCA) reverses sclerosing cholangitis in the experimental Mdr2/Abcb4 knockout mouse (Mdr2/Abcb4−/−) cholangiopathy model for (primary) sclerosing cholangitis (PSC) while UDCA aggravates bile infarcts in cholestatic conditions with (complete or partial) biliary obstruction. Notably, neither norUDCA nor its mother compound UDCA have relevant affinities for dedicated bile acid receptors such as FXR or TGR5, although norUDCA has recently been shown to increase FXR acetylation by attenuation of SIRT-1. Moreover, norUDCA has anti-lipotoxic, anti-proliferative, anti-fibrotic as well as anti-inflammatory effects which complement stimulation of bile acid detoxification and induction of alternative bile acid export via the basolateral membrane. norUDCA also stimulates autophagy and attenuates liver injury in a mouse model of A1AT deficiency. A recent study demonstrated beneficial effects of norUDCA (but not UDCA) on granuloma size and hepatic fibrosis in a mouse model of Schistosoma mansoni infection as world-leading cause of hepatic fibrosis and portal hypertension. The anti-inflammatory properties of norUDCA were directed to MHC class II protein expression on dendritic cells and macrophages and norUDCA reduced T-lymphocyte proliferation and serum levels of pro-fibrogenic Th2 cytokines IL-13 and IL-4. Preliminary data suggest immunomodulatory effects of norUDCA (but not UDCA) on CD8 T-cell immunity by inhibition of mTOR signalling in the lymphocytic choriomeningitis virus (LCMV) model of CD8 T-cell driven hepatic immunopathology and in the Mdr2/Abcb4−/− cholangiopathy model for PSC. Such mechanisms could also contribute to direct anti-inflammatory and anti-fibrotic effects of norUDCA independent form its anti-cholestatic effects.
Based on these encouraging experimental data in preclinical models and successful completion of phase I studies, norUDCA was recently tested in a double-blind, randomized, placebo-controlled phase II trial in the treatment of PSC which demonstrated improvement of cholestatic liver enzymes (without induction of pruritus) irrespective of previous exposure and response to UDCA. Based on these encouraging results, a large phase III trial in PSC has recently been initiated.

Due to its broad mechanisms of action, norUDCA has considerable clinical potential in a wide range of cholestatic conditions/cholangiopathies beyond PSC, in particular conditions where defects of the biliary bicarbonate umbrella or MDR3/ABCB4 (directly corresponding to the Mdr2/Abcb4<sup>−/−</sup> mouse model) as well as biliary toxicity may be involved. This may include primary biliary cirrhosis (PBC), various forms of secondary sclerosing cholangitis, prevention/treatment of non-anastomotic strictures after liver transplantation, progressive familial intrahepatic cholestasis (in particular PFIC-3 and LPAC-syndrome caused by MDR3/ABCB4 defects) and cystic fibrosis among others.

In addition to cholestatic disorders, norUDCA also has beneficial therapeutic effects in metabolic liver diseases such as non-alcoholic fatty liver disease (NAFLD). As such, norUDCA has shown beneficial effects in various genetic and dietary mouse models of NAFLD/NASH including NEMO<sup>−/−</sup> mice (spontaneously developing NASH) and ApoE<sup>−/−</sup> mice on Western diet (developing both hepatic steatosis and atherosclerosis). Since enhanced mortality due to cardiovascular disease is of major prognostic relevance in patients with NAFLD/NASH, therapeutic strategies aiming at both disorders are of key interest. norUDCA significantly reduced hepatic triglyceride content, hepatic inflammation and aortic plaques surface area in Western chow-fed ApoE<sup>−/−</sup> mice. Recently a double-blind, randomized, placebo-controlled, phase II dose-finding study comparing different doses of norUDCA with placebo in the treatment of NAFLD has been completed which demonstrated improvement of liver enzymes, hepatic steatosis and liver stiffness, encouraging further long-term studies in NAFLD/NASH.

In summary norUDCA represents the first in class cholehepatic drug which has shown efficacy in preclinical mouse models of cholestatic and metabolic liver diseases and phase II studies in PSC and NAFLD. Importantly, cholehepatic drugs can be combined with enterohepatic drug (e.g. FXR ligands) and enterohepatic blockers (e.g. ASBT inhibitors resins) to combat complex cholestatic and metabolic liver diseases.
Serum biomarker development demonstrating the transformation of fatty liver to steatohepatitis in association with diabetes mellitus

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Introduction: There are currently no “liver-specific” non-invasive prognostic markers of non-alcoholic steatohepatitis (NASH) capable of showing ‘early’ inflammation prior to or with inflammatory cell infiltrate. Using 2 steatosis mouse models, a human correlate, and hepatocyte culture, we offer a proof-of-concept for detection of an increase in oxysterols as liver specific non-invasive predictors of CYP7B1 suppression driven by early inflammation. Also, we demonstrate a connection to diabetes mellitus; major risk factor for progression of NAFLD.

Methods: B6/129 (NAFL model) and C57Bl/6 mice were fed a WD or high fructose diet for 2–6 weeks to achieve early fatty liver. Mouse and human non-alcoholic steatosis liver Cyp7b1 mRNA, oxysterol, inflammatory marker levels, and liver histology were determined.

Results: The 2-week WD feeding of mice led to a 69% repression of Cyp7b1 mRNA levels coupled to increases in (25R)-26-hydroxycholesterol and biochemical evidence of early inflammation (increased IL-1β/ALT). By 6 weeks, Cyp7b1 suppression persisted with WD feeding, with further increases in oxysterol/ALT levels and visible inflammatory infiltrates. The 32-week WD feeding led to overt inflammatory cell infiltrate. Similarly, CYP7B1 mRNA levels in human non-alcoholic steatosis were repressed (Δ62%; n = 3) as compared to healthy livers; and associated with a correlative oxysterol increase. 4-week of WD feeding or high fructose diet led to > 60% (p < 0.001) repression of Cyp7b1 mRNA levels. cAMP, a product of glucagon stimulation, down-regulated Cyp7b1 in isolated primary hepatocytes.

Discussion/Conclusion: Human CYP7B1 deficiency increases hepatic (25R)-26-hydroxycholesterol and causes inflammation-driven fibrosis within first year-of-life. NAFLD takes similar course to this disease progression. With diabetes mellitus, paradoxical elevation of glucagon occurs. We offer evidence that hyperglucagonemic repression of CYP7B1 leads to persistently elevated oxysterol levels which drive hepatic inflammation. Oxysterol measurement in serum, in the presence of increased inflammatory markers, could serve as non-invasive markers of early inflammation.
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POSTER ABSTRACTS

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Obeticholic acid increases cholesterol saturation and FGF19 in human gallbladder bile

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Introduction: Obeticholic acid (OCA) is the first in class steroidal agonist of the nuclear farnesoid X receptor (FXR) and was recently approved for the treatment of primary biliary cholangitis (PBC). We aimed to explore the molecular actions of OCA in human liver, gallbladder, and bile in patients awaiting laparoscopic gallstone surgery.

Methods: As part of the investigator-initiated randomized placebo-controlled OCABSGS trial (NCT01625026), 20 patients with gallstones but otherwise healthy were randomized to OCA (25 mg/kg/day) or matching placebo for three weeks before surgery. Serum liver enzymes, lipids, bile acids (BAs), the BA synthesis marker C4 (\(7\alpha\)-hydroxy-cholest-4-ene-3-one), FGF19 and insulin resistance (HOMA-IR) were measured before and after treatment. During surgery, biopsies were taken from the liver and gallbladder for molecular biology investigations. Gallbladder bile was investigated for lipid composition and FGF19.

Results: All patients completed the study per protocol. OCA vs. placebo groups were well matched by gender (80% women in both groups), age (48.8 ± 8.9 vs. 50.8 ± 13.3 years, all data mean ± SD), BMI (27.9 ± 4.9 vs. 28.7 ± 4.5 kg/m\textsuperscript{2}), and HOMA-IR (2.4 ± 1.5 vs. 2.7 ± 1.8). OCA patients showed minor but significant increases in LDL-cholesterol and alkaline phosphatase and decreases in \(\gamma\)GT. In serum, OCA significantly (p < 0.05) increased FGF19 (from 95.0 ± 8.5 to 234.4 ± 35.6 ng/l), and decreased C4 (from 31.4 ± 7.6 to 2.8 ± 1.3 nmol/l) and endogenous BAs (from 1312 ± 236 to 518 ± 179 nmol/l). No changes occurred in controls. At surgery, BAs in bile of OCA patients were significantly (p < 0.05) lower (98.6 ± 76.9 vs. 186.6 ± 100.9 mmol/l) than in controls. This reduction resulted in an increased Cholesterol Saturation Index (5.2 ± 2.2 vs. 3.7 ± 1.0; p = 0.07). OCA (including conjugates) contributed to 46.3 ± 13.9% and 16.9 ± 6.4% of the total BA levels in serum and bile, respectively. FGF19 was present in gallbladder bile in the OCA group at levels three times higher than controls (403 ± 165 vs. 135 ± 131 ng/l, p < 0.005). RNASeq confirmed induction of FXR target genes in the liver and gallbladder and the biliary origin of FGF19.

Discussion/Conclusion: This is the first report showing enrichment of FGF19 in human bile during OCA treatment. Although the physiological role of FGF19 in the human biliary tree is unknown, we speculate that it might trigger relaxation of the gallbladder which, in connection with increased cholesterol oversaturation by OCA, would further enhance susceptibility for gallstones.
Rapid regulation of hepatic lipid metabolism by modulation of bile acid fluxes in humans

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Changes in bile acid (BA) turnover may rapidly influence cholesterol and energy metabolism in humans. We explored how BA fluxes may influence BA and cholesterol syntheses, FGF signaling and lipoprotein levels under basal and experimental conditions.

Serum levels of individual BAs, markers of cholesterol and BA synthesis, FGFs 19 and 21, PCSK9, and lipoprotein pattern were followed over 24 h in healthy humans, both in the basal state and following treatments with cholestyramine, statin, and FXR agonists.

BA and cholesterol syntheses both show distinct but asynchronous diurnal variations. Conjugated and unconjugated BAs show different circadian rhythms. Following meals, transintestinal flux of conjugated BAs regulates circulating FGF19. Rapid suppression of BA synthesis by FXR agonists is not fully explained by elevated FGF19 levels. Inhibiting intestinal BA uptake by cholestyramine may have different effects on liver metabolism in fed and fasting conditions, probably indicating a diurnal variation of the normal intestinal microflora.
In vitro rescue of ABCB11 non-sense mutations: Induction of a readthrough of premature stop codons

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Introduction: Progressive familial intrahepatic cholestasis type 2 (PFIC2) is due to mutations of ABCB11, encoding the canalicular bile salt export pump (BSEP). Non-sense mutations (premature termination codon, PTC) are responsible for severe forms of the disease. The aim was to assess the ability of drugs to induce readthrough of PTC of ABCB11.

Methods: Six PTC identified in PFIC2 patients were studied (p.Y354X, p.R415X, p.R470X, p.R1057X, p.R1090X and p.E1302X). Readthrough levels of human and rat PTC in their nucleotide environment were first quantified before and after treatment with aminoglycosides (G418 [200 μg/ml]), gentamicin (800, 1200 μg/ml) and Ataluren® (15 μM) using dual reporter assay (luciferase/galactosidase) in NIH3T3. After transfection in HEK293, MDCK and Can10, cells were treated with gentamicin. Expression and cellular localization of full-length Bsep protein resulting from readthrough were studied by epifluorescence and confocal microscopies. Stable MDCK clones co-expressing BsepWT or BsepR1090X and Ntcp were used to study vectorial transport of [³H]taurocholate.

Results: In NIH3T3, aminoglycosides significantly increased readthrough levels of all human and rat mutations while Ataluren® only slightly increased readthrough level of human p.E1302X. In HEK293, gentamicin induced readthrough of 4 PTC (p.R415X, p.R470X, p.R1057X, p.R1090X). Resulting full-length proteins localized within the cytoplasm, except BsepR1090X that was also detected at plasma membrane. In Can10, gentamicin treatment significantly increased readthrough of R1090X PTC and the resulting full-length protein was detected mainly in the cytoplasm but also at the canalicular membrane. At 27°C, proportion of BsepR1090X full-length protein localized at the canalculus significantly increased. In MDCK, functional study of BsepR1090X full-length protein performed after gentamicin-induced readthrough showed a significant increase in transport of [³H]taurocholate.

Discussion/Conclusion: This study constitutes a proof of concept for readthrough therapy in selected patients with PFIC2 due to PTC of ABCB11, such as p.R1090X and suggests that combination with chaperone therapy might further improve the rescue.
Ubiquitination of Lys-340 reduces NTCP-mediated bile acid uptake and NTCP plasma membrane expression

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Introduction: Transient overexpression of the sodium taurocholate cotransporting polypeptide (NTCP) can lead to its aggregation in the endoplasmic reticulum, ubiquitination and subsequent proteosomal degradation. ER-associated degradation (ERAD) mainly involves misfolded proteins where impaired folding is due to excessive overexpression. The aim of the present study was to determine whether ubiquitination affects NTCP localization and function upon modest overexpression (in absence of ERAD) and to identify the site(s) of NTCP ubiquitination.

Methods: NTCP ubiquitination was verified by immunoprecipitation in combination with western blot analysis in U2OS cells transiently expressing NTCP. Five conserved lysine residues in close proximity (K1-5R; position 309, 311, 314, 316 and 318) and a sixth lysine (K340) further towards the C-terminus of NTCP were mutated to arginine by site-directed mutagenesis resulting in four constructs; NTCPWT, NTCPK1-5R, NTCPK1-6R, NTCPK340R. These constructs were stably expressed in HepG2 cells or transiently in U2OS cells. NTCP-mediated bile acid uptake was measured using tritium-labelled taurocholate (TCA). NTCP plasma membrane expression was determined by cell surface biotinylation. Effect of the lysine mutations on NTCP mRNA and protein expression was measured using RT-PCR and western blot analysis, respectively.

Results: NTCP was expressed at the plasma membrane and fully glycosylated in stably transfected U2OS cells, and ubiquitinated. Cells expressing NTCPK340R and NTCPK1-6R displayed an increased NTCP plasma membrane expression and an increased bile acid uptake compared to NTCP-WT and NTCPK1-5R. Furthermore, NTCPK340R and NTCPK1-6R protein expression was increased while NTCP mRNA levels were unaffected.

Discussion/Conclusion: These results suggest that ubiquitin-modification at a conserved lysine on position 340 targets NTCP for endocytosis or degradation. Regulated ubiquitin-modification of this lysine residue would allow cells to modulate NTCP stability, plasma membrane localization and bile acid uptake.
Sex hormone-dependent intestinal expression of ASBT determines the sclerosing cholangitis phenotype and the response to pharmacological disruption of enterohepatic circulation of bile acids in Mdr2\(^{-/-}\) mice

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Introduction: Several liver conditions exhibit sex predilection for onset and disease progression. Here we examine the sex bias for the sclerosing cholangitis (SC) phenotype and response to anti-cholestatic therapy in Mdr2\(^{-/-}\) mice.

Methods: The SC phenotype was determined in 45-day old Mdr2\(^{-/-}\) mice in BALB/cJ background. Male Mdr2\(^{-/-}\) mice were treated with 5 µg/kg of 17-b-estradiol (in corn oil) daily i.p. for 5 days starting at day 40. SC-435, a minimally absorbed ASBT inhibitor, admixed to the chow was administered for 14 days beginning at day 30.

Results: Compared with males, female Mdr2\(^{-/-}\) mice displayed significantly higher serum levels of alkaline phosphatase (ALP), total bilirubin (TB), ALT, higher scores for bile duct proliferation, necrosis of hepatocytes, and liver fibrosis, increased collagens deposition on Sirius Red-stained sections, and higher frequency of liver infiltrating Kupffer cells and monocyte derived macrophages. While bile acid concentration in liver, plasma and bile were higher in females than males, RNAseq and candidate qPCR studies showed downregulation of genes associated with pathways of bile acid (BA) metabolism and cholesterol synthesis in livers of female mice prompting investigation on sex-dependent regulation of intestinal BA re-uptake. ASBT, a facilitator of enteral BA re-uptake, was upregulated in female compared with male Mdr2\(^{-/-}\) mice which corresponded with decreased fecal BA excretion (mean 0.43 ± 0.08 vs. 1.43 ± 0.39 umol/g body weight/day; \(p = 0.01\)). Treatment with estradiol down-regulated FXR-expression in the terminal ileum and significantly increased serum ALT and TB levels in male Mdr2\(^{-/-}\) mice. Treatment with SC-435 (10 mg/kg/day) was associated with greater reduction of ALP, TB and ALT in female than male mice compared with untreated sex-matched control Mdr2\(^{-/-}\) mice.

Discussion/Conclusion: Female Mdr2\(^{-/-}\) mice display an aggravated SC phenotype which is associated with increase bile acid pool size due to sex hormone-dependent expression of intestinal FXR and ASBT. The latter also determines to anti-cholestatic therapy with ASBT inhibitors.
The role of microbiota in sex-specific regulation of lipid metabolism

Previous studies have documented that lipid metabolism is influenced both by sex and gut microbiota, but these factors were assessed independently. In the present study, we investigated the contribution of gut microbiota to sex-specific differences in lipid metabolism. We measured ileal gene expression by whole-transcriptome microarray analysis in germ-free (GF) and conventional (Conv) male and female mice. We also determined serum bile acids and faecal microbiota composition. Comparing biological functions and gene sets between GF males and females demonstrated a strong sex-specific effect in immune regulated pathways, such as antimicrobial and inflammatory responses. In Conv mice, the most striking sex-specific effect was found in the biological function lipid metabolism. Exploring the sub-functions of lipid metabolism showed that, in presence of gut microbiota, cholesterol and lipid related gene sets were mostly affected in the distal ileum by sex differences. However, in GF mice, the sex differences in expression of lipid related genes were blunted and thus appeared to be dependent of presence of microbiota. Next, we explored the role of bile acids as mediators in the crosstalk between microbiota and host lipid metabolism. In female mice primary and secondary serum bile acid concentrations were higher compared to males and this not dependent on the presence of microbiota. As expected, sex-specific effects in secondary serum bile acid levels were dependent on presence of microbiota. Microbiota composition analysis showed sex-specific differences in Conv mice, which supports the notion that bile acids are mediators of the crosstalk between microbiota and host lipid metabolism. In conclusion, our data show that the presence of gut microbiota influences sex-specific regulation of lipid metabolism in mice.
Complex treatment of uncomplicated cholelithiasis at the stage of cholecystolithiasis

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Introduction: Cholecystolithotomy is actual organ-saving surgical care for uncomplicated cholelithiasis in the cholecystolithiasis stage.

Aim: To improve outcome of organ-saving cholecystolithotomy by usage of electromyostimulation (EMS) of gallbladder (GB) in combination with ursodeoxycholic acid (UDCA) treatment in the postoperative period.

Methods: 83 patients had undergone organ-saving surgery by the laparoscopic endoscopically assisted cholecystolithotomy (LEACHLT), mean age 21–70 (44.3 ± 11.5) years, compared to 20 patients with normal. Biochemical parameters such as a cholesterol, bile acids and phospholipids levels were determined before the operation in "B" and "C" bile duodenal portions. The motility function of GB was measured with ultrasound (breakfast with sorbit). GB increase ≥ 40% was accepted as a normal level. After LEACHLT the motility of GB was EMS since the 3rd day. One-week course was repeated every month (3–6 months) and combined with Ursofalk® treatment.

Results: Before the operation with reduced cholato-cholesterol and phospholipid-cholesterol coefficients: 4.4-fold and 8.4-fold (p < 0.01) in the "B" portion, 3.8-fold and 4.2-fold (p < 0.01) in the "C" portion and 3.5-fold and 5.7-fold (p < 0.01) in bile collected from GB at operation. The mean values of bile secretion efficiency before the operation 39.76 ± 2.05% was lower in comparison with normal 47.82 ± 3.07%, (p < 0.05). Before surgery 56.6% of patients had normotonia, 43.4% – hypotonia. After EMS course bile secretion efficiency was 59.34 ± 4.11%, (p < 0.001), was increased in 1.4 times higher. Long-term results were studied in 66 (79.5%) patients in terms of up to 10 years. After LEACHLT the frequency of GB normotonia increased to 75.7%, and the incidence of GB hypotonia decreased to 24.3% (p < 0.05). Increase of cholato-cholesterol and the phospholipid-cholesterol coefficients in the "B" portion – 2.5-fold and 2.6-fold (p < 0.01) and "C" portion – 1.4-fold and 1.7-fold (p < 0.01). Recurrence of cholecystolithiasis was noted in 5 (7.5%) patients with the initial GB hypotonia.

Discussion/Conclusion: The LEACHLT combined with EMS and UDCA course in the postoperative period is the optimal method for treatment of uncomplicated cholelithiasis in the cholecystolithiasis stage.
The use of SeHCAT scans in patients with undiagnosed chronic diarrhea

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Introduction: Bile acid malabsorption (BAM) is a condition caused by an underlying disease such as ileal Crohn’s disease (CD) (type 1), can be idiopathic (type 2), or be the consequence disease processes such as post-cholecystectomy (type 3). The selenium homocholic acid taur ate (SeHCAT) retention test is used for diagnosis and costs £436–486 per patient.

Methods: We retrospectively studied 48 patients, who underwent a SeHCAT study for undiagnosed chronic diarrhoea. This included 13 CD patients and 35 patients without CD. Patients had undergone all relevant investigations beforehand. Results were tested for statistical significance using the Chi-squared test.

Results: The SeHCAT scan had a positive predictive value (PPV) of 58.3%. Out of those with CD, the PPV was 84.6% (n = 11), with non-CD patients having a PPV of 51.4% (n = 19) (p = 0.07). Of those CD patients with a diagnosis of BAM, 6/9 had terminal ileal disease, or had an ileal resection, 5/9 (55.5%) had severe BAM, whereas the proportion of non-CD patients with BAM, only 8/19 (21.6%) had severe BAM (p = 0.16). Also of note, 11 positive SeHCAT scans were for patients without predisposing risk factors (22.9%), with a presenting complaint of either diarrhoea, abdominal pain, bloating or a combination of all three. 8 out of 12 patients with cholecystectomies had BAM (66.6%).

Discussion/Conclusion: We conclude SeHCAT scans have a high PPV in CD patients. There is a case for the empirical usage of bile acid sequestrants in patients who are post-cholecystectomy with diarrhoea. This could result in cost-savings, preventing unnecessary scans and future outpatient visits. Future studies could identify a scoring system so that SeHCAT scans could be used selectively.
The role of nuclear receptors and histone deacetylases in the regulation of bile acid synthesis in humans: Effects of drug treatment

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Introduction: Despite an impressive body of experimental evidence, the knowledge on the molecular regulation of bile acid synthesis in humans is still limited. In particular, the role of histone deacetylases (HDACs), which has been well characterized in animal models, is unknown. Aim of the present work is to investigate some of the mechanisms potentially involved in the control of bile acid synthesis in humans by means of pharmacological manipulations.

Methods: Surgical liver biopsies were obtained in untreated subjects, and in patients receiving drugs known to affect bile acid metabolism: cholestyramine (5 patients), CDCA (9) and UDCA (7); mRNA levels of cholesterol 7alpha-hydroxylase (CYP7A1), related nuclear receptors/coactivators and histone deacetylases were assayed by quantitative real-time RT-PCR. Serum levels of 7alpha(OH)-4-cholesten-3-one (C4), a marker of in vivo bile acid synthesis, were analysed by GC-MS. C4 analysis was also performed in 10 patients receiving valproic acid (VPA), which is known to affect HDAC function and bile acid synthesis in animals.

Results: Serum C4 levels were markedly reduced in patients receiving CDCA, and increased after cholestyramine. No effects were detected with UDCA treatment. Such changes were paralleled by corresponding alterations in hepatic expression of CYP7A1. No significant changes were observed in tissue expression of the main nuclear receptors and coactivators, in particular FXR, SHP, HNF-4, nor in the expression of HDAC3 and HDAC7. Treatment with VPA did not associate with significant changes in plasma lipids and C4 levels.

Discussion/Conclusion: Our data cannot provide direct evidence on a relevant regulatory effect of nuclear receptors and of HDACs on CYP7A1 expression in human liver. In vivo findings also do not support a significant role of HDACs on bile acid synthesis and on plasma cholesterol; still we have to consider the poor specificity of HDAC inhibition induced by VPA and the low pre-treatment cholesterol levels.
Hepatoprotective impact of TGR5: Central role of gallbladder function and bile acid pool modulation

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Introduction: The bile acid (BA) receptor TGR5 protects the liver against BA overload in mice. As TGR5-KO mice have a more hydrophobic BA pool and as TGR5 is highly expressed in the gallbladder (GB), we studied TGR5 impact on GB function and BA pool composition.

Methods: WT and TGR5-KO mice were submitted to: 90% hepatectomy (EH) upon normal, UDCA (ursodeoxycholic acid, 0.5%)- or cholestyramin (CT2%)-enriched diet; bile duct ligation (BDL); cholecystectomy (CCT); TGR5 agonist (RO5527239 (RO) 10 mg/kg/day, Roche) treatment. Survival, liver injury (H&E, plasma ALT and Bilirubin) and regeneration (liver weight, PH3, Ki67), BA pool composition (plasma, liver; LC-MS) were analyzed. GB volume and filling (99Tc Mebrofenin scintigraphy) were measured, as well as GB and hepatic bile pH.

Results: In TGR5-KO as compared to WT mice, post-EH survival was reduced (25% vs. 64% at day 9), peribiliary necrosis and BA overload were exacerbated while regeneration parameters were similar. CT treatment (BA overload reduction) and UDCA (highly hydrophilic BA pool) strikingly improved survival.

In TGR5-KO as compared to WT mice, GB volume was smaller, and GB filling deeply impaired. RO treatment induced rapid GB dilation in WT mice only, while the BA pool was shifted towards a more hydrophilic composition in these mice. CCT abrogated the BA pool shift in RO-treated mice, suggesting that this effect was GB-dependent. Upon RO treatment, GB but not hepatic bile pH was acidified in WT mice, thus facilitating BA transport towards the blood, instead of intestinal passage.

In the BDL model, GB had a protective effect in WT but not TGR5-KO mice, as shown by more body weight loss, liver injury and inflammatory infiltration observed in CCT versus sham operated mice.

Discussion/Conclusion: TGR5 significantly impacts GB function and thereby BA pool composition, potentially supporting TGR5 hepatoprotective properties under BA overload conditions.
Impact of miR-24 on a MEN1 and SMAD3 gene expression in primary biliary cholangitis

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Introduction: Primary biliary cholangitis (PBC) is a cholestatic disorder characterized by the inflammatory-destructive process of intrahepatic bile ducts and progressive fibrosis which may lead to cirrhosis. Small non-coding micro RNAs, are known to play a role in modulation of liver fibrogenesis. miR-24 was found to suppress expression of Menin 1 (MEN1) which promotes transcription of genes engaged in the hepatic fibrosis by binding to SMAD3. The aim of the study was to analyze expression of miR-24 and its potential effect on MEN1/SMAD3 pathway in PBC.

Methods: Human liver tissue were collected from patients with early stages PBC (F0–F3; n = 18), cirrhotic PBC (F4; n = 10), and control tissue (n = 10). The expressions of miR-24, MEN1 and SMAD3 were analyzed by TaqMan® Real-Time PCR Assays.

Results: Hepatic expression of miR-24 was enhanced in F2 stage of fibrosis (3-fold increase vs. controls, p = 0.05). In comparison to control tissue no difference was noticed in the levels of MEN1 and SMAD3 mRNAs in early-stages of fibrosis (F1–F3). However, in cirrhotic PBC (F4), miR-24 expression was significantly reduced (2.5-fold decrease vs. controls, p = 0.0008) what was accompanied by an elevated level of SMAD3 mRNA 2-fold increase vs. controls; p = 0.004).

Discussion/Conclusion: The high level of miR-24 with unchanged MEN1 and SMAD3 expressions in early-stage (F2) of PBC may suggest an adaptive mechanism protecting against fibrosis. In advanced PBC (F4) this mechanisms seems to be lost with levels of miR-24 substantially reduced and simultaneously increased expression of SMAD3.

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Early onset of increased hypercholanemia during pregnancy correlates with higher risk of meconium-stained fluid

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Introduction: Intrahepatic cholestasis of pregnancy (ICP) is the commonest gestational liver disease. The risk of adverse fetal outcome has been associated with the severity of maternal hypercholanemia after diagnosis. The aim was to investigate whether there is a relationship between the severity and timing of onset of hypercholanemia and the risk of meconium-stained amniotic fluid (MSAF) and adverse neonatal events.

Methods: The study included 382 pregnancies complicated by ICP managed at a referral hospital in Buenos Aires (Argentina) between June 2009 and December 2013. The patients were classified into three groups according to the severity of hypercholanemia at diagnosis; mild (10–19.9 µmol/l), moderate (20–39.9 µmol/l) and severe (≥ 40 µmol/l). Their clinical characteristics and pregnancy outcomes were investigated in a prospective observational study.

Results: Higher risk of MSAF was observed when ICP appeared early in gestation or when hypercholanemia was more severe. Taking both parameters into account an MSAF risk factor (MRF) was defined. Based on a model of positive/negative predictive values, a cut-off point of MRF = 3 was selected, which prioritized sensitivity versus specificity. In ICP patients with MRF > 3, the probability of MSAF was enhanced 4-fold. An increase in the frequency of MSAF was also associated with higher serum levels at diagnosis of alanine transaminase, alkaline phosphatase and direct bilirubin.

Discussion/Conclusion: The risk of MSAF is associated not only with the magnitude of hypercholanemia at diagnosis but also with the early gestational onset of raised maternal serum bile acids.
sGC stimulation and PDE5 inhibition decrease sinusoidal resistance and reduce fibrosis in rats with biliary cirrhosis

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Introduction: Chronic liver disease, such as fatty liver, alcohol abuse, or viral hepatitis, can cause scarring of the liver tissue and portal hypertension (PHT). The development of liver disease highly corresponds to the disturbance in cGMP signalling and nitric oxide availability (NO). We aimed to investigate the effects of the soluble guanylyl cyclase (sGC) stimulator riociguat (RIO) and activator cinaciguat (CINA) and phosphodiesterase-5 inhibitor tadalafil (TADA) in rats with cholestatic biliary cirrhosis.

Methods: 50 Male Sprague-Dawley rats underwent BDL or sham-operation (SO). Starting one week after surgery, RIO (0.5 mg/kg), CINA (1 mg/kg), TADA (1.5 mg/kg) and vehicle (VEH) were gavaged for 3 weeks. Portal pressure (PP), mean arterial pressure, heart rate and splanchnic/portal blood flow were measured. Liver fibrosis, hepatic inflammation and hepatic cGMP levels were assessed.

Results: Cirrhotic BDL-VEH rats showed significantly higher values of PP (13.07 ± 0.97 mmHg) in comparison to healthy controls. PP was decreased by RIO (9.96 ± 0.7 mmHg, p = 0.021) and TADA treatment (10.27 ± 0.86 mmHg, p = 0.050), without affecting systemic hemodynamics. RIO decreased intrahepatic vascular resistance (2.86 ± 0.25 vs. 4.85 ± 0.54 mmHg/min*ml, p = 0.005). Both, RIO and TADA treatment reduced hepatic hydroxyproline content (RIO: 350 ± 30 µg/g, p = 0.003, TADA: 282 ± 50 µg/g, p = 0.003 vs. BDL-VEH: 503 ± 20 µg/g liver) and liver fibrosis (Chrome-aniline-blue stained area: RIO: 2.14 ± 0.3%, p = 0.011; TADA: 3.27 ± 0.73%, p = 0.342; vs. BDL-VEH: 4.15 ± 0.53%). Liver transaminases were decreased by RIO (AST: -36%, p < 0.001; ALT: -32%, p = 0.035) and TADA (AST: -24%, p = 0.006; ALT: -27%, p = 0.053). Moreover, hepatic Il6 expression was decreased in BDL-RIO (-56.2%, p = 0.053), indicating less necroinflammation. Livers from BDL-RIO animals presented higher hepatic cGMP levels. In cirrhotic rats, treatment with CINA at 1 mg/kg caused weight loss, increased lactate levels and arterial hypotension.

Discussion/Conclusion: The sGC stimulator riociguat and the PDE5 inhibitor tadalafil show beneficial effects in cirrhotic rats by reducing liver fibrosis and decreasing portal hypertension.
PORTAL PRESSURE

INTRAHEPATIC VASCULAR RESISTANCE

HYDROXYPROLINE

FIBROTIC AREA (CAB)
Bile acids induce hepatic chemokine expression by activating Ca^{2+}/NFAT signaling

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The inflammatory response plays an important role in cholestatic liver injury where bile acid (BA) induction of proinflammatory cytokines in hepatocytes may initiate this event. However, the signaling pathways involving BA stimulation of cytokine production remain elusive. Our previous study (JCI Insight, ‘17) demonstrates that the intracellular DNA sensor Tlr9 takes part in this stimulation. However, the data also indicate that Tlr9 independent signaling exists. Here we report that Ca^{2+}/NFAT (Nuclear Factor of Activated T cells) signaling is involved in BA induction of chemokines in mouse hepatocytes and cholestatic livers. We found that: 1) Inhibitors involved in Ca^{2+}/calmodulin/Calcineurin/NFAT signaling pathway, Cyclosporine A (CsA), KN-62, and FK506, all greatly repressed BA induction of chemokine Cxcl1, Cxcl2 and Cxcl10 expression in mouse hepatocytes; 2) At pathophysiological levels, taurocholic acid (TCA) significantly increased Ca^{2+} levels in the nucleus but not in the cytosol of mouse hepatocytes; 3) Nfatc3 nuclear translocation (a critical step in its activation as a transcription factor) is associated with BA induction of chemokines in mouse hepatocytes, where CsA, KN-62, and FK506 also blocked Nfatc3 nuclear translocation and repressed chemokine induction by bile acids; 5) When the expression of Nfatc3 was knocked down using siRNA in mouse hepatocytes, BA induction of Cxcl1 and Cxcl2 was markedly reduced; 6) The nuclear translocation of Nfatc3 was also detected in vivo in the livers of cholestatic mouse models (i.e. Mdr2^{−/−} mouse and 7-day bile duct ligated mouse), where increased Cxcl2 mRNA expression was also detected. Therefore, we conclude that Ca^{2+}/NFAT signaling plays an important role in the BA induced inflammatory response in cholestasis, a novel mechanism of cholestatic liver injury.
Modification of the intestinal intraluminal bile acid pool composition upon bariatric surgery in a preclinical minipig model

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Introduction: Bile acids (BAs) are signaling molecules modulating metabolic homeostasis. They are synthesized and conjugated in the liver, de-conjugated and modified by the gut microbiota, and reabsorbed in the distal ileum to follow the entero-hepatic cycle. The metabolic improvements following bariatric surgery could happen in part due to modifications in circulating BAs. However, the intestinal intra-luminal BA composition and its modification by bariatric surgery are unknown.

Methods: The Gottingen-like minipig model presents similar metabolic responses to bariatric surgery as humans. Bile and intestinal content were sampled from different segments of the gastro-intestinal tract upon SHAM surgery or distal one anastomosis gastric bypass (dOAGB) (n = 6/group). dOAGB is a new bariatric surgery variant presenting better metabolic effects than RYGB. BA composition was analyzed by LC-MS/MS. The proportion of biliary and intestinal BA species in the different segments was compared between both groups.

Results: In SHAM, the conjugated:free and the primary:secondary BA ratios progressively decreased from proximal to distal throughout the gastro-intestinal tract, reflecting the known microbial modifications on BAs. The dOAGB group presents similar modifications in the conjugated:free ratio, but the primary:secondary BA ratio is higher upon dOAGB due to increased HCA and decreased HDCA in all intestinal segments, suggesting changes in gut microbiota or selective intestinal BA reuptake upon bariatric surgery.

Discussion/Conclusion: We show for the first time the composition of the BA pool all along the gastrointestinal tract and in bile, in physiology and its modification by bariatric surgery (dOAGB) in a human-sized, omnivorous mammal. Bariatric surgery modulates BA metabolism in the intestine, but also in the liver in a complex inter-organ communication. This data will be completed with mechanistic studies aiming to identify the origin of BA modifications and a potential link between BA modifications and metabolic effects of the surgery.
Both intestine-specific and renal/hepatic inhibition of the bile salt transporter ASBT ameliorates cholestatic liver injury

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Introduction: Intestine-specific pharmacologic inhibition of the apical sodium-dependent bile salt transporter (ASBT) reduces pruritus and serum bile acid concentrations in patients with primary biliary cholangitis, but also leads to diarrhoea due to increased presence of bile acids in the colon. We hypothesize that extraintestinal inhibition of ASBT will increase renal bile acid clearance while dampening the increased faecal excretion thus leading to less diarrhoea, with similar liver protection. Here, we investigated whether ASBT deficiency ameliorates cholestatic liver damage in mice, under conditions where faecal bile acid excretion as well as intestinal bile acid uptake is negligible.

Methods: Bile duct ligation was performed in ASBT deficient mice (ASBT KO) and wild-type littermates (control group). Histology and biochemistry markers for hepatobiliary injury were then assessed. Bile acid concentrations in plasma and urine was analyzed by HPLC.

Results: A 2.5-fold increase of urinary concentration and 62% decrease in plasma of bile salt levels was found in bile duct ligated ASBT deficient mice compared to control littermates. ASBT deficient mice showed significantly reduced serum alkaline phosphatase (48%) and total bilirubin (56.8%). Furthermore, a tendency towards reduced alkaline aminotransferase (86%, p = 0.06) and aspartate transaminase (87%, p = 0.05) levels was observed. Liver histology of ASBT deficient mice improved clearly.

Discussion/Conclusion: Both intestinal and extraintestinal inhibition of ASBT are effective in attenuating cholestatic liver damage suggesting that systemic ASBT inhibitors might provide benefits compared to the recently developed non-absorbed ASBT inhibitors by increasing renal excretion of bile salts to reduce the risk of colonic overexposure and diarrhoea.
FXR and TGR5 signaling crosstalk and the gut microbiota in liver metabolism and diseases

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Introduction: The gut to liver axis plays a critical role in regulation of bile acid synthesis and hepatic lipid and glucose metabolism. Bile acid-activated receptors, FXR and TGR5 are co-expressed in the enteroendocrine L cells. The role of intestinal FXR in metabolic regulation is controversial. Both FXR agonists and antagonists have been shown to reduce weight and improve insulin tolerance in mice. The gut microbiota plays an important role in bile acid synthesis, composition and pool size. The aim of this study is to unveil the mechanism of FXR and TGR5 signaling in improving hepatic metabolism and metabolic disorder.

Methods: Wild type, Fxr-/−, Tgr5−/− and db/db mice were oral gavage with an intestine-restricted FXR agonist fexaramine (FEX, 30 mg/kg) to study its effect on bile acid synthesis, GLP-1 secretion, and gut microbiome. Mice were also treated with antibiotics to test the role of gut microbiota on FEX-stimulated metabolic effects.

Results: FEX stimulated lithocholic acid (LCA) production and increased secretion of GLP-1, FGF15 and FGF21, improved insulin and glucose tolerance and promoted white adipose tissue browning in mice. Analysis of 16S ribosomal RNA sequence of the gut microbiome identified FXR-induced and LCA-producing bacteria Acetalifactor and Bacteroides. Antibiotic treatment completely reversed the FEX-induced metabolic phenotypes. FEX treatment effectively improved lipid profiles, increased GLP-1 secretion, improved glucose and insulin tolerance and promoted adipose tissue browning, while antibiotic treatment reversed the beneficial effects of FEX in obese and diabetic db/db mice.

Discussion/Conclusion: This study uncovered a novel mechanism in which activation of intestinal FXR shaped the gut microbiota to activate TGR5/GLP-1 signaling to improve hepatic glucose and insulin sensitivity and increase adipose tissue browning. The intestine FXR-gut microbiota-TGR5-GLP-1 axis plays a critical role in regulation of liver metabolism and homeostasis. Activation of FXR/TGR5/GLP-1 signaling through modulation of the gut microbiota may have therapeutic potential for treating non-alcoholic fatty liver disease, diabetes, and obesity.
Role of a high-fructose diet in early stages of cholelithiasis

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Background: Obesity and metabolic syndrome pandemics are associated with increased dietary fructose consumption, but few information is available on its effect on biliary lipids. Biliary cholesterol is transported mainly by vesicles and micelles. The first stage in the formation of gallstones corresponds to biliary cholesterol crystallization, derived from the vesicular transporters. The aim of this study was to investigate the influence of consuming a high-fructose diet on serum lipids, and determine its implication in gallstones formation.

Methods: The experimental design was quantitative, and 2 groups of BALB/c mice were formed: one control (n = 5), and the other (n = 5) treated with a high-fructose diet (30% fructose in drinking water). After 2 months, the animals were sacrificed, and blood and bile samples were obtained. We determined serum glucose and the corresponding lipid profiles. In bile samples, cholesterol and phospholipids levels were analyzed, and cholesterol transporters (vesicles and micelles) were separated by gel filtration chromatography.

Results: Treated animals showed: 1) no change in body weight (control: 31.4 ± 1.4 g vs. treated: 32.5 ± 2.6 g); 2) increase in glycemia (control: 90±8 mg/dl vs. treated: 135 ± 18 mg/dl; p < 0.001); 3) no alteration in serum triglycerides (control: 423 ± 186 mg/dl vs. treated: 341 ± 77 mg/dl); 4) no change in serum total cholesterol (control: 81 ± 11 mg/dl vs. treated: 99 ± 26 mg/dl); 5) no change in HDL-cholesterol and LDL-cholesterol; 6) no alteration in biliary lipids (cholesterol control: 3.6 mM, cholesterol treated: 5.3 mM and phospholipids control: 33.9 mM, phospholipids treated: 40.8 mM); 7) no change in vesicular and micellar phospholipids.

Conclusions: A high-fructose diet increase only the glycemia, without altering serum or bile lipids. We did not observe changes in either biliary lipid concentrations or biliary cholesterol transporters. We conclude that fructose apparently does not alter the gallstone formation process in our experimental model.
Role of the bile acid receptor TGR5 (GPBAR1) in cholangiocarcinoma (CCA)

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Introduction: The membrane bound G-protein coupled bile acid receptor TGR5 (GPBAR1) is expressed in epithelial cells of the liver and was found to be overexpressed in human cholangiocarcinoma (CCA) and cell lines generated from CCAs. Especially secondary bile acids (BAs) play a role in the development of different malignant tumors in the gastrointestinal tract, including liver. TGR5 activation triggers secretion, proliferation and anti-apoptotic effects in normal cholangiocytes and CCA cell lines. Whether TGR5 activation also promotes invasiveness and metastasis development of CCA is unclear.

Methods: Crispr/Cas9 mediated TGR5 knockout was achieved in the human CCA cell line TFK-1 using sgRNAs that specifically mutate the transmembrane domain 3 (TMD3). Cells were transfected via nucleofection while empty vector served as control. Puromycin selected clonal cells were analyzed for their TGR5 genotype and phenotype by Sanger sequencing and immunofluorescence staining, and the generated TGR5 variant was analyzed by homology modelling. Proliferation, migration and invasiveness were studied in response to bile acid stimulation using BrdU-incorporation as well as transwell cell migration and invasion assays.

Results: Using Crispr/Cas9 technique, we generated a TGR5 deletion variant delta89-110, lacking 57 bp within TMD3. While the mutated TGR5 protein was still detected in the plasma membrane, bile acid and agonist induced cell proliferation and migration was completely abolished in TGR5delta89-110 expressing cells. Modelling of the truncated receptor revealed obstruction of the receptor binding pocket by the shortened TMD3, rendering receptor activation impossible.

Conclusion: Together, these data indicate that TMD3 is essential for BA mediated TGR5 activation. Furthermore, TGR5delta89-110 expressing CCA cells were resistant to BA induced cell proliferation, migration and invasion, underscoring the role for TGR5 in CCA progression.
Whole genome sequencing of 278 patients with intrahepatic cholestasis of pregnancy: initial findings

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Introduction: The rapid decrease in sequencing costs has led to a position where it is possible to sequence and analyse whole genomes both in research and diagnostic settings. The NIHR Bioresource Rare Diseases project was established to pilot the feasibility of routine, nation-wide genomic testing. The cohorts of 15 rare diseases included severe, early-onset cholestasis of pregnancy (ICP), defined as onset of disease before 32 weeks and maternal serum bile acids > 40 µmol/l.

Methods: Following recruitment, whole blood samples were sent for processing to the Cambridge translational genomics lab for DNA extraction, quantification and quality control (QC). Samples passing QC were submitted to Illumina for whole genome sequencing (WGS) to a clinical standard (30x coverage), and variant calling.

Results: To date, 278 whole genome sequences (c. 3 x 10⁹ base pairs per individual) have been returned. Initial analysis consisted in identifying pathogenic or likely pathogenic variants. Variants were prioritised based on their minor allele frequency (< 3% in control populations), consequence (splice region, high or moderate effect), presence in Human Gene Mutation Database (HGMD), and location in the biliary transporter genes ABCB4 and ABCB11, known to cause ICP. Each of the resulting 117 variants, in 93 individuals, were discussed in a Multi-Disciplinary Team meeting in the context of the patients’ phenotype. In the end, 39 variants, in as many individuals, were deemed causal; 54% of which have never been described. Interestingly, some variants reported in the literature as pathogenic or likely pathogenic in ICP were discarded as too frequent in the control populations.

Discussion/Conclusion: The NIHR Bioresource project has demonstrated the feasibility of routine whole genome sequencing as part of clinical care pathways in ICP. However, approximately 80% of the ICP cases remained unexplained, showing there is still more to discover, and this will be better achieved by WGS.
Inhibition of hepatic bile acid uptake improves obesity-related metabolic dysfunctions in mice

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Introduction: Known for their role in fat absorption, bile acids are now also recognized as signalling molecules that regulate glucose metabolism, inflammation, and energy expenditure via activation of their receptors FXR and TGR5. Hepatic uptake of bile acids is mediated by the sodium taurocholate co-transporting polypeptide (NTCP) and members of the organic anion-transporting polypeptide (OATP) family. Here, we propose that (partially) inhibiting hepatic bile acid transport delays hepatic clearance of bile acids from portal and peripheral blood, thereby prolonging bile acid signalling and ameliorate obesity-related metabolic dysfunctions.

Methods: Effects of prolonged bile acid signalling on metabolic parameters were studied in diet-induced-obesity NTCP KO mice and by treating OATP1a/1b KO (+hOATP1B1) mice with Myrcludex B, a pharmacological inhibitor of NTCP. Furthermore, the SGBS pre-adipocytes and the GLP-1 secreting GLUTag cell line were used to study bile acid signalling in vitro.

Results: NTCP KO mice were partially protected to deleterious effects of a high fat diet with 26 ± 15% reduced body weight gain, less liver steatosis, and smaller adipose tissue deposits. Indirect calorimetry revealed increased energy expenditure linked to brown adipose tissue thermogenesis. Food intake and locomotor activity were unchanged. Myrcludex B treatment of OATP1a/1b KO mice induced rapid reduction of body weight (16 ± 2% within 3 weeks), increased body temperature and mice had higher fasting serum GLP-1 levels (increase of 9.74 pM, p = 0.003). The increase in serum GLP-1 is likely explained by direct TGR5 activation in L-cells, as both the bile acid TCDCA and the TGR5 agonist TC-G 1005 increased GLP-1 secretion respectively 2- or 6-fold in GLUTag cells. Furthermore, TCDCA and TC-G 1005, but not FXR agonist GW4064, amplified mitochondrial respiratory uncoupling in SGBS pre-adipocytes, linked to increased UCP1 and PGC1α mRNA expression.

Discussion/Conclusion: Targeting hepatic bile acid uptake provides a novel strategy to pharmacologically exploit increased bile acid signalling to treat obesity-related metabolic disorders.
The influence of gut microbiota-mediated bile acid metabolism on the transport of P-glycoprotein substrates across the intestinal epithelium

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Introduction: To date, pharmacokinetic investigations at the host-microbe interface have primarily considered effects on drug metabolism \cite{1, 2}. Microbial bile acid (BA) metabolism, deconjugation and dehydroxylation of the steroid nucleus by the gut bacteria, has been shown to impact BA solubilization capacity for poorly water-soluble drugs and hence may affect drug absorption \cite{3}. Previous work involving germ-free and conventionalized mice (each group possessing distinct BA signatures) identified altered transcriptional expression of genes encoding intestinal transporters involved in lipid translocation \cite{4}. This work investigated possible effects on intestinal drug transporter functioning \textit{in vitro}.

Methods: The impact of the conjugation and hydroxylation state of the BA nucleus on the gene expression of common efflux transporters (including ABCB1, P-glycoprotein) in Caco-2 and T84 cells was assessed. The ability of host (conjugated) and microbial (deconjugated/dehydroxylated) BAs to affect drug uptake was investigated using the P-glycoprotein substrates, cyclosporine A (CsA) and rhodamine 6G. CsA treatment was associated with a concentration-dependent decrease in cell viability; hence, altered cell viability was used as a marker of BA-modulated CsA uptake. Potential mechanisms by which BAs could affect P-glycoprotein functioning were evaluated using ATPase and bidirectional transport assays.

Results: Unconjugated BAs significantly augmented CsA toxicity and reduced Rhodamine 6G efflux, compared to their conjugated counterparts (p < 0.05). These effects could not be explained by changes to ABCB1 mRNA transcripts. BAs were determined to inhibit, rather than stimulate, basal P-glycoprotein ATPase activity suggesting a non-competitive interaction with the protein.

Discussion/Conclusion: Microbial BA metabolism was shown to affect the uptake/activity of efflux transporter substrates. The physicochemical properties of unconjugated BAs, including their capacity for passive non-ionic diffusion, likely underpins their preferential attenuation of P-glycoprotein-mediated efflux.
References:


Neutraceutical targeting of the bile acid receptor, farnesoid X receptor, for intestinal disease

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Introduction: Dysregulated bile acid metabolism has been linked to the pathogenesis of intestinal disorders, including inflammatory bowel disease (IBD) and cancer. The nuclear bile acid receptor, farnesoid X receptor (FXR), represents a promising target for treatment of these diseases. Previous studies suggest that a class of plant-derived phytochemicals are modulators of FXR activity. The aim of this study was to investigate the effects of a lead natural compound, denoted KFS1, on FXR activation in colonic epithelial cells.

Methods: Polarised monolayers of T84 colonic epithelial cells were treated bilaterally with the FXR agonist, GW4064 (5 µM) and KFS1 (1–100 µM) for 24 h. Expression of FXR and FGF-19 were measured by RT-qPCR. Transepithelial electrical resistance (TEER) was measured using the EVOM² Voltohmmeter.

Results: GW4064 (5 µM) induced an 1100 ± 457 fold increase in FGF-19 mRNA expression in T84 cells (n = 22, p < 0.05). At 50 µM and 100 µM, KFS1 increased levels of FGF-19 expression by 218.28 ± 196.81 and 93.66 ± 75.16 fold, respectively (n = 3). KFS1 also reduced TEER by 60.24% ± 5.02% and 64.35% ± 6.18%, respectively (n = 7), suggesting it exerts toxic actions at these concentrations. Interestingly, at lower concentrations that did not alter TEER, KFS1, but not GW4064, increased FXR mRNA expression. KFS1 (5 µM and 10 µM) increased FXR expression by 4.16 ± 0.37 fold and 3.28 ± 0.35 fold, respectively (p < 0.05; n = 4). Moreover, treatment with KFS1 (1, 5, or 10 µM) for 1 h prior to treatment with GW4064 potentiated GW4064-induced FGF-19 expression by 2.5 ± 0.76 fold; 2.86 ± 0.21 fold; p < 0.01, and 4.64 ± 0.5 fold; p < 0.01, respectively (n = 6).

Conclusion: Our data suggest that KFS1 modulates FXR signalling in colonic epithelial cells. At high concentrations, KFS1 may act as an FXR agonist, which appears to induce cellular toxicity. At lower concentrations, KFS1 increases FXR expression, which may prime cells for agonist-induced FXR activation. These findings suggest that foods, or food supplements, rich in these plant-derived phytochemicals have potential for development as FXR-targeted neutraceuticals.
What is the cost of delayed diagnosis of bile acid malabsorption?

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Introduction: Bile acid malabsorption (BAM) is accurately diagnosed using a ⁷⁵selenium taurocholic acid (SeHCAT) scan which also defines treatments patients require. BAM can cause chronic, often debilitating symptoms including loose stool, faecal incontinence and abdominal pain. Primary BAM affects 1% of Britons yet is frequently misdiagnosed as IBS. A further 1% have BAM secondary to other conditions. The 2012 NICE DG7 review of SeHCAT included a cost-effectiveness evaluation based on assumptions without supporting evidence. This study is to evaluate the cost of delayed diagnosis of BAM.

Methods: The notes of all patients undergoing SeHCAT scanning in our Trust over a one-year period were reviewed retrospectively. The number of abnormal scans and patient response to treatment were recorded. Costs of additional clinics/tests/procedures performed before the diagnosis of BAM were calculated using NICE costing templates.

Results: 19 men and 37 women were referred for SeHCAT scanning between June 2016–May 2017. Of these, 64% were abnormal: 13 demonstrated severe (< 5% 7-day SeHCAT retention), 13 moderate (5–10%), 5 mild (10–15%) and 5 borderline (15–20%) BAM. If SeHCAT scanning was ordered at first consultation (n = 11), patients reported 24 months (median) of symptoms (range 6–360) and the mean diagnostic package of care cost was €1020.80. If the SeHCAT scan booked 2nd line or later (n = 25), patients reported symptoms for 30 months (median, range 0.5–360) and mean cost was €1660.78.

Discussion/Conclusion: Late diagnosis of BAM is associated with markedly increased costs, unnecessary demands for other services and treatment delay for patients. National data on SeHCAT usage suggest that our findings will apply to most other Trusts.
Chronic ursodeoxycholic acid treatment protects against acute ischaemia-induced arrhythmias and improves conduction velocity in adult hearts

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Introduction: Intrahepatic cholestasis of pregnancy (ICP) is associated with an increased incidence of stillbirths, attributed to fetal arrhythmias. Ursodeoxycholic acid (UDCA) is currently used to treat ICP, and has been shown to be antiarrhythmic in the fetal hearts. In the adult myocardium, acute Ischaemia-Reperfusion (IR) can lead to potentially lethal arrhythmias. The effect of UDCA against ischaemia-induced and reperfusion-induced arrhythmias was investigated.

Methods: Experiments were conducted on adult male Sprague-Dawley rats (250–300 g), divided into 3 groups: control (n = 8), acute UDCA treatment (1 µM perfusion; n = 8), and chronic UDCA treatment (two weeks pre-treatment with 150 mg/kg/daily plus 1 µM perfusion; n = 8). Hearts were explanted and Langendorff-perfused. After equilibration, regional ischemia was induced by ligation of the left anterior descending (LAD) artery for 10 minutes, followed by reperfusion. Arrhythmia incidence was quantified in accordance with Lambeth Convention guidelines. Optical mapping of transmembrane voltage was also performed to compare conduction velocity (CV) and action potential duration (APD) before and after UDCA perfusion.

Results: Chronic UDCA administration reduced the incidence of acute ischemia-induced arrhythmias (p = 0.03), and the total number of ventricular ectopic beats during the ischemic phase compared with control (10 ± 3 vs. 52 ± 16, p = 0.04). No antiarrhythmic effect was observed in the acute UDCA administration group. Neither acute nor chronic UDCA treatment altered the incidence of reperfusion arrhythmias. UDCA treatment improved CV (Cycle length (CL) 150 ms: 46 m/s vs. 39 ± 1 m/s, p = 0.004; CL 130 ms 45 ± 1 m/s vs. 39 ± 1 m/s, p = 0.02), with no effect on APDs.

Conclusion: Chronic UDCA treatment reduced the incidence of acute-ischaemia induced arrhythmias. Acute UDCA treatment only conferred no anti-arrhythmic protection. The anti-arrhythmic mechanism of UDCA, which remains still unclear, may be partially mediated by an improvement in CV. Chronic UDCA administration may represent a potential novel antiarrhythmic strategy against ischaemia-induced arrhythmias.
Cross-species molecular imaging of bile salts and lipids in liver: Identification of molecular markers of structural elements of the mammalian liver

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Introduction: The liver is the primary organ involved in handling of bile salts, a class of amphipathic molecules with beneficial signaling activities as well as detrimental detergent action. Here we studied the spatial distribution of bile salt species in the liver. We furthermore aimed to identify specific lipid markers that define the structural elements of the liver.

Methods: Matrix-assisted laser desorption/ionization-mass spectrometry imaging (MALDI-MSI) was used to monitor the spatial distribution of bile salts and lipids in liver sections of rat, dog, and patients with unaffected and cholestatic parenchyma (primary sclerosing cholangitis, PSC).

Results: MALDI-MSI in negative ion mode showed the local presence of a variety of (keto) bile salts, predominantly taurine-conjugates, as localized patches of varying sizes (representing the bile ducts) throughout the liver tissue. Specific molecular markers were identified for the connective tissue (a phosphatidic acid), the liver parenchyma (a phosphatidylinositol), and the bile ducts (a hydroxyl-sulfatide [ST-OH]). The sulfatide (m/z 906.6339) was found to be uniquely localized in a thin lining on the inside of the bile duct, co-localized with cytokeratins, and encased luminal bile salts.

A similar distribution of aforementioned sulfatide was observed, albeit in constricted ductular structures, in the liver of a patient with PSC and a mild clinical phenotype. In contrast, sulfatides were virtually absent in the liver of a patient with advanced PSC, with (atypical) bile salts abundant in the extra-ductular space. The latter is reminiscent of regurgitation of bile salts from leaky bile ducts into the portal tract, which was observed in an animal model of PSC.

Discussion/Conclusion: The distinct structural elements of the mammalian liver are characterized by classes of specific lipids. We propose that (hydroxyl-)sulfatides are specific molecular markers of the bile duct. The identified atypical cholanoids may have value in monitoring PSC progression.
N-(4-[\textsuperscript{18}F]fluorobenzyl)cholylglycine, a novel tracer for positron emission tomography of enterohepatic circulation of bile acids: Proof-of-concept study in rats

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**Introduction:** Enterohepatic circulation (EHC) is an important physiological process for bile acids to function as detergents and signal carriers, and for regulation of their intracellular concentrations. We hypothesized that N-(4-[\textsuperscript{18}F]fluorobenzyl)cholylglycine ([\textsuperscript{18}F]FBCGly) would be a suitable tracer for positron emission tomography (PET) of the EHC of conjugated bile acids, and we report here a proof-of-concept study in rats.

**Methods:** [\textsuperscript{18}F]FBCGly was investigated \textit{in vivo} by dynamic PET/MR in anaesthetized rats (Sprague-Dawley). The rats were untreated or treated with endogenous cholyltaurine or the Bsep-inhibitor rifampicin. Possible \textit{in vivo} metabolites of [\textsuperscript{18}F]FBCGly was investigated in plasma and bile samples, and the stability of [\textsuperscript{18}F]FBCGly towards enzymatic de-conjugation by cholylglycine hydrolase was tested \textit{in vitro}.

**Results:** PET/MR studies showed that \textit{i.v.} injected [\textsuperscript{18}F]FBCGly was rapidly taken up by the liver, secreted into bile, and underwent EHC within 40–60 min after administration. Cholyltaurine and rifampicin both inhibited the hepatobiliary secretion of [\textsuperscript{18}F]FBCGly. Cholyltaurine also inhibited the EHC of [\textsuperscript{18}F]FBCGly. No fluorine-18 labelled metabolites of [\textsuperscript{18}F]FBCGly were observed.

**Discussion/Conclusion:** We have developed a radiosynthesis of [\textsuperscript{18}F]FBCGly and shown by PET/MR that [\textsuperscript{18}F]FBCGly undergoes EHC in rats without metabolizing. Inhibition studies suggest that the Bsep is likely to be a major canalicular transporter of [\textsuperscript{18}F]FBCGly in hepatocytes. To the best of our knowledge, [\textsuperscript{18}F]FBCGly is the first PET-tracer shown to undergo EHC. This novel tracer may prove useful for PET studies on the effect of drugs or diseases on the EHC of conjugated bile acids.
Loss of BSEP/ABCB11 protects MDR2/ABCB4 KO mice from cholestatic liver injury by altering bile acid profile and signaling

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Introduction: Cholestasis is characterized as intrahepatic accumulation of potentially cytotoxic bile acids (BAs) subsequently leading to liver injury reflected by disruption of hepatocellular integrity, inflammation, fibrosis, cirrhosis and increased risk for cancer. Bile salt export pump (Bsep/Abcb11) is the main canalicular BA transporter and rate limiting step for hepatobiliary BA secretion. Here we aim to investigate the role of Bsep in development of liver injury in a mouse model of sclerosing cholangitis – the Mdr2KO mouse.

Methods: To explore the consequences of Bsep loss in a mouse model of sclerosing cholangitis, Mdr2/Bsep double knockout (DKO) mice were generated. WT mice subjected to bile duct ligation (BDL) as well as Mdr2 KO mice were fed with a tetrahydroxylated bile acid (THBA). Gene expression profile of inflammatory and fibrotic markers, serum biochemistry, liver histology, immunohistochemistry (IHC) and serum BA composition were investigated. Amount of RORgt+CD4+CD3+ T cells as well as FOXP3+CD4+CD3+ T cells in livers of Mdr2 KO and DKO mice were assessed by FACS analysis.

Results: In contrast to Mdr2 KO mice, DKO mice were protected against liver and bile duct injury, reflected by serum biochemistry and H&E staining. Gene expression of inflammatory markers F4/80, Tnfa and Mcp1 remained unchanged (compared to WT Ctrls) in DKO mice, while in Mdr2 KO mice these markers were increased (4-fold, 6-fold and 8-fold, respectively; p < 0.05). Fibrosis markers were increased in Mdr2 KO mice (Desmin 9-fold, Col1a1 24-fold; p < 0.05) but remained unchanged in DKO mice. mRNA expression of Cyp3a11 and Cyp2b10, two enzymes involved in BA hydroxylation/detoxification were increased 5-fold and 100-fold in DKO mice, respectively, while Mdr2 KO mice showed unchanged levels. In line, 67% of serum BAs in DKO mice were polyhydroxylated, with tetrahydroxylated BAs being most prominent. In Mdr2 KO mice, polyhydroxylated BAs were completely absent. Within the CD4+CD3+ T cell population about 50% are RORgt+ and 5% FOXP3+ in Mdr2 KO mice versus 10% RORgt+ and 30% FOXP3+ cells in DKO mice. Notably THBA feeding profoundly reduced expression levels inflammatory markers (IL1b and Cxcl1 by 50%) and fibrotic marker (Col1a2 by 80%) in Mdr2 KO. In line, THBA feeding improved inflammation in WT BDL mice, reflected by reduced number of F4/80 positive cells and improved gene-expression profile (F4/80 by 50%, Cxcl1 by 55% and Cxcl2 by 75%; p < 0.05) compared to WT BDL mice.
**Discussion/Conclusion:** Loss of Bsep results in increased expression/activity of enzymes involved in BA hydroxylation/detoxification, thereby modifying CD4+CD3+ T cell population, thus protecting Mdr2 KO mice from development of cholestatic liver disease. Therefore, THBA may be a new potential treatment strategy for cholestatic liver diseases.
Serum biomarker development demonstrating the transformation of fatty liver to steatohepatitis in association with diabetes mellitus

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Introduction: There are currently no “liver-specific” non-invasive prognostic markers of non-alcoholic steatohepatitis (NASH) capable of showing ‘early’ inflammation prior to or with inflammatory cell infiltrate. Using 2 steatosis mouse models, a human correlate, and hepatocyte culture, we offer a proof-of-concept for detection of an increase in oxysterols as liver specific non-invasive predictors of CYP7B1 suppression driven by early inflammation. Also, we demonstrate a connection to diabetes mellitus; major risk factor for progression of NAFLD.

Methods: B6/129 (NAFL model) and C57Bl/6 mice were fed a WD or high fructose diet for 2–6 weeks to achieve early fatty liver. Mouse and human non-alcoholic steatosis liver Cyp7b1 mRNA, oxysterol, inflammatory marker levels, and liver histology were determined.

Results: The 2-week WD feeding of mice led to a 69% repression of Cyp7b1 mRNA levels coupled to increases in (25R)-26-hydroxycholesterol and biochemical evidence of early inflammation (increased IL-1β/ALT). By 6 weeks, Cyp7b1 suppression persisted with WD feeding, with further increases in oxysterol/ALT levels and visible inflammatory infiltrates. The 32-week WD feeding led to overt inflammatory cell infiltrate. Similarly, CYP7B1 mRNA levels in human non-alcoholic steatosis were repressed (162%; n = 3) as compared to healthy livers; and associated with a correlative oxysterol increase. 4-week of WD feeding or high fructose diet led to > 60% (p < 0.001) repression of Cyp7b1 mRNA levels. cAMP, a product of glucagon stimulation, down-regulated Cyp7b1 in isolated primary hepatocytes.

Discussion/Conclusion: Human CYP7B1 deficiency increases hepatic (25R)-26-hydroxycholesterol and causes inflammation-driven fibrosis within first year-of-life. NAFLD takes similar course to this disease progression. With diabetes mellitus, paradoxical elevation of glucagon occurs. We offer evidence that hyperglucagonemic repression of CYP7B1 leads to persistently elevated oxysterol levels which drive hepatic inflammation. Oxysterol measurement in serum, in the presence of increased inflammatory markers, could serve as non-invasive markers of early inflammation.
Circulating fibroblast growth factor 21 is increased during cholestasis and correlates with hepatic expression of genes involved in regulating bile acid homeostasis

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Introduction: Fibroblast growth factor 21 (FGF21) is a member of the fibroblast growth factor family of signaling molecules. Secreted by the liver and adipose tissue, it is involved in modulating glucose and lipid metabolism. In vitro, FGF21 expression and secretion are induced by activation of either Farnesoid X Receptor (FXR) or the Peroxisome proliferator-activated receptor α. In humans, little is known on the cross talk FGF21 and bile acids. Aim of the present study was to investigate the changes induced by obstructive cholestasis on FGF21 homeostasis.

Methods: Nine subjects with obstructive cholestasis undergoing abdominal surgery were investigated, and compared with 24 non-cholestatic controls. Liver biopsies and serum samples were collected. Expression of the main nuclear receptors involved in transcriptional regulation of bile acid synthesis/transport and of biliary transporters was analysed by real time RT-PCR. Circulating levels of FGF21 were measure with commercial ELISA kit.

Results: Circulating levels of FGF21 were significantly increased in cholestatic patients (p < 0.05) but no differences were detected regarding its hepatic mRNA expression between the two groups. In the liver, expression of cholesterol 7alpha-hydroxylase (CYP7A1), the limiting enzyme of bile acid synthesis, was significantly reduced during cholestasis, together with an increased mRNA expression (p < 0.01) of the short heterodimer partner (SHP), a target of the bile acid receptor FXR. Expression of genes coding for canalicular biliary transporters, such as ABCB4 and ATP8B1 (p < 0.05) were also increased. Circulating levels of FGF21 directly correlated with hepatic FXR and SHP expression and inversely with CYP7A1 expression.

Discussion/Conclusion: In human obstructive cholestasis, circulating FGF21 is markedly increased and correlates with hepatic expression of nuclear receptors and hepatobiliary transporters involved in bile acids metabolism. Such findings provide insight into a cross talk FGF21/bile acids, suggesting FGF21 as possible circulating marker for hepatic bile acids homeostasis.
Low-dose ursodeoxycholic acid in association with low caloric diet in the long-term treatment of non-alcoholic steatohepatitis in obese patients

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Introduction: In this study we assessed comparatively the effects of UDCA monotherapy, simvastatinum and combination between UDCA and vitamin E in the treatment of NASH.

Methods: We studied 53 patients with NASH and obesity. We excluded patients with viral or autoimmune hepatitis, diabetes mellitus or drug abuse. Liver biopsy was performed before and after therapy. We evaluated liver function tests, serum lipids and BMI at the beginning of therapy, after 6 and 12 months. The A group composed of 18 normolipidemic cases, treated with UDCA 13–15 mg/kg/day, B group consist of 15 hyperlipidemic cases which received simvastatin 20 mg/day and C group (20 patients) with UDCA and vitamin E (400 IU twice a day) therapy.

Results: A number of 39 patients had elevated serum aminotransferase level, but 14 had normal values. In B group, lipide profile was: 7 cases with hypercholesterolemia, 4 cases with hypertriglyceridemia and 4 with both. In the A group, mean value of serum ALT-level was decreased from 88.3 ± 21.7 U/l at baseline, to 52.12 ± 17.5 U/l at 6 months. In B group, serum ALT was reduced (in mean with 19.3 ± 7.2 U/l) after 6 months and cholesterolemia was significantly improved in 8 cases (72.7%). In two cases we increased simvastatinum dose at 40 mg/day. In C group mean ALT and AST levels was more decreased: in mean with 49.3 ± 5.2 U/l. After one year, aminotransferase levels reach normal range only in C group. Comparatively, in A and B groups the normalisation rates of ALT was lower (89.7% and 73.33%). The association between UDCA and vitamin E had a significant and positive effect in the improvement of steatosis, lobular inflammation and fibrosis. After 12 months the rate of the improvement of steatosis grade was significantly better in C group: 73.3% in A group, 58.7% in B group and 89.1% in the C group. We could not establish a correlation between the values of serum aminotransferases and other parameters, but multivariate analysis showed that the BMI > 28 kg/m² and elevation of serum ALT were associated with steatosis grade. Patients which associated combined therapy with change of the lifestyle and low caloric diet had a good and rapid response.

Discussion/Conclusion: The combination between UDCA and vitamin E significantly improves liver function tests and steatosis grade in long-term therapy and is very well tolerated. The combined therapy and low caloric diet still remains first line therapy in patients with NASH and obesity.
Combined budesonide-UDCA therapy versus UDCA mono-therapy in the treatment of the primary biliary cholangitis

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Introduction: We assessed the efficacy and monitored the incidences of the side-effects of two years combined therapy (UDCA plus budesonide) comparative with UDCA alone therapy, in patients with primary biliary cirrhosis (PBC).

Methods: This comparative study was performed on 33 patients with PBC, from stages I to III, structured in two groups: the A group consist of 20 patients which received UDCA (10–15 mg/kg/day) and the B group composed of 13 patients treated with UDCA and budesonide (9 mg daily divided in 3 doses). We evaluated liver histology, serum levels of aminotransferase, Bb, AP at 6, 12 and 24 months. Also, we monitored the titers of antimitochondrial antibodies (AMAs), immunoglobulin M and bone mineral density (BMD).

Results: In the A group, clinical symptoms significant improved in 25% of cases after 6 month, in 60.7% after 12 months and 89.28% after 24 months. The mean value of serum bilirubin concentration was reduced from 6.7 ± 2.5 mg%, at baseline, to 2.8 ± 1.3 mg% at 6 months and to 1.7 ± 0.7 mg% at 12 months. Aminotransferases values were reduced more quickly comparative with bilirubin and AP levels: with 44.6% at 6 months and 63.2% at 12 months. In B group, aminotransferase values reduced more slowly, but significant decrease AP activity after one year (p = 0.001). The Ig M level decreased in both group, but the AMAs titer did not change. Changes in BMD on the femoral neck and lumbar spine after 2 years were significantly observed in B group.Inflammatory activity was significantly reduced in the combined therapy (6 cases, 46.15%) and in 4 cases (20%) with mono-therapy. Fibrosis decreased in group B in 5 cases, but in the A group only in one case. After 24 months, histological stage of disease improved only in B group (3 cases). In the A group, we observed side-effects at one patient (diarrhoea) and in B group two patients presented hyperglycemia, two mild hirsutism and 4 osteoporosis.

Discussion/Conclusion: Combined therapy with UDCA and budesonide improved liver histology and liver enzymes, where as the effect of UDCA monotherapy was mainly on liver function tests.
Testosterone reduces circulating PCSK9 but does not influence cholesterol or bile acid synthesis in healthy males

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Introduction: Human cholesterol metabolism is influenced by age and gender. LDL cholesterol increases with age, and is lower in premenopausal females than in males, while HDL cholesterol is increased by estrogen. The synthesis of cholesterol is higher in males, as are bile acid synthesis and pool size. Estrogen stimulates hepatic LDL receptors, and appears to lower circulating PCSK9. The role of testosterone for these gender differences is less clear.

We tested the hypothesis that increased levels of testosterone will increase bile acid and cholesterol syntheses and PCSK9 levels in normal males.

Methods: Serum lipoproteins, PCSK9, lathosterol (marker of cholesterol synthesis), 7α-hydroxy-4-cholesten-3-one (C4; marker of bile acid synthesis), fibroblast growth factor 19 (FGF19) and individual serum bile acids were measured in 25 male volunteers before and after treatment with 250 and 500 mg of testosterone.

Results: Testosterone treatment resulted in a dose-related reduction in circulating PCSK9, but had no effects on lathosterol, C4, individual serum bile acids or FGF19 levels. Increased VLDL and lowered LDL and HDL cholesterol levels were seen 14 days after treatment.

Discussion/Conclusion: Our results indicate that testosterone reduces PCSK9 levels, further supporting that this modulator of LDL receptors is under hormonal control. However, differences in testosterone are unlikely to explain known gender differences in bile acid and cholesterol synthesis.
T cell-mediated cholangitis alters bile acid metabolism

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Introduction: It is becoming increasingly clear that lipid metabolites such as bile acids affect lymphocyte function. It is unknown whether lymphocytes in turn are able to regulate bile acid metabolism. Both mechanisms could contribute to cholestatic liver inflammation. We therefore aimed to investigate the effect of T cells causing antigen dependent cholangitis on bile acid metabolism in a newly developed mouse model of cholangitis.

Methods: Mdr2ko mice were crossed with transgenic K14-OVAp mice, which express an MHC class I restricted ovalbumin peptide on biliary epithelial cells (Mdr2xK14-OVAp). Antigen dependent T cell damage was induced by the adoptive transfer of T-cell receptor transgenic OT-1 CD8-positive T cells. Bile acid metabolism was assessed by quantification of bile acids in liver and serum using a targeted liquid chromatography – mass spectrometry based approach and by analyzing the expression of rate limiting enzymes and bile acid transporters.

Results: Transfer of antigen specific CD8-positive T cells into Mdr2xK14-OVAp mice led to a significant increase in serum ALT levels and severe peribiliary inflammation. Liver infiltrating T cells produced increased levels of the proinflammatory cytokines IFN gamma and TNF alpha. T cell mediated cholangitis resulted in significantly increased levels of total conjugated bile acids in serum and livers of recipient mice, whereas unconjugated bile acid levels were reduced. In the liver, T-cell transfer led to a significant downregulation of genes responsible for bile acid synthesis and uptake and an increased expression of the bile salt export pump. CD8-positive T cells alone were able to induce these changes, as demonstrated in Mdr2xK14-OVAp recipient mice on the RAG1 knockout background. This effect was at least in part mediated by the proinflammatory cytokines IFNgamma and TNF.

Discussion/Conclusion: By using a novel mouse model which combines antigen dependent T cell and bile acid induced liver injury, we demonstrate that CD8-positive T cell suffice to significantly downregulate bile acid metabolism. Understanding this interplay may have implications for designing combined treatment strategies for cholestatic liver diseases.
Intestinal and liver crosstalk in control of cholesterol homeostasis by FXR

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Introduction: Bile acids influence cholesterol homeostasis at multiple sites. They are essential for intestinal cholesterol absorption, regulate cholesterol synthesis and control biliary as well as transintestinal cholesterol excretion. In this study we investigated the specific roles of the intestinal and the hepatic bile acid receptor FXR in regulation of cholesterol homeostasis.

Methods: C57Bl/6J WT and a panel of FXR transgenic mice as well as Abcg8 knock-out mice were fed chow diet with or without the FXR agonist PX20606 (10 mg/kg/day) for two weeks. Bile acid and cholesterol fluxes were determined using stable isotope based technology. Lipid parameters were determined in blood and feces.

Results: PX20606 (PX) treatment of WT mice decreased plasma cholesterol and triglyceride by 40% and increased fecal neutral sterol excretion (FNS) tenfold. Both plasma and fecal effects were abrogated in whole body FXR⁻/⁻ mice. The increase in FNS and decrease in plasma lipids was also abrogated in intestinal specific FXR⁻/⁻ mice, suggesting the essential role of intestinal FXR activation for these effects. In support of this indication, the PX-induced increase in FNS excretion could be rescued in intestinal FXR transgenic mice on a FXR-deficient background. Interestingly, in these mice no significant effect on plasma lipids was observed indicating that activation of hepatic FXR was required to decrease plasma cholesterol and triglyceride. Uncoupling of PX effects on FNS and plasma lipids was also observed in Abcg8⁻/⁻ mice. The effect of PX on FNS was strongly inhibited in these mice yet plasma cholesterol decreased substantially upon PX treatment.

Discussion/Conclusion: Intestinal and hepatic FXR control cholesterol fluxes in a complex manner. Intestinal FXR drives fecal neutral sterol excretion while hepatic and intestinal FXR act in concert to control plasma cholesterol and triglyceride concentration.
A novel fibroblast growth factor 15-dependent and bile acid-independent promotion of liver regeneration in mice

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Introduction: The role of intestine-derived factors in promoting liver regeneration after partial hepatectomy (PHx) are not clear, but bile acids (BAs) and fibroblast growth factor 15 (Fgf15) that is highly expressed in the ileum have been proposed to promote hepatocyte proliferation. Fgf15 strongly suppresses the synthesis of BAs and emerging evidence gathered from our and other groups indicate that Fgf15 is important for liver regeneration. Although the mechanisms by which Fgf15 promotes liver regeneration are unclear, Fgf15 may do so by reducing BA levels, and/or by directly promoting cell proliferation. However, it's unknown whether these two mechanisms are independent or integrated.

Methods: We have generated a new line of Fgf15 tet-off, transgenic mice (Fgf15 Tg) that had Fgf15 overexpression but very low BA levels resulted from Fgf15-mediated suppression of BA synthesis. Furthermore, we have overexpressed Fgf15 by AAV-fgf15 transduction and by treatment with recombinant Fgf15 protein. These animal models were used to study the role of Fgf15 in liver regeneration following PHx. The degree of cell proliferation and liver regeneration were determined by standard molecular biological technologies.

Results: Overexpression of Fgf15 promote cell proliferation. Furthermore, overexpression of Fgf15 led to an earlier and greater activation of MAPK, Stat3, and NF-κB signalling pathways, minutes after the PHx.

Discussion/Conclusion: Direct in-vivo evidence is provided that Fgf15 is critical in stimulating the priming phase of liver regeneration via activating early signalling pathways that are critical for cell survival, independent of BA levels.
Increased risk of adverse pregnancy outcomes in gestational diabetes mellitus complicated by intrahepatic cholestasis of pregnancy

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Introduction and aim: To compare maternal and perinatal outcomes of pregnancies associated with both intrahepatic cholestasis of pregnancy (ICP) and gestational diabetes mellitus (GDM) with those with ICP alone, recent studies having identified a link between the two.

Methods: A retrospective cohort review was conducted of all pregnancies associated with a diagnosis of ICP (definition: pruritus with serum bile acids ≥ 10 µmol/l) managed in public clinics at two main teaching hospitals in South Australia between 2001 and 2010, with information about perinatal outcomes and associated pregnancy comorbidities. After detailed case-note review, the cohort was divided into two based on whether there was also a diagnosis of GDM during the pregnancy.

Results: 315 women (353 pregnancies) were identified with ICP during the 10-year period: incidence 0.7% pa. 308 pregnancies were affected by ICP only, and 45 (12.7%) were affected by ICP and GDM (odds ratio 3.06, 95% confidence interval 2.23–4.18). Key findings: women with ICP and GDM had a greater BMI (p < 0.001), were diagnosed with ICP at an earlier gestational age (p < 0.001) than the ICP-only cohort, and tended to have more severe ICP (p = 0.08). Babies were also born earlier to ICP and GDM women (p = 0.008), more often following obstetric intervention than spontaneous birth, and had more perinatal complications, with more feeding difficulties (p = 0.03), more need for mechanical ventilation (p = 0.002) and more admissions to special care (p = 0.03), as well as a tendency to increased birthweight (p = 0.099): there were no stillbirths.

Discussion/Conclusion: This is the first study to assess outcomes in mothers and offspring affected by both ICP and GDM, compared with those affected by ICP only, and shows an increased risk of perinatal complications. Further studies are needed to elucidate the relationship between the two conditions, and to consider whether earlier screening for GDM is warranted in this population.
The number needed to treat with ursodeoxycholic acid to prevent one liver transplantation or death in patients with primary biliary cholangitis varies between subgroups

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Introduction: No previous studies have evaluated the absolute risk reduction of death or liver transplantation (LT) associated with ursodeoxycholic acid (UDCA) treatment in primary biliary cholangitis (PBC). We therefore aimed to assess the number needed to treat with UDCA to prevent one LT or death within 5 years (NNT5y).

Methods: Long-term follow-up data of UDCA-treated and untreated patients included in the Global PBC Study group were used. Both the cumulative risk of LT/death in untreated patients as well as the hazard ratio (HR) of UDCA on LT-free survival was assessed using Cox regression analyses, adjusting for baseline differences with inverse probability of treatment weighting (IPTW), overall as well as in subgroups. The NNT was estimated as follows; NNT = (1/(LT-free survivaluntreated[t]HRUDCA)-(LT-free survivaluntreated[t])).
Results: We included 3902 patients (90% UDCA-treated, 91% female, mean age 54 ± 11.9). The cumulative IPTW-adjusted 5-year LT-free survival without UDCA was 86% (95% CI: 84–87), and the adjusted HR of UDCA for LT/death was 0.46 (95% CI: 0.40–0.52, p < 0.001). Consequently, the overall NNT5y to prevent one LT/death was 11 (95% CI: 9–13). Despite a significant difference in HR between young (≤ 46 yrs, HR = 0.19, 95% CI: 0.12–0.28) and elderly (> 63 yrs, 0.45, 95% CI 0.33- 0.62) patients, the NNT5y was quite similar (9, 95% CI: 7–14 vs. 14 (95% CI: 9–28), respectively). On the contrary, while the HR was stable over the biochemical stage of disease (0.37 [95% CI: 0.30–0.47], 0.32 [95% CI: 0.25–0.40] and 0.50 [95% CI: 0.37–0.70] in early, intermediate and advanced disease, respectively), the NNT5y was substantially higher among those with early disease (22, 95% CI: 17–32) than among those with intermediate (5, 95% CI: 4–6) or advanced disease (5, 95% CI: 3–8), as a result from differences in the 5-year cumulative incidence of LT/death.

Discussion/Conclusion: In general the NNT5y with UDCA to prevent one LT/death in PBC is low, but varies according to the patients’ characteristics.
Differences in contractile and signalling responses to bile acids and their respective conjugates in neonatal cardiomyocytes: Role of Gi protein, muscarinic receptors and TGR5

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Introduction: In cholestatic disorders deranged bile acid homeostasis results in accumulation of these molecules in the circulation and this affects peripheral organs. In neonatal and fetal hearts elevated bile acids are known to trigger arrhythmia, but there is limited knowledge about the underlying mechanisms. We aimed to delineate mechanisms underlying fetal heart rhythm disturbances in neonatal rat and mouse cardiomyocytes exposed to bile acids.

Methods: The level of cAMP was measured by FRET microscopy in cells isolated from transgenic mice expressing a FRET sensor pEPAC1-cAMPs. The contraction rate of myocytes isolated from wild type and TGR5KO mice was manually recorded. Mitochondrial toxicity was measured using membrane-bound dye. Acute stimulation for 15 minutes with 100 µM bile acids and specific TGR5 agonist, INT-777 was used.

Results: The unconjugated bile acids CDCA, DCA and UDCA and, to a lesser extent, CA were found to elicit substantial cAMP release, whereas all glyco- and tauro-conjugated bile acids were weak in generating cAMP response. The bile acid-induced cAMP production does not lead to an increase in contraction rate, and seems to be mediated by the RI isoform of adenylate cyclase, unlike adrenaline-dependent release which is mediated by the RII isoform. In contrast, bile acids elicited slowing of neonatal cardiomyocyte contraction indicating that other signalling pathways are involved. The conjugated bile acids were found to be partial agonists of the muscarinic M2, but not sphingosin-1-phosphate-2, receptors, and act partially through the Gi pathway.

Discussion/Conclusion: High level of cAMP produced in response to unconjugated bile acids is mainly attributed to TGR5 activation, but does not translate into an elevated contraction rate (unlike adrenaline), which suggests different signalling compartmentation. The contraction-slowing effect of unconjugated bile acids at higher concentrations may occur due to cytotoxicity.
Transcriptional regulation of FGF19 in human intestinal cells by nuclear receptor agonists

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Introduction: Pharmacological agonists of the nuclear bile acid receptor FXR are currently in development for the treatment of chronic liver disorders. The intestinal hormone fibroblast growth factor 19 (FGF19) is a known FXR target gene and is considered to mediate a part of the physiological and pharmacological effects of FXR signalling. In this in vitro study, we investigate the cross-talk of FXR with other nuclear receptors (i.e. PPARs) to control FGF19 gene expression.

Methods: Human HT-29 colon carcinoma cells were either treated with the FXR agonist CDCA, a PPAR agonist (fenofibrate-PPARα; rosiglitazone-PPARγ; GW501516-PPARβ/δ) or a combination of both for 4 and 24 hours. Gene expression of FXR and the FXR target FGF19 was analysed by qPCR as well as Western blot and ELISA, respectively.

Results: PPAR agonist treatment of HT-29 cells led to a time-dependent induction of FXR mRNA expression. This effect was most pronounced for the PPARγ agonist rosiglitazone resulting in a more than 20-fold induction of FXR mRNA expression after 4 and 24 hours. This induction of FXR expression after rosiglitazone treatment was also observed on protein level. Interestingly, treatment of HT-29 cells with rosiglitazone alone decreased FGF19 mRNA and protein levels. However, co-treatment of rosiglitazone together with the FXR agonist CDCA resulted in a strong increase of FGF19 mRNA expression after 24 hours and also resulted in a more pronounced induction of FGF19 protein levels than treatment with CDCA alone.

Discussion/Conclusion: These results provide preliminary in vitro evidence for a putative cross-talk of FXR with nuclear receptors of the PPAR family to control FGF19 expression in intestinal cells. More specifically, we hypothesize that up-regulation of FXR by the PPARγ agonist rosiglitazone could potentiate the action of FXR agonists. Our results warrant further analysis of such a cross-talk and its potential pharmacological down-stream effects in vivo.
Gut bacteria of the family Coriobacteriaceae influence lipid metabolism in mice

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Introduction: Coriobacteriaceae are dominant members of the human gut microbiome and can metabolize cholesterol-derived metabolites such as bile acids. However, consequences for the host are unknown. The major goal of the present study was to characterize the effects of Coriobacteriaceae on lipid metabolism in vivo.

Methods: Male germ-free (GF) C57BL/6N mice were colonized with a minimal consortium of four Coriobacteriaceae (Corio) and fed either a control, high-fat (HFD), or bile acid-supplemented (BA) diet for 16 weeks. GF and specific-pathogen free (SPF) mice were used as controls.

Results: BA diet fed mice stayed lean, but CORIO colonized had 75 to 150% more white adipose tissue (WAT) (GF, 37 ± 15; CORIO, 65 ± 15, SPF, 26 ± 16 mg/g body weight, p < 0.001). No adipocyte size difference was observed, suggesting that hyperplasia rather than hypertrophy was responsible for the phenotype. Proteome analysis of epididymal WAT revealed several CORIO mice-specific proteins related to lipid metabolism, including CIDEC, a protein involved in fatty acid trafficking in lipid droplets. Increased WAT in CORIO mice fed the BA diet was associated with signs of metabolic disturbances, including increased systemic insulin (GF, 0.8 ± 0.3; CORIO, 1.8 ± 1.0; SPF, 1.2 ± 1.0 ng/ml; p < 0.05) and leptin levels (GF, 3.3 ± 2.9; CORIO, 11.5 ± 8.0; SPF, 2.0 ± 1.4 ng/ml; p < 0.001). These changes were accompanied by systemic hypercholesterolemia (GF, 92 ± 40; CORIO, 141 ± 40; SPF, 56 ± 23 µM; p < 0.05), which was also observed in CORIO mice fed CD and HFD.

Discussion/Conclusion: To test if these results can be reproduced in the presence of native communities of commensals in the gut, colonization studies are being performed using the minimal microbiota OligoMM [Brugiroux et al. 2016 Nat Microbiol]. Furthermore, the role of Coriobacteriaceae-derived lipases is under investigation.
Mitochondrial oxysterol biosynthetic pathway gives evidence for CYP7B1 as controller of regulatory oxysterols

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Introduction: The well-described mitochondrial CYP27A1 initiated pathway of metabolism first oxidizes cholesterol to both (25R)-26-hydroxycholesterol (26HC) and 25-hydroxycholesterol (25HC) which are subsequently metabolized to CDCA and βMCA in rodents. We found that selective increased expression of a mitochondrial cholesterol transporter, StarD1, in biliary diverted mice led to the increased synthesis of not only CDCA/βMCA, but the CA. In the present study, we used in vitro and in vivo lost- and gain-of-function approaches to more completely study the pathway.

Methods: Liver oxysterols/bile acids in B6/129 (Wild Type), Cyp7b1−/− and Cyp27a1−/− mice with/without StarD1 overexpression were analysed by HPLC. Primary hepatocytes and mitochondria were also prepared from these mice and were incubated with [3H]-Cholesterol or [3H]-24HC. Formed [3H]-Oxysterols and/or [3H]-bile acids were analysed by HPLC. mRNA levels were determined by RT-qPCR.

Results: StarD1 overexpression in B6/129 mice increased hepatic 26HC and 25HC over controls. Unexpectedly, an elevated hepatic 24HC (2.5-fold vs control, p ≤ 0.01) and a marked down-regulation in Cyp7b1 mRNA were found. Brain 24HC levels were unchanged. Primary B6/129 hepatocytes converted radiolabelled-cholesterol to three oxysterols, 24HC, 25HC and 26HC (ratio, 3:1:10). However, Cyp27a1−/− hepatocytes did not form 24HC and 26HC, and only formed trace 25HC likely through ER 25-hydroxylase. In addition, isolated B6/129 mitochondria converted radiolabelled-cholesterol to three oxysterols including 24HC demonstrating mitochondrial CYP27A1 is responsible for 24HC production. Furthermore, the radiolabelled-25HC and 26HC were converted mainly to CDCA/βMCA in primary B6/129 mice hepatocytes. Interestingly, 24HC was converted not only to CDCA/βMCA, but also to CA, HCA, MDCA, and HDCA. In primary CYP7B1−/− hepatocyte culture, the 24HC was largely converted to HDCA and MDCA.

Discussion/Conclusion: This study outlines the discovery of a mitochondrial-initiated pathway of oxysterol/bile acid biosynthesis. The result also suggests CYP7B1 to be highly regulated, and the major enzyme controlling cellular levels of 3 vital regulatory oxysterols.
Oncomir microRNA-346 is upregulated in ascending but not sigmoid colon in patients with primary sclerosing cholangitis (PSC) and ulcerative colitis (UC)

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Introduction: MicroRNAs are short non-coding RNAs that play a critical role in regulation of gene expression. Oncogenic properties of miR-346 have been recently reported. Patients with PSC and UC have higher risk of colorectal neoplasia when compared to healthy subjects or patients with UC without PSC. Colitic changes in patients with PSC are more pronounced in right colon. In this study we investigated expressions of miR-346 and its two target genes VDR and TNFα, which are known to modulate carcinogenesis.

Methods: Biopsies from ascending and sigmoid colon were obtained from patients with: (i) PSC and UC [PSC(+)/UC(+); n = 10]; (ii) PSC but no UC [PSC(+)/UC(-); n = 10]; (iii) no PSC and UC [PSC(-)/UC(+); n = 10]; (iv) healthy controls (n = 10). Expression of miR-346, TNF-α mRNA and VDR protein levels were analyzed.

Results: miRNA-346 expression was significantly increased in ascending colon of PSC(+)/UC(+) when compared to other analyzed groups (p< 0.001 for all). In contrast, in sigmoid colon expression of miRNA-346 in PSC(+)UC(+) was not different than in PSC(-)UC(+). In PSC(-)/UC(+) group an exceptionally low colonic (both ascending and sigmoid) expression of miRNA-346 was associated with massive increase of VDR and TNFα expressions when compared to other analyzed groups (negative correlations with miRNA-346; Rho = -0.8 and Rho = -0.5, respectively).

Discussion/Conclusion: Expression of miR-346 is enhanced in ascending but not sigmoid colon of patients with PSC and UC. This was not seen in patients with UC but not PSC. In the latter an extremely low expression of miR-346 in colon resulted in a very strong induction of TNFα cytokine which is known to be cytotoxic to tumor cells at high concentration. miRNA-346 may play a role in colonic neoplasia inhibiting VDR and TNFα expressions in patients with PSC and UC.
Effect of ursodeoxycholic acid on biochemical markers of cholestasis in children with Alagille syndrome

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Introduction: Treatment of Alagille syndrome (AS) in children is a very complex problem. One of the main components of complex therapy is the use of ursodeoxycholic acid (UDCA).

The scope and methods of research: Twenty-three children with AS were under observation: 10 boys and 11 girls aged 1 month to 14 years 5 months (mean age 5 years ± 1 year).

All children received UDCA at a dose of 12.5 to 24 mg/kg/day (16.6 ± 3.0 mg/kg/day). A biochemical blood test was performed before the treatment and 3 weeks after the beginning of the treatment. The parameters of total and direct bilirubin, gamma-glutamyltranspeptidase (GGTP), alanine aminotransferase (ALT), aspartate aminotransferase (AST), cholesterol, alkaline phosphatase (AP) were evaluated. The level of bile acids in the blood serum was determined.

Results: Before the treatment of UDCA, the level of total bilirubin was 17.0 ± 1.6 μmol/l, direct bilirubin – 7.5 ± 0.7 μmol/l; the level of cholesterol was 5.8 ± 0.6 mmol/l, GGTP 127.7 ± 12.3 U/l, AP 381.0 ± 36.4 U/l, ALT 101.0 ± 10.1 U/l, AST 115.0 ± 11.0 U/l. The level of bile acids in the blood serum was 198.0 ± 18.7 μM/l.

Three weeks after the start of treatment with UDCA, the level of total bilirubin decreased to 12.0 ± 1.2 μmol/l (p = 0.0167), direct bilirubin – to 2.2 ± 0.2 μmol/l (p = 0.0000); level the cholesterol 4.7 ± 0.4 mmol/l (p = 0.1352); GGTP 87.7 ± 8.6 mmol/l (p = 0.0111). The level of AP was 360.4 ± 34.2 U/l (p = 0.6823), the level of ALT was 58.8 ± 5.6 U/l (p = 0.0008), AST 68.9 ± 6.9 U/l (p = 0.0010). The level of bile acids in the blood serum decreased, which amounted to 144.2 ± 14.1 μMol/l (p = 0.0271).

Conclusions: The use of UDCA in the treatment of SA in children significantly improves biochemical indicators that are markers of cholestasis and reduces the level of cytolytic activity (ALT and AST).
Treatement with S-adenosyl-L-methionine (SAMe) may affect immune responses in primary biliary cholangitis (PBC) via its antioxidant properties

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Introduction: The synthesis of S-adenosyl-L-methionine (SAMe), a major glutathione (GSH) precursor is impaired in PBC. Overexpression of aberrant PDC-E2 in PBC cholangiocytes result in induction of anti-mitochondrial antibodies (AMA) production. Production of AMA was previously associated with S-glutathionylation¹. In this project, we analyzed the effect of SAMe supplementation on antioxidant and immune responses both in patients with PBC and in human cholangiocytes in culture.

Methods: Seventeen patients with PBC treated with UDCA were supplemented with SAMe (1200 mg/day) for 6 months. Serum AMA/M2 titers, FGF-19, MnSOD, S-glutathionylation, TNFα, TGFβ and IFNγ levels were analyzed at the beginning and end of the study. H69 cholangiocytes were incubated with 50, 100, 250 and 500 µM of SAMe. Additionally, the expression levels of crucial enzymes in GSH turnover, i.e. glutamate cysteine ligase (GCL), GSH synthetase (GS), glutaredoxin (Grx), and TNFα were studied.

Results: Nine patients (significantly younger at the diagnosis and with longer duration of the disease) esponded to SAMe with elevation of MnSOD and S-glutathionylation. This phenomenon was associated with a significant reduction of AMA/M2. Increased protein S-glutathionylation showed a negative correlation with AMA/M2 titers and TNFα (r = -0.67 and r = -0.68 respectively). AMA/M2 titers correlated positively with IFNγ (r = 0.71) but negatively with TGFβ and FGF-19 (r = -0.75 and r = -0.7, respectively). In H69 cells, SAMe caused a significant elevat ion of GCL, GS, Grx levels in a dose dependent manner. This was also associated with decreased TNFα at the dose of 100 µM of SAMe.

Discussion/Conclusion: SAMe exerts a profound effect facilitating antioxidant responses both in patients with PBC and cultured human cholangiocytes. SAMe may also modulate immune responses in PBC by decreasing AMA/M2 titers, likely related to its antioxidant and S-glutathionylation properties. These findings may provide new insights into the molecular events promoting the development and progression of PBC.

¹Hu et al (2012).
Isolation and characterization of infant BSH active bacterial isolates

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Introduction: Bile acids (BA) have a major role in helping the gastrointestinal system to digest, absorb and metabolise nutrients. BA are also signalling molecules that can alter a range of different metabolic processes. Bile altering enzyme, bile salt hydrolase (BSH), is widespread among gut resident bacteria. These enzymes initiate the gateway reaction for BA metabolism and can dictate the BA profile of the host, a profile that can be altered in a number of gut disease states translating to altered host metabolism.

Methods: Here, we describe the screening process of infant bacterial isolates for BSH activity. 59 BSH active isolates out of 600 showed both glyco and tauro deconjugation ability in agar assays.

Results: RAPD PCR revealed 4 distinct specific isolates. De novo sequencing revealed that each isolate encodes 2 distinct BSH enzymes. The collective activity of these enzymes, for each strain, was examined by UPLC-MS confirming BSH activity and revealing a unique BA profile for each strain based on de-conjugation preference towards a number of specific moieties.

Discussion/Conclusion: Once fully characterised these strains may be applied to amend disease associated or inducing bile acid adjustments of the GIT.
Difference between two mice strains changes their bile acid composition, gut microbiota, and metabolic regulation system

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Introduction: Obesity and metabolic syndrome progressions are dependent on their living environment, genetic background, and their interactions. According to some research to evaluate these factors, they show that 129S6/Sv mice were less to obesity in comparison with B6 mice. These results arise from the difference of genetic background and gut microbiota between two strain mice. Additionally, recent studies suggest that alteration of the bile acid (BA) composition by microbiota improved metabolic syndrome. Thus, we thought BA metabolism is associated with these phenotypic differences. By comparing strain of the mice, we explore the relationship among BA, gut microbiota, and metabolic regulation system.

Methods: Six-week-old B6 and 129X1/Sv (129) mice were fed on a high-fat diet (HF) and HF diet mixed CA (choric acid). After two months, we took blood, feces, and liver from these mice to investigate lipid levels and BA composition. Additionally, by the use of their fecal samples, we performed pyrosequencing of the V1–V2 region of 16S rRNA genes.

Results: In B6 mice, BA improved obesity and hepatic steatosis. However, in 129 mice, BA couldn’t show these effects. Lipogenic gene and BA synthesis gene expression levels were different between HF+BA treated B6 and 129 mice in the liver. Moreover, BA treatment induced energy expenditure in brown adipose tissue (BAT) by TGR5/M-BAR activation in B6, but not 129 mice. Furthermore, we found that BA composition and total BAs levels were different between strains. FXR antagonistic TBMC levels decreased in HF+BA treated 129 mice. Microbiome analysis shows that secondary BA-producing bacteria greatly increased in 129 mice supplemented with HF+BA.

Discussion/Conclusion: Our data suggest that difference of genetic background changes their gut microbiota and BA composition, influences TGR5 and FXR signaling activity, and caused metabolic phenotype differences.
Dynamic determinants of portal hypertension are identified by histological collagen proportionate area estimations

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Introduction: Portal hypertension is determined both by ‘static’ fibrosis and ‘dynamic’ hemodynamic components. The correlation of fibrosis area and portal pressure has not been systematically assessed in different animal models and in human liver disease of different etiologies. Thus, we evaluated the correlation of collagen-proportionate area (CPA) with portal pressure (PP) in animal models and with hepatic venous pressure gradient (HVPG) in patients with cirrhosis.

Methods: Carbon tetrachloride (CCl₄) or bile duct ligation (BDL) models were used to mimic toxic or biliary cirrhosis in rats, respectively. Portal pressure was measured by direct cannulation of the portal vein. Patients underwent HVPG measurements and transjugular liver biopsy. Liver samples were stained by chrome-aniline-blue (CAB) or picro-sirius-red (PSR) and CPA was quantified by ImageJ software.

Results: Portal pressure correlated with CPA both in BDL (R = 0.844, p < 0.001) and in CCl₄ (R = 0.866, p < 0.001) animals. The ‘linear fitting’ curve was steeper in BDL (PP = 2.303*CPA+4.045) as compared to CCl₄ (PP = 1.520*CPA+4.794). Animals outside the 75% confidence interval of the expected PP (based on CPA) might have pronounced ‘dynamic’ components, e.g. in endothelial dysfunction, splanchnic blood flow or portosystemic shunting.

Similarly, in (n = 18) patients, HVPG correlated with CPA (R = 0.339, p = 0.17) yielding a ‘predicted’ HVPG (mmHg) of 0.663*CPA+11.8. In patients with 25% higher HVPG as expected by the CPA-formula, we recorded higher vWF-Ag (368 ± 3 vs. 233 ± 50%; p = 0.048), IL6 (23 ± 8 vs. 8 ± 4 ng/l; p = n.s.), and bile acids (34 ± 18 vs. 7 ± 3 µmol/l; p = n.s.) than in patients with 25% lower HVPG as expected. More patients will be presented at congress.

Discussion/Conclusion: CPA as the ‘static’ component of PHT correlates with PP/HVPG, with model-specific and etiology-dependent correlation estimates. Outliers to this curve imply profound alterations of ‘dynamic’ components of PHT, such as endothelial dysfunction, significant collateralization, bacterial translocation or a deranged gut-liver axis. Identification of outliers refines the assessment of vascular and hemodynamic dysfunction and may allow for personalized therapy of PHT.
Figure 1

Figure 2
Investigation of the modulation of the ATPase activity of human multidrug resistance protein 3 (MDR3/ABCB4) by bile acids

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Introduction: The human ABC-Exporter MDR3 is mainly localized in the canalicular membrane of hepatocytes. MDR3 is required for flopping lipids of the phosphatidyl choline (PC) family from the inner to the outer leaflet of the membrane. Here, PC is taken up into mixed micelles by secreted bile acids (transported by BSEP/ABCB11) and cholesterol (transported by ABC G5/G8). Since PC lipids are necessary to avoid membrane solubilisation by the high concentrated bile acids in the bile canaliculi, we suggest a cross-talk between MDR3 and substrates of BSEP.

Methods: MDR3 was heterologous expressed in Pichia pasotris cells and purified by a tandem affinity chromatography (IMAC followed by CBP). The malachite green assay was performed to determine the modulation of the ATPase activity of MDR3 by different bile acids.

Results: We are able to purify up to 6 mg heterologous expressed human MDR3 from 100 gram of wet cell weight. Functionality of purified MDR3 is granted since only PC lipids such as dioleyl-glycerol-phosphatidyl-choline (DOPC) could stimulate the ATPase activity of MDR3.

Different bile acids used in the micromolar range caused either stimulation (e.g. taurocholic acid) or reduction (e.g. taurolithocholic acid) of ATPase activity in presence and absence of DOPC. Interestingly, tauroursodeoxycholic acid showed no modulation of the activity.

Discussion/Conclusion: We can demonstrate an effect of bile acids on MDR3 ATPase activity. Our data suggest a correlation between the critical micelle concentration of bile acids and their effect on MDR3.
Anti-apoptotic actions of lithocholic acid on colonic epithelial cells: Implications for treatment of inflammatory bowel disease

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Introduction: Although inflammatory bowel disease (IBD) represents a significant health and economic burden, current therapies are often ineffective. Dysregulated intestinal barrier function has been well-documented to contribute to IBD pathogenesis, with increased epithelial apoptosis being apparent. We have previously reported that ursodeoxycholic acid (UDCA) attenuates intestinal inflammation in the DSS model of colonic inflammation in mice, and that its efficacy is dependent on bacterial metabolism into lithocholic acid (LCA) (Ward et al, 2017). Here, we set out to examine the effects of LCA on apoptosis in colonic epithelial cells.

Methods: C57BL/6 mice were administered dextran sodium sulphate (DSS, 2.5%) in their drinking water for 5 days, with or without treatment with lithocholic acid (30 mg/kg) via IP injection each day. T84 colonic epithelial cells were treated with LCA (30 µM) or the farnesoid X receptor (FXR) agonist, GW4064. Apoptosis was induced by treatment of the cells with a combination TNF-α (20 ng/ml) and INF-γ (40 ng/ml) for 24 hrs and was assessed by measuring levels of cleaved Poly (ADP-ribose) polymerase (PARP). miRNA profiling was performed on GW4064 (5 µM; 6 hrs)-treated T84 cells, with TargetScan being used to identify miRNA targets. Reverse transfection of T84 cells was performed using lipofectamine RNAimax and protein expression was assessed by western blotting or qPCR.

Results: In the in vivo model of DSS-induced colonic inflammation, LCA treatment reduced levels of cleaved-PARP in the mucosa from 19.9 ± 10.9 to 3.9 ± 1.1-fold of that in non-DSS treated mice (n = 3–4; p ≤ 0.01). Similarly, in cultured monolayers of T84 cells, LCA prevented cytokine-induced apoptosis, an effect that was mimicked by the FXR agonist, GW4064. miRNA array profiling of GW4064-treated T84 cells revealed increased levels of miR-29a-3p. miR-29a3p is predicted to target PTEN, a pro-apoptotic protein, with a 92% context score as ascertained by TargetScan. Treatment of T84 cells with GW4064 resulted in decreased PTEN mRNA (n = 8; p ≤ 0.05) and PTEN protein levels (n = 3; p ≤ 0.05). Similarly, transfection of T84 cells with a miR-29a-3p mimic, but not a scrambled control, decreased PTEN levels (n = 3; p ≤ 0.01).

Discussion/Conclusion: Our data suggest that bacterial metabolism of UDCA to LCA in the colonic lumen may promote barrier function by prevention of cytokine-induced apoptosis. The mechanism by which LCA exerts anti-apoptotic actions may involve activation of FXR, induction of miR29a3p, and reduced expression of the pro-apoptotic protein, PTEN.

Multiple cholephilic compounds involved in cholestatic itch inhibit autotaxin activity

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Introduction: Cholestatic diseases, such as PBC, PSC and intrahepatic cholestasis of pregnancy (ICP), lead to increased serum concentrations of cholephilic compounds, that are normally secreted into bile. Pruritus is a common symptom in cholestasis and correlates with serum activity of autotaxin (ATX) which converts lysophosphatidylcholine into lysophosphatidate (LPA). The bile salt analogue obeticholic acid (OCA) can cause itch when administered at high doses. We have recently shown (Keune et al. Nat Commun. 2016;7:11248) that bile salts lacking a 12-OH group (UDCA, CDCA) inhibit ATX activity. Here, we explored modulation of ATX activity by other cholephilic compounds.

Methods: The effect of compounds on ATX activity was determined by the amount of liberated choline, as detected by an enzymatic fluorometric analysis. Fractions from bile of cholestatic patients with itch were generated by HPLC and analyzed for inhibition of ATX activity.

Results: OCA and its analogue INT-767 showed marked inhibition of ATX activity (Ki = 0.9 µM and 2:0 µM, respectively) whereas INT-777, which contains a 12-OH group was less effective (Ki = 231 µM). Taurolithocholate and its 3-sulfated conjugate had identical inhibitory effects (Ki = 3 µM). Among sulfated progesterone metabolites, implicated in ICP, 5α-pregnan-3α,20α-diol-3,20-disulfate (PM2diS) strongly inhibited ATX activity (Ki = 1.3 µM), while 5β-pregnan-3α,20α-diol-3-sulfate (PM3S) was less effective (Ki = 30 µM). The steroid dehydroepiandrosterone sulfate (DHEAS) also inhibited ATX activity (Ki = 45 µM) while progesterone showed only weak inhibition (Ki = 244 µM). No inhibition was induced by other uncharged steroids like pregnanediol and prednisolone. Several fractions from HPLC-fractionated bile not containing bile salts also inhibited ATX activity.

Discussion/Conclusion: Several compounds that are elevated in serum during cholestasis inhibit ATX activity, including bile salts and sulfated steroids. Multiple compounds in fractionated bile also demonstrated inhibitory capacity. These observations question the linear relationship between increased serum ATX activity and serum LPA levels.
Assessment of drugs that inhibit bile salt export pump (Bsep) in a siRNA Bsep knockdown rat model

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Introduction: Although drugs that are shown to inhibit the bile salt export pump (BSEP) may be associated with clinical drug-induced liver injury (DILI), these drugs are poorly associated with hepatotoxicity in preclinical test species. Here we present the development of a novel rat model using siRNA knockdown (KD) of Bsep, resulting in reduced Bsep expression. This model allows an in vivo evaluation in rats of potential for enhanced hepatotoxicity risk from drugs that are reported to inhibit Bsep in vitro and have been associated with clinical DILI.

Methods: Several human DILI positive drugs were evaluated in vitro for inhibition of rat Bsep, Ntcp (Na+ taurocholate cotransporting polypeptide), and Mrp 2, 3, and 4 (multidrug resistant proteins). A total of nine Bsep inhibitors that are associated with DILI, including Asunaprevir (ASN), Cyclosporine A (CSA), Benz bromarone (BBR), Bosentan (BST), Ritonavir (RTN), Lopinavir (LPN), Simeprevir (SMP), TAK-875, and the antibiotic Telithromycin (TTM) were administered to Bsep KD rats for 7 days. Additionally, three non-Bsep inhibitors, Acetaminophen (APAP), MSD-A, and Clarithromycin (CTM) were also tested in this model. Plasma and liver samples were assessed for bile acid and drug exposure measurements. Liver samples were collected for transporter protein levels and gene expression analyses.

Results: Bsep mRNA and protein levels were reduced in liver by 70% and 90%, respectively. Bsep KD alone resulted in significant and consistent changes in plasma and liver bile acids, and in a distinct liver gene expression pattern. Among the nine Bsep inhibitor treatments, ASN and TAK-875 resulted in synergistic increases of serum transaminase and total bilirubin levels in Bsep KD groups that were also associated with very large changes in plasma conjugated bile acids.

Discussion/Conclusion: Elevations of plasma conjugated bile acids appear sensitive to reductions in Bsep function, and are shown here to be enhanced in some cases with Bsep KD, but may also result from inhibition of other transporters and microbiome alterations.
Changes in plasma bile acid profiles after partial internal biliary diversion in three ABCB11-mutated (PFIC2) patients

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**Background and aims:** We ask if plasma bile acid profiles can be used to monitor the effectiveness of partial internal biliary diversion (PIBD) for treating PFIC2 patients with uncontrolled cholestasis.

**Methods:** Plasma bile acids were profiled in 3 cases of ABCB11-mutated PFIC2 children before and after PIBD compared to healthy controls.

**Results:** Before PIBD, all three patients presented with > 50-fold higher levels of total plasma bile acids, 2–7-fold higher ratios of taurine:glycine conjugated primary bile acids, and unchanged secondary bile acids levels compared to healthy controls. After PIBD, one patient showed relief of cholestasis with a bile acid profile shift toward that of healthy controls including a 5-fold reduction in total plasma primary bile acids and a reduced taurine:glycine conjugate ratio. However, the secondary bile acids DCA and LCA increased 26- and 12-fold, respectively, consistent with a direct drainage of biliary bile acids into the colon after PIBD and an elevated conversion to secondary bile acids by the gut microbiome. None of these changes were seen in the non-responders. One year later, the responding patient suffered a recurrence of cholestasis, and the bile acid profile shifted back to a more pre-PIBD-like profile.

**Conclusions:** Plasma bile acids have the potential to serve as sensitive biomarkers for monitoring treatment efficacy of PIBD. Relief of cholestasis after PIBD is associated with significantly increased circulating toxic secondary bile acids and this may limit the utility of PIBD.
Probiotic potential of new *Lactobacillus salivarius* isolate with regard to BSH activity

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**Introduction:** *Lactobacillus* species are commonly applied as probiotics with the prerequisite that they can metabolise bile acid (BA). This metabolic activity is dependent on the presence of bile salt hydrolase (BSH) enzymes which initiate the gateway reaction for BA metabolism in the gut by commensal microbes. These bile altering microbes govern the overall BA composition of the host which has been shown to be altered in a variety of disease states such as IBD.

**Methods:** This study describes a new isolate and subspecies of *L. salivarius* of porcine origin. This strain was characterised genetically by de novo sequencing to detect the presence of BSH enzymes. UPLC-MS determined the *in vitro* activity and range of these BSH enzymes following incubation with 32 individual BAs. This *L. salivarius* strain was also assessed for EFSA characteristics and studied *in-vitro* (gut simulation model) and *in-vivo* (mouse model) to examine its probiotic potential with an emphasis of the alteration of the host BA pool.

**Results:** Our new BSH positive strain of *L. salivarius* contained three distinct BSH sequences. Their collective activity revealed that these enzymes are active in deconjugation with preference towards a number of specific BA to yield a unique profile for this isolate. Gut simulation assays, EFSA and *in-vivo* assessment indicate this strains ability to influence the composition of the host BA pool under different diet conditions.

**Discussion/Conclusion:** We question whether strains with fully characterised BSH activity such as our *L. salivarius* can be applied as a probiotic to ameliorate effects of specific BA alterations seen in certain disease conditions.
Histomorphological assessment of hepatic fibrosis progression with accompanying pronounced ductular proliferation in chronic thiacetamide-induced experimental liver fibrosis/cirrhosis in young rats

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Background: It is assumed that liver fibrosis results from a dynamic process, characterized by the excess synthesis and accumulation of extracellular matrix (ECM) components (i.e. fibrogenesis) over its removal (i.e. fibrolysis). Although hepatic fibrosis is a major histopathological finding associated with most forms of chronic liver disease, its morphogenesis and development have not been sufficiently elucidated.

The study objective was the histological and histochemical analysis of the dynamics of the liver fibrotic process due to chronic application of thioacetamide (TAA), a selective hepatotoxin, in young rats in an experimental model of liver fibrosis.

Methods: Group I (n = 30) consisted of rats exposed to the pharmacological model of liver fibrosis (TAA-model) via intraperitoneal (i.p.) administration of TAA (Thioacetamide, Sigma Aldrich), twice a week, in a dose of 200 mg/kg b.w., dissolved in 0.5 ml 0.9% NaCl (n = 30). The animals were sacrificed after 4, 8 and 12 weeks of the experiment in inhalation anaesthesia with isoflurane without damaging the integuments, 10 animals in each experimental subgroup (Ia, n = 10; Ib, n = 10; Ic, n = 10). The administration mode and the dose were determined based on literature data. Group II – animals, in which inhalation anaesthesia with isoflurane was applied, with no damage to integuments (comparative group) (n = 6). Group III – animals given 0.5 ml 0.9 % NaCl i.p., twice a week for 12 weeks (comparative group) (n = 6). Liver samples were obtained and fixed in buffered formalin solution, and embedded in paraffin. Necro-inflammatory injuries and fibrosis were assessed by Mayer’s hematoxylin and eosin (H&E) stain. Moreover, fibrosis (staining for collagen fibers and reticulin fibers) was determined by the Sirius-red, Masson’s trichrome blue, Masson’s-Goldner stains and reticulin according to Gomori’s stain. Fibrosis stage and inflammation grade were assessed by the Batts and Ludwig histological scoring system (1995).

Results: Chronic i.p. application of TAA caused gradual enhancement of liver fibrosis ranging from mild portal fibrosis (after 4 weeks) to cirrhotic nodules in the liver (after 8 and 12 weeks). The process of fibrogenesis was concomitant with followed necro-inflammatory injuries, mainly affecting the limiting plate of the liver lobule as “piecemeal necrosis” of hepatocytes, with the presence of inflammatory cell infiltrations composed of lymphocytes and plasma cells.
Moreover, intrahepatic cholestasis with coexisting distinct ductular proliferation was also a common finding. Enhanced proliferation of small ductal structures was found to accompany marked portal inflammation. The 8-week TAA group showed bridging and septal fibrosis connecting portal areas and central veins in portal to portal, portal to central, and/or central to central patterns, focally including the formation of regenerative hepatic nodules; on the other hand, the 12-week TAA group demonstrated fully developed cirrhotic changes. Striking collagen deposits were present in the periportal areas and those with bridging fibrosis in the TAA treated groups, whereas only mild inflammatory cell infiltration was found around some portal areas in controls, with no collagen deposits or fibrous septum formation.

**Discussion/Conclusion:** Our data indicated that chronic TAA administered to young rats resulted in progressively increasing liver fibrosis with coexisting distinct ductular proliferation, finally leading to fully developed cirrhotic changes. The pattern of liver fibrosis/cirrhosis obtained in our study using the experimental animal model with selective hepatotoxin thioacetamide closely resembles panlobular and parenchymal fibrosis that is found in most human chronic liver diseases.
**Evaluation of serum fibroblast growth factor 19 (FGF19) and total free fecal bile acids in stool as markers of bile acid malabsorption in patients with chronic diarrhea: A pilot study**

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**Introduction**: Excessive amounts of bile acids (BA) entering the colon due to bile acid malabsorption cause chronic bile acid diarrhea (BAD). Fibroblast growth factor 19 (FGF19) is the ileal hormone providing feedback inhibition of BAs synthesis in the liver. Little is known about the mechanisms of BA dysregulation in different groups of patients with chronic diarrhea like inflammatory bowel disease (IBD), irritable bowel syndrome (IBS-D) and microscopic colitis.

**Methods**: The AIM is to evaluate median serum levels of FGF19 and fecal bile acids in patients with chronic diarrhea who we believe to have BAD (IBD after right hemicolectomy, active IBD, IBD in remission and IBS-D). Fasting serum FGF19, total free fecal bile acids and fecal calprotectin (FC), were measured using ELISA test in 40 patients divided into 3 groups: 14 patients with elevated FC (as a marker of mucosal inflammation), 21 patients with normal FC levels and 5 patients after IBD surgery. Non-parametric statistics were used and results are expressed as medians with interquartile ranges.

**Results**: Median FC levels among the 3 groups were 845 vs. 30 vs. 185 µg/g, respectively (p = 0.002). Median serum FGF19 were 34.03 vs. 113.43 vs. 0.004 pg/ml, (p = 0.043). Median fecal total free bile acids levels among the 3 groups were 31.7 vs. 41.0 vs. 23.8 µmol/l, (p = 0.26). We estimate higher median FGF19 levels in IBD patients in remission and lower FGF19 in IBD patients after surgery. All median fecal free bile acid levels among the groups were elevated, but no differences between the groups.

**Discussion/Conclusion**: BAD is very under-diagnosed and should be considered in patients with chronic diarrhea. Overall results from this pilot study suggest that FGF19 is lower in patients with BAM and higher in IBD in remission, but inter-individual variation of FGF19 is larger. Further bigger studies are needed to establish the efficacy of FGF19.
Does the placenta contribute to the enhanced risk of pruritus during maternal hypercholanemia?

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Introduction: Intrahepatic cholestasis of pregnancy (ICP) is frequently accompanied by pruritus, whose etiology has been associated with an enhanced production of lysophosphatidic acid (LPA) by the combined action of phospholipase A1/A2 (PLA1/PLA2) and autotaxin (ATX). Here we have investigated whether the placenta is involved in LPA release to maternal circulation during ICP.

Methods: ATX and LPA were determined in serum by ELISA and total bile acids by a colorimetric technique. The expression of ATX and LPA was determined by RT-qPCR in human and rat placenta and by western blot and immunofluorescence in human placenta.

Results: Serum levels of ATX and LPA were elevated in women with ICP, and a correlation between both parameters was found. No relationship between serum levels of ATX or LPA and bile acids was found. Placenta ATX but not PLA2 was significantly upregulated in ICP. A correlation between serum ATX and placental ATX mRNA levels was found. In human placenta at term, ATX was clearly detected in Hofbauer cells, but only faintly in trophoblast cells. In pregnant rats, the expression of Atx and Pla2 in placenta was lower than in liver. When obstructive cholestasis was imposed by bile duct ligation from day 14 of gestation until term, placenta Atx and Pla2 expression was markedly enhanced.

Discussion/Conclusion: The placenta substantially participates in LPA production during gestation. This contribution is markedly higher during maternal cholestasis and may hence be involved in ICP-associated pruritus.
A novel non-immunosuppressive cyclosporine analog inhibits NTCP and shows potential for treatment of metabolic diseases in mouse models

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Introduction: Bile acids (BAs) composition in circulation regulates multiple metabolic pathways in organs. Dysfunction of NTCP leads to altered BA profiles in both humans and mice. Here, we developed a novel non-immunosuppressive derivative of Cyclosporin A, CsA055, as an NTCP inhibitor and evaluated its potential in animal models with metabolic abnormalities.

Methods: In vitro taurocholic acid (TCA) uptake assay was conducted and the chemical was also evaluated for immunosuppressive effect. Wildtype C57BL/6 mice were given intraperitoneally or orally to examine the levels of circulating BAs. The effects of CsA055 were investigated in methionine-choline deficient (MCD) diet induced mouse model of nonalcoholic steatohepatitis (NASH), atherosclerosis-prone apolipoprotein E-deficient (Apoe−/−) mice and the leptin-deficient (ob/ob) mice.

Results: CsA055 significantly blocks TCA uptake and shows non-immunosuppressive effects. Treatment with CsA055 increases circulating BAs level in mice. Oral CsA055 treatment ameliorates hepatic inflammation and liver damage in MCD-induced NASH model. CsA055 also lowers the serum cholesterol levels in Apoe−/− mice fed with chow or high-fat diet. For the ob/ob mice, CsA055 averts the glucose intolerance and partially deters the liver steatosis but has little effect on the body weight.

Discussion/Conclusion: We developed a novel NTCP inhibitor CsA055 and used it to evaluate the consequence of NTCP inhibition in a few mouse models with metabolic abnormalities. Our results shows inhibition of NTCP by CsA055 may provide a means to alleviate liver damage, hepatic lipotoxicity and improve metabolic syndrome.
Bile acid 7-dehydroxylation by *Clostridium scindens* in vitro and in vivo

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**Introduction:** The microbially-mediated modification of bile acids (BAs) impacts their affinity to mammalian receptors and consequently host signalling, for instance the regulation of host metabolism. Bile acid 7-dehydroxylating bacteria are commensals of particular importance as they carry out the 7-dehydroxylation of liver-derived primary BAs to 7-dehydroxylated secondary BAs, which represent a major fraction of the secondary bile acid pool. However, the dynamics of their bile acid transformations is not fully understood. *Clostridium scindens* is one such organism in the human and murine gut. This study aims to characterize BA transformations performed by *C. scindens* in vitro and in vivo and to investigate its colonization in the murine gut.

**Methods:** A gnotobiotic mouse line devoid of 7-dehydroxylating strains was complemented with ¹⁵N isotopically labeled cells of *C. scindens*. Nanoscale Secondary Ion Mass Spectrometry (NanoSIMS) was used to track *C. scindens* along the intestinal tract and identify locations where colonization and growth occur. *In vivo*, BA transformations by *C. scindens* were characterized using targeted metabolomics. Additionally, the same strain was incubated in batch cultures and the transformation of primary BAs monitored as a function of time to identify secondary BAs produced by *C. scindens*.

**Results:** *In vitro*, *C. scindens* exhibits not only 7α-dehydroxylating capabilities but also oxidizes C3 and C12 hydroxyl groups in primary and secondary bile acids. Of note, we observe the production of 12-oxoLCA, the 7-dehydroxylated, 12-oxo species. Using NanoSIMS, we demonstrate that the large intestine constitutes a niche for *C. scindens*, where it efficiently 7-dehydroxylates cholic acid to deoxycholic acid.

**Discussion/Conclusion:** This work revisits paradigms of bile acid 7-dehydroxylation in vitro and in vivo, and provides direct evidence for the colonization and growth of 7-dehydroxylating bacteria in the large intestine. It also evidences the formation of an oxidized and dehydroxylated bile acid by *C. scindens* through an unknown mechanism.
OCA ameliorated dyslipidemia but not insulin resistance in a mouse model of gestational diabetes mellitus

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Introduction: Pregnancy is associated with profound metabolic changes including altered glucose, lipid, and bile acid homeostasis. Late gestation is characterised by progressive insulin resistance, dyslipidemia, and raised serum bile acids. The bile acid sensor FXR, known to be functionally suppressed in pregnancy, modulates glucose and lipid metabolism. Obeticholic acid (OCA), a potent FXR agonist, has been shown to improve insulin sensitivity in type 2 diabetic patients with non-alcoholic fatty liver disease. We therefore hypothesised that administration of OCA could ameliorate disease features in a mouse model of gestational diabetes mellitus (GDM).

Methods: GDM was induced in female C57BL/6 mice by feeding a high fat diet (HFD; 60% kcal derived from fat), for 4 weeks prior to and throughout pregnancy, or equivalent for non-pregnant controls. Normal chow-fed mice were used as additional controls. From gestational day (GD) 1, mice received diets ± 0.03% OCA (approx. 40 mg/kg/day; Intercept Pharmaceuticals). Glucose and insulin tolerance tests were performed on GD16 and GD17. Mice were sacrificed at GD18. Phenotypic and biochemical parameters were measured by qPCR, plasma GLP-1 and lipid quantification assays.

Results: OCA significantly reduced plasma cholesterol in both non-pregnant and pregnant HFD-fed mice, however had no effect on insulin resistance. In non-pregnant HFD-fed mice, OCA ameliorated weight gain, reduced mRNA expression of inflammatory markers in white adipose tissue and reduced plasma GLP-1 concentrations. Furthermore, mRNA expression of hepatic lipogenesis and cholesterol homeostasis targets were significantly downregulated. However, these effects were not evident in pregnant mice.

Discussion/Conclusion: OCA administration can reduce plasma cholesterol levels in a murine model of GDM. However, the efficacy of OCA was reduced in pregnancy as several of the effects observed in non-pregnant mice were absent. This suggests that the agonistic effect of OCA is not sufficient to overcome the signals which reduce FXR activity in pregnancy.
TGR5-dependent hepatoprotection through the regulation of biliary epithelium permeability

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Introduction: TGR5, the bile acid (BA) G-protein-coupled receptor protects the liver against BA overload through yet elusive mechanisms. We explored the hypothesis that TGR5, highly expressed in biliary epithelial cells, may regulate biliary epithelium permeability.

Methods: Trans-epithelial electric resistance (TER) and fluorescent dextran transfer were measured in the NRC (Normal Rat Cholangiocyte) cell line. In vivo, fluorescent dextran was injected in the gallbladder (GB) lumen and traced in plasma. Cells and mice were treated with RO5527239, a specific TGR5 agonist. TGR5-induced signalling pathways were studied by western blot (WB). Tight junction protein (TJP) expression was investigated in NRC, in livers and GB from WT (Wild Type), TGR5-KO and Junctional Adhesion Molecule-A (JAM-A)-KO mice, under vehicle or TGR5 agonist treatment, in basal conditions or after Bile Duct Ligation (BDL).

Results: In NRC, TGR5 agonists increased TER and reduced dextran passage. Inhibition of EGFR transactivation and of PKCζ suppressed these effects. Dextran transepithelial transfer after GB injection was increased in TGR5-KO as compared with WT mice. In TGR5-KO mice, JAM-A (but not other TJP) expression and localization at TJ were reduced in bile ducts and GB epithelia, as compared with WT. In vitro, TGR5 agonists induced JAM-A stabilization and phosphorylation in NRC cells. The impact of TGR5 on TER critically required JAM-A phosphorylation, as it was abolished in JAM-A S285A (non-phosphorylatable) mutant. After TGR5 agonist treatment, JAM-A expression and phosphorylation increased (biliary tract) and dextran transepithelial transfer decreased (GB injection). After BDL (48 h), JAM-A expression and phosphorylation was increased in bile ducts and GB epithelia as compared with control animals. TGR5 agonist treatment significantly protected WT but not JAM-A-KO mice from BDL-induced liver injury.

Discussion/Conclusion: The BA receptor TGR5 regulates biliary epithelial permeability, through the modulation the TJP JAM-A expression and phosphorylation, thereby protecting liver parenchyma against BA leakage.
Deviations in peripheral blood subpopulations are connected with the presence of pruritus in primary biliary cholangitis patients

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Introduction: The role of particular peripheral blood (PB) subpopulations in the course of primary biliary cholangitis (PBC) remains still uncertain. Bile acids, endogenous opioids, autotaxin and lysophosphatidic acid seem to have a key role in the pathogenesis of cholestatic pruritus in PBC patients. The aim of our study was to assess the relationships between analyzed peripheral blood cell subsets and the presence of itch in patients with newly diagnosed PBC.

Methods: The frequencies of PB subpopulations were measured by flow cytometry in 34 previously untreated female patients with PBC. 19 participants from research group presented pruritus. Control group consisted of 20 healthy age- and sex-matched volunteers. The severity of pruritus was assessed according to Visual Analogue Scale (VAS) questionnaire and the mean result was 4.1/10 points.

Results: PBC patients complaining of pruritus had significantly lower percentages of CD3+/CD16+CD56+ NKT-like cells (p = 0.04) and CD3+ T lymphocytes (p = 0.03) than PBC patients without pruritus. Additionally, PBC patients with itch presented significantly lower absolute counts of CD4+/CD3+ cells (p=0.01) and CD3+CD25+ cells (p = 0.03) in comparison to PBC patients without itch. Percentages of CD3+/CD16+CD56+ NKT-like cells and absolute counts of CD3+CD25+ cells were significantly higher in PBC patients compared to controls (p < 0.01). There were no significant differences in percentages of CD3+ T lymphocytes and absolute counts of CD4+/CD3+ cells between research and control group.

Discussion/Conclusion: Results obtained in our survey suggest that deviations in PB subsets might be involved in the pathogenesis of cholestatic pruritus in PBC patients and this issue should be undoubtedly clarified in further studies.
Bile acid traffic across the mammary gland: Implications on lactation during maternal cholestasis

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Introduction: Intrahepatic cholestasis of pregnancy is the most frequent pregnancy-specific liver disease, being characterized by pruritus and elevated bile acid concentrations in maternal serum. Bile acids are also present in milk, but the sensitivity of their profile to maternal hypercholanemia and the transporters involved in bile acid handling by the mammary gland are poorly understood.

Aim: To investigate the role of ATP-binding cassette (ABC) proteins in the traffic of bile acids between blood and milk and the sensitivity of this process to high levels of maternal serum bile acids.

Methods: Bile acid concentrations in serum and milk were measured by HPLC-MS/MS. Gene expression was determined by RT-QPCR and immunofluorescence.

Results: Bile acid concentrations in rat and mouse were higher in serum than in milk. Bile acid profiles in these fluids were also different in both species. In mammary gland, mRNA levels of ABC pumps able to transport bile acids were: high for Bcrp, less abundant for Mrp1, Mrp3 and Mrp4 and negligible for Bsep and Mrp2. As compared with wild-type mice, bile acid concentrations in the milk of Bcrp⁻/⁻ mice were lower. Intraperitoneal administration of taurocholate (5 µmol) to mice markedly increased (20-fold) total bile acid concentrations in serum but only moderately in milk, even in Bcrp⁻/⁻ strain. Bile duct ligation (BDL) in pregnant rats markedly increased serum bile acid concentrations, which was not proportionally reflected in milk. In rat mammary tissue Mrp4 was up-regulated by BDL. In 10-day-old neonates of the BDL group, serum bile acid levels were higher (2-fold) whereas their body weight was lower. The exchange of breastfeeding mothers immediately after birth reverted this situation without affecting endogenous neonatal bile acid synthesis.

Conclusion: Bcrp is involved in bile acid secretion into milk, whereas Mrp4 participates in a blood-milk barrier that protects neonates from maternal hypercholanemia during breastfeeding.
Targeting host and microbial choline metabolism by a semi-synthetic bile acid suppresses TMA/TMAO formation and ameliorates atherosclerosis and NASH in mice

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Introduction: Microbial conversion of dietary phospholipids (PLs) produces in a sequential reaction trimethylamine (TMA) in the gut and trimethylamine-N-oxide (TMAO), a pro-atherogenic metabolite in the liver. Herein we investigated the role of biliary PLs to TMA/TMAO formation, and explored whether the biliary PL lowering effect of norursodeoxycholate (norUDCA) impacts on TMA/TMAO formation and concomitant cardiac/hepatic disease progression in ApoE−/− mice.

Methods: Wildtype (WT) and Mdr2−/− (lacking biliary PLs) mice on chow and 5%PL-enriched diet; ApoE−/− mice on (High-Fat/High-Cholesterol) diet ± 0.5% (w/w) of norUDCA. Histology, immunohistochemistry, serum/hepatic lipids, western-blots and qPCR from aortic root and liver tissue; 16S rRNA gene amplicon sequencing of intestinal microbiota; LC/MS and NMR spectroscopy to quantify biliary lipid composition, and TMA/TMAO from serum, urine, and faeces of mice and in vitro cultures of gut-derived microbiota.

Results: Colonic TMA levels were significantly reduced in Mdr2−/− compared to WT littermates, associated with a distinct shift in gut microbiota. Similarly, treatment of ApoE−/− mice with norUDCA resulted in significant reduction of biliary choline, glycerophosphocholine, and phosphatidylcholine, accompanied by reduced faecal- and urinary TMA, as well as urinary/serum TMAO levels. In anaerobic polymicrobial cultures obtained from norUDCA fed animals, or after exogenous addition of norUDCA, TMA formation from choline was inhibited. Notably, ApoE−/− mice fed norUDCA showed a substantial reduction in hepatic steatosis and inflammation, reduced aortic plaque surface area and macrophage infiltration. The anti-inflammatory effects of norUDCA could be associated to reduce TMA/TMAO levels as we demonstrate that both can antagonize LCA-induced TGR5 activity in vitro.

Discussion/Conclusion: We provide evidence that biliary PLs substantially contribute to microbial TMA formation. norUDCA by lowering PL excretion, and by interfering with microbial choline catabolism can block TMA formation in vivo and in vitro. In an animal model of atherosclerosis and NASH, norUDCA is able to ameliorate hepatic- and cardiac disease progression.
Asperuloside, the extraction of Tochu-tea improves metabolic syndrome through the induction of bile acid signaling

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Introduction: Since Tochu has been popular as one of herbal medicine. In Japan, the leaves of Tochu have been widely used to prepare as Tochu-tea which may have anti-obesity properties. We discovered whether “Asperuloside”, which is one of the glycoside contained in Tochu-tea, has anti-metabolic syndrome effect and explored its mechanism.

Methods: C57BL/6J mice into three groups which fed control diet (control), high-fat diet (HFD) and High-fat diet with 0.25% w/w asperuloside (ASP). We performed animal studies including body weight gain, food intake, OGTT, IPITT, bile acids (BA) composition analysis, oxysterols analysis, and gene expression analysis were conducted.

Results: ASP treated group was suppressed body weight gain, body fat accumulation significantly. In Brown Adipose tissue (BAT), ASP increased the expression of Dio2 gene that activated by BAs and inducing energy expenditure. As we expected, remarkably higher expression of the energy consumption related genes (PRDM16, UCP1, PGC1α) was observed in ASP group. In liver, oxysterols analysis, we observed decreasing of LXR ligand 22R-HC. This lead suppressing the expression of fatty acid synthesis genes (SREBP1c, FAS, ACC, SCD-1) in the liver administered ASP. Liver and plasma BA pool size and gene expression of Cyp7a1 were increased in ASP group. Furthermore, inflammation and fibrosis genes (TNFα, IL1b, Col1a1, Col3a1) were remarkably reduced in ASP group.

Discussion/Conclusion: ASP changes cholesterols and bile acids synthesis in liver and activates energy expenditure in the BAT. This suggested ASP improves Type2 diabetes and non-alcoholic steatohepatitis via BA signaling and gut microbiota crosstalk.
Enhanced microbial deconjugation of bile acids in pregnancy represses intestinal FXR-mediated regulation of hepatic bile acid synthesis

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Introduction: Pregnancy is associated with maternal metabolic adaptations, including progressive hypercholanaemia, hypercholesterolaemia and hypertriglyceridaemia, which can develop into maternal metabolic disease. Gut signals modify hepatic homeostatic pathways, linking intestinal content to metabolic activity. We sought to identify whether enteric signals originating in response to intestinal contents contribute to raised serum bile acids observed in human and murine pregnancies.

Methods: Following a standardised diet, we measured FGF19 and 7α-hydroxy-4-cholesten-3-one serum levels in 26 women, and measured distal ileal mRNA expression of FGF15 in C57BL/6 mice (n = 6–10), in non-pregnant and pregnant individuals. To determine the effects on FXR signalling, we exposed human terminal ileal explants and murine intestines in vivo to the hepatic FXR antagonist epiallopregnanolone sulfate. We used shotgun whole genome sequencing and UPLC-MS to determine the caecal microbiome and metabolome, performing targeted and untargeted pathway analyses to predict the effects of the altered metagenome and metabolite profiles. Finally, we supplemented a murine diet with cholic acid to determine whether the observed alterations could be overcome by the presence of an intestinal FXR agonist.

Results: Human and murine pregnancy were associated with reduced intestinal FXR signalling, with lower FGF19/15, and resultant increased hepatic bile acid synthesis. Ileal FXR signalling was not affected by epiallopregnanolone sulfate exposure. Caecal conjugated bile acids were lower in pregnancy due to elevated bile salt hydrolase-encoding Bacteroidetes. Cholic acid supplementation induced intestinal FXR signalling, which was not abrogated by pregnancy. Despite the intestine being loaded with cholic acid, the microbiota of cholic acid feeding was strikingly similar to that of pregnancy, with similar metabolite changes.

Discussion/Conclusion: The altered intestinal microbiota of pregnancy enhances bile acid deconjugation, reducing ileal bile acid uptake and FXR induction. There is, therefore, reduced FGF19/15-mediated hepatic repression of hepatic bile acid synthesis, a proportion of which enters the blood, causing hypercholanaemia.
Increased endogenously synthesized oxysterol accumulation represents an initiating step in fatty liver’s progression toward inflammation

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Introduction: To lower hepatic cholesterol/lipids, StarD1-overexpression was studied in mice to increase mitochondrial cholesterol delivery aiming to drive CYP27A1-initiated pathway of bile acid synthesis. Increased bile acid synthesis and lower liver lipids were associated with increased endogenous oxysterols by unexpected down-regulation of Cyp7b1. Western diet (WD) fed StarD1-overexpressing mice exhibited even greater Cyp7b1 down-regulation. Oxysterols, vital regulatory molecules of cholesterol/lipid homeostasis, in excess have been attributed to cell toxicity. To establish the role of oxysterols as an initiating pathway from non-alcoholic fatty liver (NAFL) to inflammation (NASH), 2 steatosis mouse models and one human correlate were studied.

Methods: B6/129, C57Bl/6 mice were fed a WD or high fructose diet for 2–6 weeks to achieve early fatty liver. Mouse and human non-alcoholic steatosis Cyp7b1 mRNA, oxysterol, inflammatory marker levels, and liver histology were determined.

Results: The 4-week feeding of a WD or high fructose diet to B6/129 or C57Bl/6 mice, respectively, led to > 60% (p < 0.001) repression of Cyp7b1 mRNA levels. Similarly, CYP7B1 mRNA levels in human non-alcoholic steatosis were repressed (~62%; n = 3) as compared to healthy livers. With human steatosis, the decrease in CYP7B1 mRNA levels was associated with a correlative oxysterol increase. Exploring a temporal pattern of progression, 2-week feeding of WD to mice led to a 69% repression of Cyp7b1 mRNA levels coupled with increases in regulatory oxysterols and biochemical evidence of early inflammation (increased IL-1β/ALT). By 6 weeks, Cyp7b1 suppression persisted with WD feeding with further increases in oxysterol/ALT levels, and visible periportal inflammatory infiltrates.

Discussion/Conclusion: Chronically suppressed Cyp7b1 with WD feeding in mice leads to increased hepatic oxysterol levels which initiate/drive inflammation. This model’s trends mimic human genetic CYP7B1 deficiency where chronically elevated oxysterols in CYP7B1’s absence leads to inflammation-driven fibrosis within the first year-of-life.
Pharmacological inhibition of the apical sodium-dependent bile acid transporter (ASBT) protects ileal enterocytes from bile acid-induced injury in adult mice

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Introduction: In neonatal mice, loss of Ostα results in an apparent bile acid-induced injury, oxidative stress and an epithelial restitution response. Germ-line inactivation of the Asbt prevented the ileal injury in Ostα null mice. However, the question remains as to whether the intestinal phenotype can be rescued only early in life. Alternatively, the morphological changes are a manifestation of ongoing bile acid-associated injury.

Hypothesis: Pharmacological inhibition of ileal bile acid absorption in adult Ostα null mice will reverse the ileal morphological and gene expression changes associated with loss of Ostα-Ostβ function.

Methods: Post-weaning, male and female WT and Ostα null mice (C57Bl/6) were fed diet containing an ASBT inhibitor (60 ppm SC435). After 4 weeks, ileal histology, tissue morphometry, and gene expression were analysed.

Results: Administration of an ASBT inhibitor to adult Ostα null mice for 4 weeks was sufficient to reverse the morphological changes resulting in an ileal histology similar to WT mice. Expression of Nrf2/anti-oxidant response element target genes NQO1, GSTa1, GSTa3, GSTa4, GSTmu1 and GSTmu3 were induced from 1- to 5-fold in the Ostα null mice (as compared to WT) and returned to WT levels following administration of the ASBTi.

Discussion/Conclusion: Short-term administration of an ASBT inhibitor to post-weaning adults reverses the ileal phenotype in Ostα null mice. The findings support the model whereby there is a cycle of ileal bile acid accumulation, injury, and repair that is perpetuated by the ongoing synthesis and secretion of bile acids into the small intestine over the life of the mouse. These findings further support a direct role of bile acids in the ileal injury phenotype and a cytoprotective function for Ostα-Ostβ.
Upregulation of the membrane-bound bile acid receptor (TGR5) in response to *Listeria monocytogenes* infection involves Krüppel-like factor 5 (KLF5)

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**Introduction:** TGR5 (Gpbar-1) is a membrane-bound bile acid receptor expressed in macrophages of several organs such as lung, liver and intestine, as well as in peripheral blood mononuclear cells (1–5). Activation of TGR5 in macrophages is associated with a reduced NF-κB-dependent inflammatory response (3, 4). Krüppel-like factors (KLFs) are zinc finger-containing transcription factors that regulate inflammatory responses in endothelial and smooth muscle cells (6, 7). KLFs mediate signalling following activation of macrophages, and could thereby control the development of acute and chronic inflammatory disorders. The aim of the present study is to determine the role of KLF5 in the up-regulation of TGR5 in an animal model for pathogen defense.

**Methods:** TGR5 and KLF5 mRNA expression in bone marrow-derived macrophages (BMDMs) as well as in mice livers were quantified by realtime PCR in relation to an endogenous control (HPRT1 or SDHA). The putative TGR5 promoter was cloned into a pGL3 luciferase expression vector. Luciferase gene expression was used to evaluate the effect of KLF5 on TGR5 expression by co-transfection. Binding of the transcription factors to the promoter was verified by Chromatin Immunoprecipitation (ChIP). Male, 8–12 week old TGR5 knockout (KO) and wildtype (WT) mice were injected intravenously (i.v.) with 8 x 10⁴ CFU/ml *Listeria monocytogenes* (L.m.) and observed for 7 days. Serum levels of AST and ALT were analyzed by using Spotchem-biochemical analyzer. Serum bile acids were analyzed by UHPLC-MS/MS. Flow cytometry has been used to examine immune cells in non-lymphoid tissues.

**Results:** TGR5 and KLF5 mRNA expression was significantly up-regulated in BMDMs as well as in livers from WT mice after L.m. infection. ChIP analysis confirmed the binding of KLF5 transcription factor to the TGR5 promoter region. KLF5 increased the TGR5 promoter activity in a concentration dependent manner. The effect was lost when the binding sites for KLF5 in the TGR5 promoter region were mutated. Our experiments demonstrate that TGR5 KO mice were more susceptible to L.m. infection as shown by increased mortality rates and elevated serum transaminases. Furthermore, TGR5 KO mice had higher liver and spleen Listeria titers, increased liver inflammation, elevated serum inflammatory cytokines and increased serum BA levels after L.m. infection.
**Conclusion:** The TGR5 and KLF5 mRNA expression was significantly up-regulated in macrophages and livers of WT animals after *Listeria* infection, indicating that TGR5 may confer protective effects in the WT animals, most likely through KLF5.

**References:**

Oxysterol sulfates alleviate injured liver function and decrease mortality in mouse models

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Introduction: There are two main pathways, the “neutral” and “acidic,” for the catabolism of cholesterol to primary bile acids in hepatocytes. In the acidic pathway, the delivery of cholesterol to mitochondria by StarD1 was found to be the rate limiting step instead of the mitochondrial enzyme, CYP27A1. Following increase in StarD1 expression, two novel sulfated oxysterols, 5-cholesten-3β,25-diol 3-sulfate (25HC3S) and 5-cholesten-3β,25-diol 3β,25-disulfate (25HCDS), were identified in hepatocyte nuclei. Administration of these oxysterol sulfates in vitro and in vivo resulted in decrease of lipid biosynthesis, suppression of inflammatory responses, and increase of cell survival. In the present study, we tested these oxysterols’ potential to treat LPS- or ATMP-induced acute liver/organ injury in a mouse model.

Methods: Acute liver failure mouse model was established by intravenous injection with LPS or ATMP. The injured liver function was treated with intraperitoneal administration of 25HC, 25HC3S or 25HCDS. Serum enzymatic activities were determined in our clinic laboratory. ELISA assays were used to detect pro-inflammatory factor levels in sera. Western blot, Real-time Quantitative PCR and RT² Profiler PCR Array analysis were used to determine levels of gene expression involved in inflammation and cell apoptosis.

Results: Administration of 25HC3S/25HCDS decreased serum liver-impaired markers; significantly reduced of cytokines and inflammatory cell infiltration in the tissues, and alleviated liver, lung, and kidney injury. Subsequently, the administration increased the survival rate in the LPS- or ATMP-induced mouse model, only 10% of the animals survived in 96 hours without 25HC3S versus 90% survival with the 25HC3S. These effects resulted from the inhibition of the expression of genes involved in the pro-inflammatory response and apoptosis and the simultaneous induction of the expression of genes involved in cell survival. Compared to 25HC, 25HC3S and 25HCDS exhibited significantly stronger effects in these activities, indicating that the cholesterol metabolites play an important role in the inflammatory response, cell apoptosis, and cell survival in vivo.

Discussion/Conclusion: 25HC3S/25HCDS have potential to serve as a novel biomedicine in the therapy of acute liver failure and acute multiple organ failure.

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Calnexin depletion by ER-stress during cholestasis inhibits the Na\textsuperscript{+}-taurocholate cotransporting polypeptide (NTCP)

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Introduction: Cholestasis-induced accumulation of bile acids in the liver leads to FXR-mediated transcriptional downregulation of the bile acid importer Na\textsuperscript{+}-taurocholate cotransporting protein (NTCP) and to induction of endoplasmic reticulum (ER)-stress. However, whether ER-stress affects bile acid uptake is largely unknown. Here, we investigated the role of ER-stress on the regulation and function of the bile acid transporter NTCP.

Methods: ER-stress was induced using thapsigargin or Subtilase cytotoxin in U2OS and HEPG2 cells, stably expressing NTCP. Cellular bile acid uptake was determined using radiolabelled taurocholate (TCA). NTCP plasma membrane expression was determined by cell surface biotinylation. Mice received a single injection of thapsigargin and effects of ER-stress on NTCP mRNA and protein were measured by RT-PCR and western blot analysis. Effect of cholestasis on NTCP and ER-stress were assessed in mice fed a normal or a 3,5-diethoxycarbonyl-1, 4-dihydrocollidine-diet for 7 days. Novel NTCP-interacting proteins were identified by mass spectrometry, interaction verified and functional relevance in relation to ER-stress assessed by co-immunoprecipitation and TCA uptake.

Results: ER-stress induction strongly reduces NTCP protein expression, plasma membrane abundance and NTCP-mediated bile acid uptake. This is not controlled via a single UPR-pathway but mainly depends on the interaction of NTCP with calnexin, an ER chaperone. In mice, expression of both Ntcp and calnexin is reduced by thapsigargin or cholestasis-induced ER-stress. Calnexin downregulation in vitro recapitulates the effect of ER-stress on NTCP.

Discussion/Conclusion: ER stress-induced downregulation of calnexin provides an additional mechanism to dampen NTCP-mediated bile acid uptake and protect hepatocytes against bile acid overload during cholestasis.
Inhibiting NTCP-mediated hepatic bile salt uptake stimulates biliary lipid excretion, independent of changes in bile salt output and hydrophobicity

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Introduction: Biliary lipid excretion is driven by the active transport of bile salts into the canaliculi, followed by excretion of phospholipids and cholesterol. Consequently, disruption of hepatic bile salt uptake, mediated by the Na+-taurocholate Cotransporting Polypeptide (NTCP), is expected to reduce biliary lipid excretion as the flux of bile salts through hepatocytes will be reduced. Interestingly, we observed an unexpected increase in biliary lipid excretion after inhibition of NTCP, using the inhibitor myrcludex B and aim to resolve the underlying mechanisms.

Methods: WT mice, Scavenger receptor B1 (SR-b1) knockout (KO) and ATP-binding cassette sub-family G member 8 (ABCG8) KO mice were injected with vehicle or myrcludex B. Biliary bile salt, cholesterol and phospholipid excretion was determined in bile, collected during gallbladder cannulation. Biliary bile salt species were determined using HPLC.

Results: Myrcludex B treatment in WT mice increased biliary phospholipid and cholesterol excretion by 20% and 50% respectively, whilst biliary bile salt output and bile hydrophobicity were unaffected. Furthermore, protein expression of the cholesterol transport proteins ABCG8 and SR-b1 was unaffected upon NTCP inhibition. In ABCG8-KO mice and SR-B1-KO mice biliary cholesterol output was respectively 60% and 15% lower than in WT mice. Administration of myrcludex B in the ABCG8 and SR-b1 KO models led to an increase in both phospholipids and cholesterol excretion of approximately 50%. The absolute increase in biliary lipid excretion in the KO models, however, was lower compared to WT mice.

Discussion/Conclusion: Inhibition of NTCP-mediated hepatic bile salt uptake in mice increases biliary excretion of phospholipids and cholesterol, independent of biliary bile salt output or hydrophobicity. Although protein levels of ABCG8 and SR-B1 were not altered by inhibition of hepatic bile salt uptake, these proteins do facilitate, in part, the observed increase in biliary lipid excretion after NTCP inhibition.
Gallbladder bile supersaturated with cholesterol in gallstone patients develops chiefly from bile acid shortage worldwide

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Introduction: Gallstone formation requires that bile be supersaturated with cholesterol, which is estimated by cholesterol saturation index (CSI) calculated from gallbladder total lipids and the mol % of bile acids (BAs), cholesterol and phospholipids. While CSI is a good measure of gallstone risk, we hypothesized that comparing mol % gallbladder lipid data creates misconceptions when searching for why CSI is increased in gallstone disease (GSD). We predicted mmol/l levels of gallbladder lipids provide more relevant information to identify which lipids in gallbladder bile that is responsible for elevated CSI in GSD.

Methods: Gallbladder mmol/l data for BAs, cholesterol and phospholipids were retrieved from a study on 232 patients (145 GSD, 87 gallstone-free [GSF]) and compared with the corresponding mol % data.

Results: BA and phospholipid mmol/l levels were 33 and 31% lower ($p < 0.0001$) in GSD patients compared to GSF, while cholesterol was unaltered. CSI was higher in GSD patients than in GSF ($p < 0.0001$) and correlated with gallbladder mmol/l levels of BAs and phospholipids ($p < 0.0001$) but not with cholesterol ($p = 0.542$). Meticulous literature scan confirmed that in 13 studies from 11 countries that gallbladder BA mmol/l levels are reduced in GSD patients while cholesterol mmol/l levels are not increased.

Discussion/Conclusion: Our findings suggest that shortage of BAs, and not increased gallbladder cholesterol is the major cause for why gallbladder bile is supersaturated with cholesterol worldwide.
The FXR agonist GS-9674 reduces fibrosis and portal hypertension in a rat model of NASH

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Introduction: FXR agonists reduced fibrosis and portal pressure (PP) in models of toxic and cholestatic cirrhosis and improve histological features of NASH in patients. We studied the effects of the non-steroidal FXR agonist GS-9674 on hepatic fibrosis and hemodynamics in rats with advanced NASH and evaluated combination therapy with beta-blockers.

Methods: Rats received a choline-deficient high-fat diet for 14 weeks with repeated NaNO2 injections (25 mg/kg i.p., 3x/week) to induce NASH. Vehicle, GS-9674 (30 mg/kg), propranolol (PROP, 25 mg/kg) or GS-9674+PROP were gavaged daily from weeks 4–14. Systemic hemodynamics, PP and superior mesenteric arterial blood flow (SMABF) were measured. Fibrosis was assessed by Sirius-Red area (SRA) and collagen expression. Hepatic stellate cell (HSC) activation was quantified by desmin immunohistochemistry. Target engagement was assessed by qRT-PCR of FXR downstream signaling.

Results: NASH rats had significant steatosis, fibrosis and portal hypertension. FXR-target genes shp, cyp7a1 and fgf15 were dose-dependently modulated by GS-9674 in hepatic and ileal tissue. In GS-9674 treated NASH rats, liver fibrosis (SRA: 4.1 ± 2.0% vs. 10.5 ± 2.9%; p < 0.001) and hepatic collagen expression (~44%; p = 0.032) were significantly reduced, compared to controls. In line, GS-9674 significantly reduced HSC activation (desmin-area: 5.9 ± 0.7% vs. 10.3 ± 0.8%; p = 0.004). Treatment with GS-9674 significantly decreased PP (8.9 ± 2.2 vs. 11.9 ± 2.1 mmHg; p = 0.020), without affecting systemic hemodynamics. The combination of FXR agonist GS-9674 with beta-blockade (GS-9674+PROP) significantly reduced SMABF (10.6 ± 3.9 vs. 14.8 ± 2.6 ml/min/100 g; p = 0.032) without further decreasing PP. However, PROP treatment was associated with decreased heart rate and lower arterial pressure.

Discussion/Conclusion: The non-steroidal FXR agonist GS-9674 reduces liver fibrosis and HSC activation in NASH rats, and decreases portal pressure without deteriorating systemic hemodynamics. The combination of GS-9674 with propranolol additionally decreases mesenteric hyperperfusion.
**Δ⁴-3-oxosteroid-5β-reductase (AKR1D1) deficiency: Responses and long-term outcomes from oral bile acid therapy**

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**Introduction:** Disorders of primary bile acid synthesis may be life-threatening if undiagnosed, or not treated with primary bile acid replacement therapy. To date, there are few reports on the management and follow-up of patients with the Δ⁴-3-oxosteroid 5β-reductase (AKR1D1) deficiency. The aim of this study was to evaluate the therapeutic responses of patients with AKR1D1 deficiency to oral bile acid therapy, specifically chenodeoxycholic acid (CDCA).

**Methods:** Twelve patients with AKR1D1 deficiency, confirmed by fast atom bombardment ionization-mass spectrometry analysis of urine and by gene sequencing, were treated with CDCA or ursodeoxycholic acid (UDCA). The clinical and biochemical responses to therapy were monitored over 0.5–6.4 years. The initial dose of CDCA ranged 5–12 mg/kg/day and dose adjustments were made based on findings from serum biochemistries and urinary bile acid profiles.

**Results:** Physical examination, biochemistries, and sonographic findings improved in all 12 patients during bile acid therapy, except one who underwent liver transplantation. Urine bile acid analysis confirmed a reduction in atypical hepatotoxic 3-oxo-Δ⁴ bile acids concomitant with clinical improvements in those patients treated with CDCA, but not with UDCA. The dose of CDCA required for optimal clinical and biochemical responses varied from 5.5–10 mg/kg per day among patients based on maximum suppression of the atypical bile acids.

**Discussion/Conclusion:** The primary bile acid CDCA is effective in treating AKR1D1 deficiency but the therapeutic dose requires individual optimization based on biochemical and clinical responses. UDCA is not recommended for long-term management of this bile acid synthesis disorder.
Treatment response of murine sclerosing cholangitis to systemic versus intestinal FXR agonists segregates with their effects on hepatic pro-inflammatory cytokine production

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Introduction: FXR agonists are potent therapeutic regulators of liver metabolic function. Their role in the treatment of inflammatory cholestatic liver disease is less defined. Here, we use Mdr2-/- mice, a model of defective canalicular excretion of phospholipids which leads to biliary precipitation of bile acids, cholangiocyte injury, sterile inflammation and fibrosis.

Methods: 45-day-old mdr2-/- female mice underwent oral gavage for 7 days with 30 mg/kg/day of systemic FXR agonist M345 or 100 mg/kg/day of intestinal FXR agonist M379, both derivatives of fexaramine, or with vehicle (corn oil) in controls. Serum liver biochemistries and bile acid levels were determined by colorimetric assays and liver and intestinal gene expression (8 h after the last oral dose of FXR agonist) was quantitated by Taqman-based qPCR.

Results: Compared to vehicle control mice, M345-treated mice displayed improved weight gain (+4.8 vs. -0.9 g compared to baseline; p = 0.00), lower serum liver biochemistries (ALT 298 vs. 981 IU/l; p = 0.01, ALP 149 vs. 197 IU/l; p = 0.03, TB 1.2 vs. 6.8 mg/dl; p = 0.01) and decreased serum bile acid levels (282 vs. 923 umol/l; p = 0.03). In contrast, M379-treated mice showed continued weight loss and no significant change in serum liver biochemistries or serum bile acid levels when compared to controls. While both M345- and M379 significantly induced intestinal Shp and Fgf15 gene expression and reduced hepatic mRNA expression of Cyp8b1 (down 91% vs. 79%; p = 0.22) and liver bile acid levels (down 38% vs. 29%; p = 0.36), only M345-treatment reduced hepatic TNFa mRNA expression when compared to controls (down-89%; p = 0.00).

Discussion/Conclusion: While both systemic and intestinal FXR agonists down-regulate transcription of key enzymes of de novo bile acid synthesis and subsequently liver bile acid concentration, only the systemic FXR agonist M345 represses hepatic TNFa expression, which is associated with attenuation of the sclerosing cholangitis phenotype.
Blood-circulating bile acids support hematopoietic recovery after chemotherapy

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Introduction: Chemotherapeutic agents and irradiation are standard treatments for different types of cancer. However, they can dampen normal hematopoiesis that often causes serious complications including myelosuppression (delayed/failed recovery of myeloid cells). Recovery from myelosuppressive conditions is crucial to avoid a delay and/or discontinuation of treatment protocols. Previously, we have shown that bile acids (BA) support proliferation of hematopoietic stem and progenitor cells (HSPC) in vitro and in fetal development by suppressing the activation of the endoplasmic reticulum (ER) stress (Miharada et al. Cell Rep. 2014; Sigurdsson et al. Cell Stem Cell. 2016). In this study, we investigated the role of BA in hematopoietic recovery after chemotherapy.

Methods: Injection of 5-fluorouracil (5-FU) to mice was used as a model of chemotherapy and recovery in blood analysed by flow cytometry. Cyp8b1 knockout (KO) mice were used as a model for altered BA composition in chemotherapy recovery. TUDCA was injected to increase the level of BA in blood circulation. BA compositions in PB and liver were analysed by mass spectrometry.

Results: Upon 5-FU treatment, BA levels were significantly increased in systemic circulation, specifically, during recovery. Analysis of BA synthetic enzymes in liver indicated that Cyp8b1 expression was significantly elevated during the recovery phase. Cyp8b1 KO mice showed a dramatic increase in (T)UDCA upon 5-FU treatment while some heterozygous mice exhibited lower enzyme (LE) expression and levels of (T)UDCA. The LE animals showed slow HSPC recovery and poor survival in serial 5-FU treatment. Conversely, injection of TUDCA or Salubrinal (an ER stress inhibitor) accelerated recovery upon chemotherapy.

Discussion/Conclusion: Our results indicate that BA are a part of the recovery systems in hematopoietic regeneration, and that they are a potential novel supportive factor for rapid and efficient recovery from myelosuppression.
Roux-en-Y gastric bypass induces elevation of plasma bile acids through disturbed intestinal transit and long-term change of synthesis control

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Introduction: Following Roux-en-Y Gastric Bypass (RYGB) surgery, patients undergo a marked change of bile acid metabolism which is characterized by a gradual elevation of plasma bile acid levels and a change of the shape of the postprandial response. As bile acids are known to affect metabolic regulation, these changes have been implicated in post-RYGB metabolic improvement. However, it remains unclear what mechanism underlies the observed changes. One of the challenges in identifying this mechanism is unraveling the effects of the multitude of simultaneous effects of the surgery in the gastrointestinal system. To identify the main contributing mechanisms, we therefore performed a number of in silico experiments in which we investigated the impact of these RYGB-induced effects on bile acid metabolism one at a time, isolating their contributions.

Methods: We performed the in silico experiments with two mathematical models of bile acid metabolism, describing the enterohepatic circulation and microbiota-bile acid interplay respectively. We systematically applied single changes to the model gastrointestinal system and compared the effects of these changes on plasma bile acid measurements with the changes observed after RYGB.

Results: The single-effect simulations demonstrate that changes of intestinal transit time resulting from altered stomach emptying are likely candidates to explain the short-term effects of RYGB on bile acid metabolism. In contrast, the long-term changes were found to be explained by an additional slow adaptation of FXR-mediated feedback of bile acid synthesis. The final combined RYGB model explains the slow elevation of plasma bile acids, the change of postprandial response and changes to bile acid synthesis markers simultaneously.

Discussion/Conclusion: By using an in silico approach to investigate the changes in bile acid metabolism observed after RYGB, we are able to isolate the effects of otherwise undistinguishable processes and isolate the most likely candidates to explain the underlying mechanism.
The role of the population of hepatic progenitor/oval cells in the process of fibrogenesis in the model of biliary fibrosis induced by bile duct ligation in young Wistar Crl: WI(Han) rats: The transmission electron-microscopic analysis

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Background: Mechanisms underlying hepatic fibrogenesis are not well understood. It is assumed that activated hepatic stellate cells (HSCs)/myofibroblasts and hepatic progenitor/oval cells (HPCs) are involved in experimental models of hepatic fibrosis and may be important to explain this pathology in human liver diseases.

The study objective was the transmission electron-microscopic (TEM) analysis of HPCs, including their relations with adherent hepatic nonparenchymal cells (NPCs), in the rat model of secondary fibrosis induced by common bile duct ligation (BDL) in young rats.

Methods: The study used 6-week-old Wistar Crl (Han) rats after BDL in inhalation anesthesia with a mixture of 2% isoflurane and oxygen for 1, 6 and 8 weeks. There were two comparative groups submitted to the same anesthetic procedure as in the study groups. Fresh small liver blocks (1 mm³ volume) were fixed in a modified Karnovsky’s solution containing 2.5% glutaraldehyde and 2% paraformaldehyde in 0.1M cacodylate buffer, pH 7.4 and routinely processed for TEM analysis.

Results: Ultrastructural analysis based on the BDL rat model demonstrated a pronounced relationship between the increasing portal hepatic fibrosis and marked proliferation of HPCs. The expansion of these cells had two morphological forms: periportal ductular reaction and intraparenchymal isolated hepatic progenitor/oval cells. HPCs formed the proliferating bile ductules or occurred as scattered cells in the already developed or being formed fibrotic foci and at the periphery of regenerative nodules. Our study allowed for distinguishing 4 main types of HPCs: type 0, type I (undifferentiated HPCs), type II - most common (differentiating towards bile duct epithelial cell line, i.e. bile duct-like cells) and type III (hepatocyte-like cells). Interestingly, in a close vicinity of HPCs, NPCs, especially activated hepatic stellate cells (HSCs)/myofibroblasts, including transitional HSCs (T-HSCs), and activated Kupffer cells/macrophages, were commonly found. The phenomenon was accompanied by intensive features of fibrosis. We also analyzed an interesting, unique ultrastructural picture of intercellular contacts between HPCs and adjacent NPCs, especially T-HSCs, playing a crucial role in the process of liver fibrogenesis, very seldom presented in literature. Interestingly, our study documented an extremely rare phenomenon of basement membrane penetration in proliferating bile ductules by
cytoplasmic processes sent by T-HSCs and formation of direct cell-cell contact with ductular epithelial cells related to HPCs. The association between the severity of liver fibrosis and marked proliferation of HPCs may indicate that these cells could send signals to HSCs and thus increase the risk of fibrogenesis in chronic liver diseases.

**Discussion/Conclusion:** Current ultrastructural study indicates that the expansion of HPCs, mainly bile-duct like cells, in the BDL model in young rats occurring in two morphological forms, i.e. periportal ductular reaction form and intraparenchymal isolated hepatic progenitor/oval cells, evidently promotes portal fibrogenesis, probably by involvement in signaling to hepatic stellate cells. Better understanding of the complex cellular interactions observed between HPCs and the adhering NPCs, especially the activated HSCs, might be a potential value in patients with chronic liver diseases and help to develop anti-fibrotic therapies.
The results of ursodeoxycholic acid use in the diagnosis of gallbladder polyps

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Background: With the frequent use of ultrasonography, the coincidental diagnosis of gallbladder polyps has increased. By frequency, gallbladder polyps are cholesterol polyps, adenomyoms, inflammatory polyps and adenoms. While the polyps smaller than 1 cm are controlled, the ones bigger than 10 mm are operated. In the case of cholesterol polyps, operations can be carried out due to the growth and confusion in the diagnosis.

Therefore, by using ursodeoxycholic acid (UDCA), we studied if there would be any changes in the pouch polyps of the patients who were diagnosed with gallbladder polyps not accompanied by cholelithiasis.

Methods: 45 cases (34 women, average age 54.16) who were coincidentally diagnosed with multiple polypoid lesions smaller than 9 mm accompanied by either one pouch or no pouch wall thickness were followed. The cases were checked by using USG in every 3 months. The patients were given 2 x 500 mg UDCA and followed for 6 months. The diameters and the numbers of the polyps were recorded.

Results: In the follow up, the diameter of the polyps did not change in 26.7% (n = 12) cases. In the diameter of the polyps, a decrease by 1.3 mm was detected in the 13.33% (n = 6) of the cases. In the control, no polyps were detected in 40% (n = 18) of the cases. 72.2% (n = 13) of the cases which were detected without polyps in the control had multiple polyps (2–4 polyps) before treatment. 8 of 9 cases were operated on. After the operation 7 of them were cholesterol polyps and the other one was adenomyomathosis. The cases which were not diagnosed after using UDCA and diagnosed after the operation (55.55%) were evaluated as cholesterol polyps. 72% of the cholesterol polyps were not observed in the control with short-term UDCA treatment.

Conclusion: More than half of the gallbladder polyps which are diagnosed coincidentally are cholesterol polyps. Growth in diameter can be detected in the cholesterol polyps in a short time. Cholesterol polyps are often found multiple. With UDCA treatment, they are not detected in the short term controls. Available cases which are thought to have cholesterol polyps not accompanied by cholelithiasis UDCA treatment can be used before the operation or during the follow up duration.
**Interleukin-8 mediates downregulation of TGR5 in biliary epithelial cells, which may contribute to progression of sclerosing cholangitis**

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**Introduction**: Genetic variants in the bile acid receptor TGR5 gene have been associated with primary sclerosing cholangitis (PSC). Biliary epithelial cells show a high expression of TGR5, where activation of the receptor promotes choleretic, anti-inflammatory and anti-apoptosis, supporting the hypothesis that the receptor may play a role in biliary diseases. The aetiology of PSC is poorly understood, but elevated IL-8 in bile and serum is observed in PSC patients. The aim of this study was to characterize the expression, localization and function of TGR5 in biliary epithelial cells from patients with PSC as well as from the Mdr2-/- mouse model of sclerosing cholangitis.

**Results**: TGR5 protein levels as measured by fluorescence intensity on confocal laser scanning microscopy were significantly reduced in the bile ducts of PSC and Mdr2-/- mouse livers as compared to control livers. TGR5 mRNA expression in isolated large bile ducts and isolated intrahepatic biliary epithelial cells showed a decrease of TGR5 mRNA in Mdr2-/- mice as compared to controls. IL-8 homologues KC and MIP-2 were significantly increased in the serum of Mdr2-/- mice. While stimulation with different bile acids had no effect on TGR5 mRNA expression in murine cholangiocytes, treatment of these cells with the IL-8 homologues significantly decreased TGR5 mRNA expression. The downregulation of TGR5 was accompanied by a reduced ERK1/2 phosphorylation. Furthermore, treatment of human cholangiocytes with IL-8 also showed a reduction in TGR5 protein levels.

**Conclusion**: Using immunofluorescence quantification we observed a downregulation of TGR5 protein in biliary epithelial cells from PSC patients and Mdr2-/- mice. Our data show that elevated IL-8 may promote decrease of TGR5 in the biliary epithelium. Preliminary data suggest that transcription factors of the Sp1 family may mediate this reduction of TGR5 which in turn reduces the cytoprotective effects of the receptor in cholangiocytes, thereby promoting disease progression.
A novel, cell-based assay for measuring bile salt transport inhibition by BSEP antibodies in sera from antibody-induced BSEP deficiency (AIBD) patients

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Introduction: Antibody-induced bile salt export pump deficiency (AIBD), an acquired form of intrahepatic cholestasis, may occur after liver transplantation for severe BSEP deficiency. AIBD is mediated via extracellular BSEP inhibition by antibodies arising against the transplanted allo-antigen. To improve diagnosis of this novel disease, we developed an assay for directly testing extracellular BSEP inhibition by patient sera.

Methods: Serum BSEP antibodies were detected by immunoblotting and immunofluorescence staining of both live and fixed BSEP-EYFP-expressing cells. HEK293 cell lines stably expressing either human NTCP-mCherry, BSEP-EYFP, or both were generated by lentiviral transduction and clonal isolation. Using these, we established an assay procedure consisting of preloading cells via NTCP with [³H]-Taurocholate (TC) followed by its BSEP-mediated export into fresh medium. Antibody-mediated BSEP inhibition was tested by preincubating HEK293-NTCP/BSEP cells either with AIBD or control sera depleted of free bile salts or with antibodies purified from these.

Results: Comparison of HEK293 and their transporter-expressing derivative cell lines showed NTCP-mediated [³H]-TC uptake and export by BSEP. The assay consists of an uptake phase dominated by NTCP and an export phase dominated by BSEP. Here, re-import of [³H]-TC by NTCP is abrogated by substituting choline for sodium in the medium. Using this assay, we demonstrated BSEP inhibition by several bile salt-free AIBD serum samples as well as purified AIBD antibodies. Moreover, we adapted assay conditions for a non-radioactive substrate, the fluorescent bile acid Tauro-nor-THCA-24-DBD.

Discussion/Conclusion: BSEP-reactive antibodies targeting extracellular epitopes of the transporter cause AIBD by effectively impairing bile salt transport into the canalicular lumen. Using the cell-based transport assay presented here, we can now test not only for the presence of, but also extracellular inhibition by BSEP-reactive antibodies. This information not only helps corroborating diagnosis of AIBD, but may also improve our understanding of AIBD disease onset and progression.
Effects of bile acid signaling and microbiota during HCC progression in NASH

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Introduction: One of the most prevalent diseases in Western societies represents non-alcoholic-fatty-liver disease (NAFLD) which may proceed to non-alcoholic steatohepatitis (NASH) and hepatocellular carcinoma (HCC). In comparison to most chronic liver diseases, HCC in NASH may occur without pre-existing cirrhosis. We have shown a correlation of serum bile acids (BA) with disease severity in NAFLD so that BA-metabolism emerged as important pathway in tumorigenesis. BA-synthesis is regulated by fibroblast-growth-factor-19 (FGF19) which has been linked to HCC connecting liver and gut. Here we compare mediators of BA-metabolism and investigate a potential liver-gut crosslink based on the microbiome (MB) in NASH and NASH-HCC with and without cirrhosis.

Methods: We analysed serum from NASH-patients without cirrhosis (NO), NASH with cirrhosis (NC), NASH-HCC without cirrhosis (HO), NASH-HCC with cirrhosis (HC), and healthy controls (C). Serum BA, FGF19, cell death markers (M30, M65), and adiponectin were measured. Fecal samples were used to analyze the MB by 16SrRNA-analysis.

Results: In NASH BA and FGF19 were significantly higher than in NASH-HCC, while adiponectin levels were significantly lower. Cell death (M65), beside GGT was significantly higher in NASH-HCC. Comparing patients with and without cirrhosis, we found that BA and FGF19 levels were significantly higher in HC vs. NO, while there was no difference in BA and FGF19 between NC and HO. Here, BA levels are associated with fibrosis, while FGF19 is associated with increased hepatic inflammation and tumour marker levels. MB results at order level unravelled a diminishing abundances of Bacteroidales during disease progression (C>NO>NC>HO>HC). On the contrary, an increasing abundance of Enterobacteriales and Fusobacteriales was observed during disease progression.

Discussion/Conclusion: In NASH-HCC, BA and FGF19 levels were significantly higher as compared to NASH. While BA levels seem to be associated with cirrhosis, FGF19 correlates with hepatocellular injury and tumour markers. Changes in the gut MB were associated with disease progression.
Bile acid alterations are associated with insulin resistance but not NASH in obese patients

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Introduction: Bile acids (BA) are signalling molecules controlling energy homeostasis which exhibit both toxic and protective activities in the liver. BA alterations have been reported in obesity, type 2 diabetes and NASH. However, whether BA alterations are associated with NASH independently of the metabolic status is still unclear. Therefore, we aimed to assess whether BA metabolism and signalling are altered in NASH patients compared to BMI- and insulin sensitivity- matched patients with a healthy liver.

Methods: Obese patients, recruited at the Antwerp University Hospital, underwent metabolic (including BMI and insulin sensitivity assessed by HOMA-IR) and hepatological (including a liver biopsy) assessments. Patients with NASH (n = 32) were matched to no-NASH patients (n = 26) for BMI and HOMA-IR. Plasma BA (21 species) and C4 were determined by liquid chromatography-tandem mass spectrometry. Transcriptomic analyses were performed on liver biopsies. Plasma fibroblast growth factor 19 (FGF19) was measured by ELISA.

Results: When compared to BMI- and HOMA-IR-matched no-NASH patients, NASH patients displayed unaltered plasma BA pool composition or concentrations. By contrast, considering the whole NASH + no-NASH population, primary BA strongly correlated with insulin resistance, as previously reported. Transcriptome analyses showed that NASH status was not strongly associated with altered hepatic BA metabolism. In line, plasma C4, a marker of BA synthesis, was similar in NASH and no-NASH patients. Also, no sign of hepatic BA accumulation or activation of the BA receptors – farnesoid X (FXR), pregnane X (PXR) and vitamin D (VDR) receptors – was found. Finally, plasma FGF19 was unchanged, suggesting unaltered intestinal FXR signalling.

Discussion/Conclusion: In obese patients, BA alterations are associated with the metabolic phenotype, especially insulin resistance, but not with of necro-inflammatory lesions.
Topical intestinal TGR5 agonists promote glucagon-like peptide-1 secretion and improve glucose tolerance

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Introduction: TGR5 is a G-protein-coupled receptor sensitive to bile acids. Its role in various organs, specifically in entero-endocrine L cells and brown adipose tissue, has made it a promising therapeutic target in several diseases, especially type 2 diabetes. However, recent studies have also shown on-target side effects of systemic TGR5 agonists, such as increased volume of the gallbladder. The objective of this project was to design orally administrated topical intestinal TGR5 agonists targeting the distal part of the intestine, where TGR5 and entero-endocrine L-cells are most abundant. These compounds with limited intestinal absorption are expected to be devoid of deleterious systemic effects while stimulating the incretin GLP-1 secreting entero-endocrine L-cells.

Methods: Using an innovative strategy of drug design, we designed chimeric compounds with a pharmacophore, responsible for the pharmacological activity, linked to a kinetophore in a mute position which is not essential for interaction with the target. The activity of the compounds was tested on human and murine TGR5 in a transitory transfection test in HEK cells. Their ability to promote GLP-1 secretion was tested in vivo in murine models.

Results: We have obtained potent (EC50~nanomolar in vitro), weakly permeable TGR5 agonists displaying a strong in vivo GLP-1 secretagogue effect along with a low effect on gallbladder volume. The increased GLP-1 secretion was accompanied by improved glucose homeostasis during an oral glucose tolerance test in diet-induced obese mice.

Discussion/Conclusion: We have obtained proof of concept that these original TGR5 agonists targeting the distal part of the intestine could be potential drug candidates for the treatment of type 2 diabetes, alone or in combination with a dipeptidyl-4 (DPP-4)-inhibitor to sustain elevated GLP-1 concentrations. These agonists are also useful pharmacological tools to decipher the pathophysiological role of intestinal TGR5 in pre-clinical models of metabolic diseases.
Evidence-based clinical guidelines for primary sclerosing cholangitis in Japan 2017

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Introduction: Sclerosing cholangitis is categorized into primary sclerosing cholangitis (PSC), IgG4-related sclerosing cholangitis (IgG4-SC), secondary sclerosing cholangitis (SSC), and therapeutic strategies are to be devised respectively. In principle, PSC should be differentially diagnosed by discriminating other two categories, and thereby to be clinically managed. Recently, we have performed a nationwide survey for PSC and IgG4-SC, and proposed the diagnostic criteria of PSC in Japan (J Gastroenterol. 2017;52:838–44). Thereafter, we have also proposed the clinical management of PSC as “Evidence-based guidelines for PSC in Japan 2017 (unpublished)”, especially focusing on the following points;
1. Characteristics in prevalence
2. Diagnostic algorithm through the stepwise imaging modalities
3. Availability and usefulness of liver biopsy
4. Therapeutic algorithm of nonsurgical and surgical strategies including managements of complications
5. Prognosis with a special interest on transplantation

Methods: References were listed through searching literatures by PubMed, Cochrane library. Modified Delphi method was employed for guideline preparation. The production committee decided guidelines, strength of recommendations and evidence level after reviewed literatures systematically, and guidelines were evaluated by The Expert panel. The Scientific Committee of the Japan Biliary Association (JBA) evaluated revised guidelines, and Public comments were collected on web site of JBA.

Results: Sixteen CQs were listed for Epidemiology/Pathophysiology, Diagnostics, Therapy and Prognosis. In addition, both diagnostic and therapeutic flowcharts were figured.

Discussion/Conclusion: Guidelines for PSC in Japan 2017 preferentially describe the algorithm for diagnosis and treatments including complications, to contribute clinical managements of PSC.

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A real-time bioluminescent method for assessing bile acid transporter activity

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Introduction: The enterohepatic circulation of bile acids is maintained by the action of bile acid transporters, including ileal ASBT and hepatic NTCP. Recent studies have demonstrated that ASBT and NTCP are rapidly regulated by several signaling pathways and by their association with lipid rafts. Traditional methods to assess transporter activity may not sufficiently capture these rapid functional changes. We have designed a reporter system for real time measurement of bile acid transporter activity relying on a novel probe: cholic acid attached to luciferin via a disulfide-containing, self-immolating linker (CA-SS-Luc).

Methods: CA-SS-Luc was synthesized in high yield from cholic acid. In vitro experiments were performed using HEK293 cells co-transfected with luciferase and either ASBT-V5, NTCP-V5, or the serotonin transporter SERT-V5. Ex vivo experiments were performed using intestinal epithelial cells isolated from transgenic mice overexpressing luciferase. Cells were incubated with CA-SS-Luc and imaged using an in vivo imaging system (IVIS) with 1 min or 5 min exposure time.

Results: Incubation of HEK293 cells with different concentrations of CA-SS-Luc (0.01–1 µM) resulted in bioluminescence with an intensity that was concentration- and time-dependent. Additionally, bioluminescence was positively correlated with the amount of ASBT or NTCP expressed in the cells. Interestingly, co-incubation of CA-SS-Luc with natural bile acids enhanced bioluminescence in a concentration-dependent manner with kinetic parameters for ASBT similar to those previously reported using conventional methods. Incubation with tyrosine phosphatase inhibitor III (PTPIII) led to a significant increase in bioluminescence in cells expressing ASBT, consistent with previous studies showing an increase in ASBT function by PTPIII. Further, native ileal enterocytes displayed significantly higher luminescence compared to jejunal enterocytes, corresponding to the expression of ASBT in ileum.

Discussion/Conclusion: We have developed a novel method that faithfully assesses ASBT and NTCP function in real time. This method has potential applications both for in vitro and in vivo studies.
Targeting organic solute transporter alpha-beta to attenuate liver damage induced by bile duct ligation

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Introduction: The organic solute transporter alpha-beta (OSTalpha-OSTbeta) facilitates bile acid efflux mainly in ileal enterocytes, liver and kidney which is expected to protect cells from an overload of bile acids and OSTbeta deficiency leads to congenital chronic diarrhea with features of cholestatic liver disease. Surprisingly, OSTalpha knockout mice displayed attenuated cholestasis-induced liver injury, by increasing urinary bile acid excretion. Since the OSTalpha and the OSTbeta subunit are differentially regulated, we analysed whether deficiency of OSTbeta in mice attenuates or ameliorates cholestatic liver injury.

Methods: OSTbeta knockout mice were generated using the CRISPR/Cas9 system. OSTbeta knockout mice, as well as their wildtype litter mates, were subjected to a bile duct ligation to induce cholestatic liver injury. Histological and biochemical parameters for liver damage were assessed.

Results: While all wildtype mice survived, 4 out of 10 (40%) of the OSTbeta knockout mice died within 5 days. Remarkably, a clear reduction was observed in plasma bilirubin (42%), cholesterol (38%) and bile acid levels (62%) in surviving OSTbeta knockout mice. In general, markers of hepatic inflammation (Mcp1), fibrosis (Timp, α-Sma, Col1a1) and proliferation (AFP) had a tendency to reduce in OSTbeta knockout mice with expression values compared to wildtype of 59%, 35%, 63%, 27%, and 71%, respectively.

Discussion/Conclusion: OSTbeta deficiency shows hepatoprotective effects during bile duct ligation induced liver injury. However, these beneficial effects may come with potential risks, as survival rate of OSTbeta knockouts was significantly lower. Therefore, it is likely that other mechanisms are affected by loss of OSTbeta as well. Solving this mystery may give us a better understanding of the (possible individual) roles of OSTalpha and OSTbeta.
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Introduction: The clinical efficacy of ursodeoxycholic acid (UDCA) in primary biliary cholangitis (PBC) remains subject to debate as definitive randomized controlled trials are lacking. We aimed to determine whether UDCA is associated with prolonged liver transplantation (LT) free survival in PBC.

Methods: Both untreated and UDCA-treated patients with PBC were included from 15 liver units in 8 European and North American countries. The primary endpoint of LT or death was assessed through Cox regression analyses, following inverse probability of treatment-weighing (IPTW).

Results: Of the 3902 patients that were included (mean age: 54.3 [SD 11.9] years/females: 3552 [94.0%]), 3529 (90.4%) were treated with UDCA. During a median follow-up of 7.8 (IQR 4.1–12.1) years, 721 UDCA-treated patients and 145 untreated patients underwent LT or died. After IPTW, the 10-year cumulative LT-free survival was 79.7% (95% CI: 78.1–81.2) with UDCA and 60.7% (95% CI: 58.2–63.4) without UDCA (p < 0.001). Overall, UDCA was associated with a reduced risk of LT/death (adjusted HR = 0.46, 95% CI: 0.40–0.52, p < 0.001). The adjusted HR remained
statistically significant in all stages of disease as well as in other subgroups based on stratified baseline characteristics. The association between UDCA and improved LT-free survival remained among patients treated with < 13 mg/kg of UDCA (adjusted HR = 0.50, 95% CI: 0.43–0.57, p < 0.001), but was stronger for those treated with ≥ 13 mg/kg (adjusted HR = 0.29, 95% CI: 0.21–0.39, p < 0.001). As compared to those untreated, even patients classified as inadequate biochemical responders after 1 year of UDCA had a lower risk of LT/death (adjusted HR = 0.56, 95% CI: 0.45–0.69, p < 0.001). Still, this association was stronger in complete responders (adjusted HR = 0.25, 95% CI: 0.20–0.30, p < 0.001).

**Discussion/Conclusion:** UDCA therapy is associated with improved LT-free survival among patients with PBC, irrespective of the stage of disease or biochemical response. Our findings support UDCA as the current standard of care in PBC.
The postoperative serum course of liver regeneration-associated signaling factors FGF19 and bile salts, in non- and postcholestatic patients undergoing liver resection

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Introduction: Systemic and hepatic bile salt (BS) levels rise shortly after partial hepatectomy (PHx) in rodents, likely providing input signals for the regenerative response. Deficiency of the bile salt receptor FXR or its target gene Fgf15/FGF19, results in delayed liver regeneration (LR) and mortality after PHx. In part, this is due to dysregulated BS homeostasis and attendant BS-toxicity in the remnant liver, and is reminiscent of impaired LR in (post)cholestatic patients undergoing resection for perihilar cholangiocarcinoma (pCCA). The role of bile salt/FGF19 signaling in human LR is unclear. Here, we studied the post-resectional course of these signaling molecules in patients with different hepatobiliary malignancies.

Methods: Data was collected of adult patients undergoing major hepatectomy (≥ 3 segments) at the Uniklinikum Aachen between 01.06.2013 and 01.02.2016 for colorectal liver metastases (CRLM, n = 50) and pCCA (n = 23). Plasma BS, FGF19 and C4 (serum marker of BS synthesis, and surrogate read-out for FGF19 signaling) were determined pre-operatively and on post-operative days (POD) 1, 3 and 7.

Results: Pre-operative bilirubin, AST, GGT and AP were higher in patients with pCCA in comparison with patients with CRLM (p < 0.001). At baseline, BS (3.0-fold) and FGF19 (2.5-fold) were higher, while C4 levels were lower, in patients with pCCA compared with patients with CRLM (p < 0.001). Longitudinal analysis revealed a post-operative increase in BS in the CRLM group. BS and FGF19 showed a marked drop at POD1 and remained low until POD7; following resection for pCCA. C4 levels on POD7 were comparable in both groups.

Discussion/Conclusion: A postoperative increase of circulating BS is apparent in non-cholestatic patients resected for CRLM, potentially providing a stimulus for the regenerative machinery. In contrast, post-resectional lowering of serum BS and FGF19 was observed in (post)cholestatic patients resected for pCCA. Latter changes may negatively impact on the early course of LR in these patients.
Elongation of the fetal PR interval associated with intrahepatic cholestasis of pregnancy is normalised by UDCA therapy

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Introduction: Intrahepatic Cholestasis of Pregnancy (ICP) is typically diagnosed by maternal pruritus and total serum bile acid (TSBA) concentrations ≥ 10 µmol/l. It is associated with an increased risk of intrauterine death and often treated with ursodeoxycholic acid (UDCA). Abnormal cardiac rhythm, e.g. supraventricular tachycardia, bradycardia and elongation of PR intervals in ICP foetuses has been reported. Bile acids are arrhythmogenic in in vitro cardiomyocyte models of the fetal heart, and co-administration of UDCA prevents this. We hypothesise that ICP causes detrimental changes in the fetal electrocardiogram (fECG) and UDCA can normalise this.

Methods: Non-invasive transabdominal overnight fECG data (Monica AN24, Monica Healthcare Ltd) at a 1 kHz sample frequency was collected from women who were ≥ 36 weeks’ gestation. Controls, defined as women with uncomplicated pregnancies, were recruited in addition to patients with untreated and UDCA-treated ICP. Monica DK software was used to select a 2-hour period with minimal fetal heartrate dropout and low maternal movement. The PR interval was measured on an average of up to 1000 fECG waveforms in 5-minute epochs. Statistical analysis was performed via a one-way ANOVA with post-hoc Bonferroni’s test.

Results: The median TSBA was 56 (17–67 µmol/l) in the untreated ICP group and 35 (12–90 µmol/l) in the UDCA-treated ICP group. The fetal PR interval was significantly elongated in patients with untreated ICP (181.7 ± 19.1 ms, n = 6) in comparison to controls (147 ± 11.2 ms, n = 12, p = 0.0003). This elongation was significantly reduced in ICP patients who had undergone UDCA treatment (151.0 ± 14.8 ms, n = 4, p = 0.0105). Results are presented ± standard deviation.

Discussion/Conclusion: We have demonstrated a significant elongation of PR interval in the fECG of patients with untreated ICP, a defect which is known to be a common indicator of atrioventricular block. UDCA treatment normalises the PR interval length.
Inactivation of the intestinal apical sodium bile acid transporter profoundly inhibits cholesterol absorption

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Introduction: Cholesterol enters the intestinal lumen via the diet, via biliary secretion or via transintestinal cholesterol excretion. Cholesterol disposal from the body is achieved by fecal excretion as neutral sterols and bile acids. Inhibition of the intestinal apical sodium-dependent bile acid transporter (ASBT) increases both fecal bile acid and neutral sterol excretion and inhibits atherosclerosis in animal models. We aimed to determine whether the increase in fecal neutral sterol excretion upon ASBT inactivation is due to decreased cholesterol (re)absorption or enhanced transintestinal excretion.

Methods: To differentiate between these two mechanisms, Asbt<sup>−/−</sup> mice and wildtype (WT) littermates were fed chow with or without 0.005% (w/w) ezetimibe, an intestinal cholesterol absorption inhibitor. We measured and calculated cholesterol fluxes originating from the diet, the bile and from across the intestine, using mass determinations and a dual stable isotope method.

Results: Fractional cholesterol absorption was profoundly lower in Asbt<sup>−/−</sup> mice compared to WT controls (5.7% vs. 46.2%). Ezetimibe treatment virtually abolished fractional cholesterol absorption in Asbt<sup>−/−</sup> and in WT mice (0.8% and 4.0%, respectively). Fecal excretion of neutral sterols (consisting of cholesterol and its intestinal metabolites) was threefold higher in Asbt<sup>−/−</sup> mice, compared with WT mice. Ezetimibe treatment to Asbt<sup>−/−</sup> mice did not further increase or affect fecal neutral sterol secretion. The strong inhibition of cholesterol absorption by ezetimibe in WT mice increased fecal neutral sterol secretion to a similar level as in untreated or in ezetimibe-treated Asbt<sup>−/−</sup> mice. Flux calculations indicated that the predominant fraction of fecal neutral sterols originated from transintestinal excretion of cholesterol that was not reabsorbed upon ezetimibe treatment.

Discussion/Conclusion: Prevention of intestinal bile acid reabsorption by ASBT inactivation is as effective as ezetimibe in inhibiting intestinal cholesterol (re)absorption and, thereby, in increasing total fecal sterol excretion Combining ASBT inhibition with ezetimibe treatment results in a higher total fecal sterol excretion which could have an additional benefit for the treatment of hypercholesterolemia and atherosclerosis.
Effect of ursodeoxycholic acid on biochemical markers of cholestasis in children with progressive intrahepatic cholestasis of type 1 and type 2

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Introduction: Treatment of progressive family intrahepatic cholestasis of type 1 and type 2 (PFIC1-2) in children is a very complex problem. One of the main components of complex therapy is the use of ursodeoxycholic acid (UDCA).

The scope and methods of research: Twenty-three children with PFIC1-2 were under observation: 17 boys and 6 girls aged 1 month to 4 years 9 months (mean age 13 ± 3.5 months).

All children received UDCA at a dose of 14 to 32 mg/kg/day (20.0 ± 6.0 mg/kg/day). A biochemical blood test was performed before the treatment and 3 weeks after the beginning of the treatment. The parameters of total and direct bilirubin, gamma-glutamyltranspeptidase (GGTP), alanine aminotransferases (ALT), aspartate aminotransferase (AST), cholesterol, alkaline phosphatase (AP) were evaluated. The level of bile acids in the blood serum was determined.

Results: Before the treatment of UDCA, the level of total bilirubin was 130.5 ± 13.07 μmol/l, direct bilirubin – 82.9 ± 7.35 μmol/l; the level of cholesterol was 5.31 ± 0.51 mmol/l, GGTP 37.5 ± 3.15 U/l, AP 439.5 ± 4.27 U/l, ALT 319.5 ± 31.6 U/l, AST 429.5 ± 42.9 U/l. The level of bile acids in the blood serum was 145.7 ± 12.34 μM/l.

Three weeks after the start of treatment with UDCA, the level of total bilirubin decreased to 80.5 ± 8.02 μmol/l (p = 0022), direct bilirubin – to 63.8 ± 5.25 μmol/l (p = 0.0403); the cholesterol and GGTP indices did not change significantly and amounted to 5.20 ± 0.52 mmol/l (p = 0.8807) and 31.5 ± 3.10 U/l (p = 0.1817), respectively. The level of AP decreased significantly, which was 329.7 ± 3.24 U/l (p = 0.0000), the level of ALT was 82.3 ± 8.28 U/l (p = 0.0000), AST 154.4 ± 15.2 U/l (p = 0.0000). The level of bile acids in the blood serum decreased, which amounted to 113.5 ± 10.04 μmol/l (p = 0.0492).

Conclusions: The use of UDCA in the treatment of PFIC1-2 in children significantly improves biochemical indicators that are markers of cholestasis and reduces the level of cytolytic activity (ALT and AST).
A physiology-based model of the distribution of individual bile acids within the enterohepatic circulation under normal and pathological conditions in humans

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Introduction: Bile acid (BA) distribution and biotransformation within the enterohepatic circulation (EHC) are influenced by multiple factors including surgical procedures (i.e. ileostomy), cholesterol-lowering treatments, gut microbiota, and various disease entities (i.e. cholestasis and diarrhea). We investigated the effects of such perturbations of the EHC, as well as normal inter-individual variation in BA fluxes, on levels and dynamics of cholic (CA), chenodeoxycholic (CDCA) and deoxycholic (DCA) acids in different organs, using a quantitative, physiology-based model.

Methods: The proposed model consists of a system of differential equations describing free and conjugated CA, CDCA and DCA distributions within the systemic circulation, the hepato-portal region, and the gastro-intestinal (GI) tract. The core model was taken from previously published work (Hofmann et al, 1983) updated with recent mechanistic insights, e.g., farnesoid-X receptor (FXR)-mediated regulation of BA synthesis. The model was calibrated using fasting BA levels in the systemic circulation, portal vein, liver, duodenal bile and GI tract, as well as daily dynamics of BA, FGF-19 and C4 in serum of healthy subjects.

Results: The calibrated model was used to simulate different experimental scenarios. Model simulations performed for healthy subjects with variable GI motility (± 50% intestinal transit variability) showed that GI transit time positively correlates with total BA levels and negatively correlates with time of BA maximum peak after meal intake. Simulations of an intestinal BA malabsorption scenario (absorption variation from 0 to default value) showed that FXR downregulation, followed by strong activation of BA synthesis, represents a key mechanism of BA pool maintenance, but at the same time enhances colonic BA accumulation.

Discussion/Conclusion: Hence, the proposed model can be used to study BA distribution under various conditions of perturbations. It also represents a predictive tool allowing delineation of mechanisms determining inter-patient variability in BA dynamics.
Obeticholic acid compassionate use therapy for severe primary bile acid diarrhoea

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Introduction: Bile acid diarrhoea (BAD) is caused by excess levels of unabsorbed bile acids in the colon. Primary, idiopathic, BAD is thought to result from increased hepatic bile acid synthesis, and reduced levels of the ileal hormone FGF19, which regulates synthesis, have been shown. FGF19 is an FXR-responsive gene. Obeticholic acid (OCA), a semi-synthetic FXR agonist drug, at 25 mg daily, was previously shown in a two-week open trial to stimulate FGF19 and to improve symptoms in primary BAD. A prolonged response to OCA at 10 mg is now reported.

Methods: Patients with severe, unresponsive BAD have been treated under a compassionate use scheme from Intercept Pharmaceuticals. Symptom diaries, including stool frequency, type (Bristol stool form scale), urgency and bloating were recorded daily. The first patient is a 60-year-old woman with chronic watery diarrhoea for more than 5 years. Her SeHCAT test was in the severe range at 1.3%. The second patient is a 66-year-old woman who had variable diarrhoea for 30 years with a SeHCAT 0.5%. Both had normal colonoscopy with biopsy, no other major problems and had tried various bile acid sequestrants without significant benefit.

Results: The total stools/week improved, from a baseline of 76 to 59 at week 4 in patient 1, and from 42 to 21 in patient 2. Improvements in mean stool type also occurred: 7.0 to 6.4, and 5.9 to 4.6. With this 10 mg OCA dose, little change was found by week 2, unlike the response seen before with 25 mg. Further improvements occurred in patient 1 up to week 10, although this was accompanied with an increase in bloating and mild abdominal pain.

Discussion/Conclusion: Patients with severe BAD can respond to OCA 10 mg/day, although the onset of this response is slower than with 25 mg. Further trials of FXR agonists are warranted.
Expression of mir-21 and mir-150 in patients with primary biliary cholangitis (PBC)

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Introduction: PBC is a cholestatic disease characterized by the presence of AMA autoantibodies. PBC development is accompanied by the expansion of lymphocytes into liver, followed by liver fibrosis. MicroRNA-21 is engaged in hepatic fibrogenesis as well as in immune regulation via Ras-guanyl-nucleotide releasing protein-1 (RasGRP1). Mir-150 is known to shape lymphocytes biology. Expression of mentioned above factors was assessed in livers, peripheral blood mononuclear cells (PBMCs) and sera of PBC patients.

Methods: Serum samples were collected from patients with PBC (n = 76) and controls (n = 19). PBMCs were isolated from 15 patients with PBC and 8 controls. Hepatic specimens were obtained from explanted PBC livers (n = 21) and control tissue (n = 14). Mir-21, mir-150 and RASGRP1 expressions were assessed by Real-time PCR TaqMan Assays.

Results: In PBC livers, expression of mir-150 was increased (5.4-fold, p < 0.001, vs. control) while the level of mir-21 was unchanged. In contrast, in PBMCs from PBC mir-150 expression remained at the control values but mir-21 showed enhanced expression (1.7-fold p = 0.002 vs. control) associated with decrease of RASGRP1 mRNA (2.5-fold, p = 0.002). Overall serum levels of both microRNAs were comparable in PBC and controls. However, mir-21 was significantly elevated in non-cirrhotic PBC vs cirrhotic (2.7-fold, p = 0.02). Moreover, expressions of both mir-21 and mir-150 were significantly increased in AMA-negative patients in comparision to AMA-positive (5.7-fold and 1.9-fold, respectively). Unlike in AMA-positive, in AMA-negative patients there was a strong correlation between mir-21 and mir-150 (R = 0.83, p < 0.001).

Discussion/Conclusion: There is a divergent profile of mir-21 and mir-150 expressions in livers and PBMCs of PBC patients. Expression of mir-21 was negatively related to progression of the disease. Difference between AMA-negative and AMA-positive patients in terms of analyzed miRNAs’ requires further investigations.

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Porphyran, a functional ingredient of Japanese “Nori”, improves visceral obesity and non-alcoholic fatty liver disease via alteration of bile acids and intestine interactions in mice and humans

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Introduction: Porphyran (PP) is a major component of “Nori”, the typical Japanese food made from red algae. Previous studies have been shown that the PP protected from fat accumulation and progression of insulin resistance. To investigate the detailed mechanism of these effects of PP, we focused on bile acids signaling pathway and gut microbiota interactions. We have also conducted randomised clinical trials.

Methods: C57BL/6J mice were fed on high-fat diet mixed with 2% w/w PP for 13 weeks. Liver, ileum and colon were collected. Total RNA was extracted from each tissues and the microarray was performed. To clarify the effects of PP on the intestinal barrier function and gut microbial populations, 16S rRNA gene sequences were analysed by using the feces. In addition, randomised clinical trials have conducted to confirm effects of PP in humans.

Results: In the PP group, the bile acids signaling pathway was changed and deoxycholic acid level in the feces were decreased in PP group. This reduction was also observed in humans. Analysis of microarray suggested that the signal pathway of sphingolipid synthesis was inhibited in liver and C16-ceramide contents were lower in PP group by the LC/MS/MS methods. Increase of T-β-MCA, acts as an intestinal FXR antagonist may cause the inhibition of the ceramide synthesis related genes expression. In the intestine, O-glycan biosynthesis and tight junction pathway were upregulated in PP group. We also clarified that PP markedly elevated the amount of fecal mucin as an intestinal barrier function. The analysis of gut microbiota identified secondary bile acid synthesis was significantly decrease in the PP group.

Discussion/Conclusion: In summary, we have shown that PP changed the BA composition and improved intestine environment. Effects of PP on obesity, fatty liver and insulin resistance partially be explained by bile acids and intestine interaction pathway.
Humanized bile acids lead to increased fibrosis in a toxin-induced mouse model of extrahepatic bile duct injury

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Introduction: Biliary atresia (BA) is a progressive fibro-obliterative disease of the extrahepatic bile duct (EHBD) that affects neonates. Recent data suggest that EHBD injury begins in utero. Our lab previously identified a toxin, bilatresone, which is responsible for large outbreaks of BA in neonatal livestock after maternal exposure. We administered bilatresone to pregnant mice and observed EHBD damage in pups; however, this damage appeared to be reversible as no pups progressed to complete EHBD obstruction. We hypothesized that the toxicity of specific bile acids contributes to recovery or progression from the original injury. By humanizing the bile acid composition in mice, we predicted increased EHBD damage.

Methods: Pregnant BALB/c mice were fed chow with 0.3% cholic acid (CA) or 0.3% glycochenodeoxycholic acid (GCDC) starting at gestational age 12–13 and until weaning. They were treated with bilatresone once a day for two days starting at gestational age 15 or 16. Pups were euthanized at days 7 and 21 and EHBDs examined by immunostaining for damage.

Results: The cholangiocyte monolayer was disrupted in 7 day old pups but not in 21 day old. Pups from mothers fed with GCDC or CA and treated with bilatresone had small litter sizes and increased early death. EHBDs in some but not all pups showed increased submucosal fibrosis. Mothers were unaffected, with normal EHBD and liver histology.

Discussion/Conclusion: The neonatal EHBD is susceptible to cholangiocyte monolayer disruption after administration of the toxin bilatresone to mothers during gestation. Fortifying chow with humanized bile acids increased early death and EHBD fibrosis. This suggests that EHBD damage in this model may be a 2-hit phenomenon and highlights the importance of bile acid toxicity in progression of EHBD damage.
Dietary protein quality and quantity changed energy metabolism via liver and intestine interactions signals

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**Introduction:** Caloric restriction (CR) without malnutrition is one of the most promising methods for anti-aging and decrease various diseases including metabolic syndrome. It has been suggested that the balance of macronutrients rather than calorie intake determines the effect of CR on improving metabolic syndrome. However, detailed mechanisms has not been clarified.

**Methods:** C57BL/6J mice were fed on different protein balance (8, 15, 30\% of energy) and protein quantity (soy, casein and edible insects) with controlled fat intake (30\% of energy) for 22 weeks. Glucose metabolism was explored by oral glucose tolerance test (OGTT) and insulin tolerance test (IPITT). Bile acids composition, gut microbiota by analysing 16S rRNA gene sequences and metabolite by CE-TOF-MS in cecum and plasma were analysed.

**Results:** Compared with normal protein (15\%), low protein (8\%) diet reduced body weight and fat accumulation. Also, blood glucose control and insulin resistance were improved in low protein diet and soy protein group. Fecal BA excretion was dramatically increased (more than four times) in edible insects group. The analysis of gene expression using qPCR has shown that expression patterns of BA synthesis related genes were differed according to the protein quality and quantity. In addition, FGF21 in liver was increased and FGF15 in small intestine were reduced in low protein group. An urinary urobilinogen was increased in soy and edible insects group which imply modified gut microbiota composition. It was confirmed by 16S rDNA gene analysis. The results of enrichment and metabolite analysis identified increased amount of polyamine which has recently emerged as exhibiting anti-aging properties in cecum in low protein group than normal protein group.

**Discussion/Conclusion:** Our findings provide new insights into mechanisms behind health benefits of dietary protein quality and quantity and potential role in anti-aging.
The mechanistic target of rapamycin complex 1 (mTORC1) regulates bile acid biosynthetic and transporter gene expression via activity of the farnesoid X receptor (FXR)

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Introduction: Bile acids (BAs), via the nuclear receptor FXR, regulate a complex transcriptional program to maintain fat, glucose, and protein metabolism. The mTORC1 signalling pathway integrates diverse nutrient/hormonal signals into FXR-related metabolic outputs, raising the question whether these transcriptional and posttranslational cascades are intertwined to maintain cellular homeostasis. We tested the hypothesis whether mTORC1 regulates FXR-dependent gene transcription.

Methods: We therefore explored (i) the effect of mTORC1 using different mTORC1 inhibitors (mTORC1i); (ii) FXR signalling by using different hepatocyte and intestinal cell lines and natural (CDCA), semi-synthetic (INT-747) and synthetic (GW4064) FXR ligands. RNA-seq was performed followed by unbiased pathway analysis; qPCR to validate gene expression. mTORC1 activity was investigated by Western blot. Cycloheximide (CHX) and Actinomycin D (ActD) were used to assess protein and mRNA stability.

Results: RNA sequencing and gene expression analysis of immortalized human hepatocytes after mTORC1i revealed significant negative enrichment of differentially regulated genes for bile acid metabolism and cholesterol homeostasis. Moreover, mTORC1i selectively blocked stimulated FXR target gene expression (i.e. Shp, Bsep, Fgf19) in several hepatocytes and intestinal cell lines treated with CDCA, INT-747 or GW4064. Notably, mTORC1i resulted in reduced FXR protein. Blocking protein synthesis with CHX in the presence of mTORC1i did not further reduce FXR protein, arguing for a mechanism independent of protein degradation. Remarkably, mTORC1i reduced FXR mRNA over time and could not additively be reduced by ActD indicating that mTORC1 controls FXR protein via transcriptional regulation of FXR mRNA levels.

Discussion/Conclusion: Our data demonstrate that mTORC1 substantially affects bile acid metabolism and cholesterol homeostasis at the level of gene transcription, which in part are controlled through the regulation of FXR activity.
Renal lesions in HSD3B7 deficiency resolved with primary bile acid replacement therapy

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Background and aims: 3-Hydroxy-5-C27-steroid oxidoreductase (HSD3B7) deficiency is the most common bile acid synthesis defect, typically presenting as neonatal cholestasis, liver injury, fat-soluble vitamin malabsorption and rickets. Atypical bile acids with an incomplete modified cholesterol nucleus accumulate and are cleared by renal excretion in urine. These atypical bile acids injurious to the liver but their effects on kidneys is not appreciated. By retrospective and prospective studies of patients with HSD3B7 deficiency and BSEP deficiency (PFIC-2), renal lesions were observed as an important feature of HSD3B7 deficiency and furthermore were relieved by primary bile acid replacement therapy.

Methods: Genetically confirmed HSD3B7 deficiency patients (n = 22) were compared to patients with BSEP deficiency (n = 40) for serum biochemistries and renal ultrasounds or other images. Clinical data were collected retrospectively from medical records or prospectively at each clinical visit.

Results: In patients who received kidney imaging study, renal lesions were documented in eight HSD3B7 deficiency patients comparing to one BSEP deficiency (8/14 vs. 1/39, p = 0.0012). In HSD3B7 deficiency patients, one presented with multiple small cystic high signal in bilateral renal medulla by MRI (P2, age at first clinic visit, 1.5y, with nearly normal liver function tests), one multiple abnormal echoes in the calyx (P5.5mo), one renal stones (P8 4.5y), one renal cysts with calcification (P15, 6.5y), one renal cysts with stones (P6, 17y), one progressively abnormal signals (P16, 5mo and 6mo), one calcareous infarct (P21, 11mo), one simple renal cysts (P22, 5yr); In patients with BSEP deficiency, the patient presented with left sided mild hydronephrosis by renal ultrasounds, that is different from the manifestation of HSD3B7 deficiency. 7 patients with HSD3B7 deficiency were initially treated with ursodeoxycholic acid, atypical bile acids in urine and renal lesions persisted, though LFTs normalized in 3 of them. With chenodeoxycholic acid administration (n = 4), both LFTs and urinary bile acid profile were normalized, as well resolved renal lesions.

Conclusion: To our knowledge, this is the first report to document a significant association of renal lesions in HSD3B7 deficiency, and to demonstrated that renal lesions could be reversed by bile acid replacement therapy. These findings make a case for screening for bile acid synthesis disorders in patients with both renal lesions and liver disease.
Taurocholate upregulates IncRNA H19 in cholangiocytes and activates hepatic stellate cells via exosome release under cholestatic conditions

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Introduction: Accumulation of bile acids, especially conjugated bile acids, plays important roles in the pathogenesis of cholangiopathies. Long non-coding RNA (IncRNA) is a novel class of master regulators of gene expression and involved in regulating many physiological and pathological processes. Our recent studies showed that cholangiocyte-derived exosomal IncRNAH19 is an important player in cholestasis. However, the effect of bile acids on IncRNAH19 expression and hepatic stellate cell (HSC) activation remains unclear and is the focus of the current study.

Methods: Cultured mouse large cholangiocytes (MLE), human H69 and LX2 cells, primary cholangiocytes and HSCs from wild type (WT), Mdr2⁻/⁻ and H19⁻/⁻ mice were used this study. Exosomes were isolated from culture media and mouse serum by ultracentrifugation and characterized. The MLE-derived exosomes were labelled with PHK67 to track the uptake by different hepatic cells. The collagen gel contraction assay was used to determine HSC contraction. Both Mdr2⁻/⁻ and bile duct ligation (BDL) mouse models of cholestatic liver injury were used.

Results: Taurocholate (TCA) dose- and time-dependently induced the expression of IncRNAH19 in MLE cells. Hepatic IncRNAH19 level was correlated to the activation of HSCs and cholestatic liver injury in both Mdr2⁻/⁻ and BDL mouse models. Cholangiocyte-derived exosomes were taken up by all hepatic cells in the following order: Kupffer cells>HSCs>Hepatocytes. Cholangiocyte-exosomal H19 significantly induced activation and contraction of HSCs. Knocking-down H19 in MLE cells had no effect on exosome release, but blocked TCA-induced activation of HSCs. In contrast, H19 overexpression-derived MLE exosomes significantly induced activation of HSCs. Furthermore, BDL-induced cholestatic liver injury was significantly reduced in H19⁻/⁻ mice.

Discussion/Conclusion: The current study shows that bile acid-induced H19-containing exosomes from cholangiocytes play a critical role in cholestatic liver injury by activating HSCs. This study suggests that serum cholangiocyte-derived H19-containing exosomes can be used as a potential biomarker for cholangiopathies.
Immunomodulatory mechanisms of the novel therapeutic bile acid 24-nor-ursodeoxycholic acid

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Introduction: Primary sclerosing cholangitis (PSC) presents a progressive immune-mediated liver disease, currently lacking effective medication. In a recent European multicenter phase II clinical study for PSC, nor-ursodeoxycholic acid (norUDCA), a side chain-shortened derivative of UDCA improved cholestasis irrespective of previous response to UDCA. However, potential immunomodulatory mechanisms of norUDCA are still poorly understood. Since CD8 T-cell trafficking along the gut-liver axis is an important pathophysiological factor in PSC, we aimed to explore how norUDCA impacts on CD8 T-cell immunity.

Methods: The potential modulatory effects of norUDCA on CD8 T-cell immunity were assessed in vitro by proliferation and differentiation assays as well as in vivo, in lymphocytic choriomeningitis virus (LCMV) model of CD8 T-cell driven hepatic immunopathology and in Mdr2 (Abcb4) KO mouse model of PSC.

Results: In vitro, norUDCA but not UDCA reduced CD8 T-cell proliferation and lymphoblast formation by 53% compared to untreated controls (p < 0.05). Mechanistically, mTOR(Ser2448) phosphorylation of CD8 T-cells was suppressed by norUDCA by 40% (p < 0.005). In line, norUDCA reduced mTORC1 and mTORC2 kinase activities as reflected by lower phosphorylation level of P70 S6 Kinase(Thr421/Ser424) and AKT(Ser473) by 41% (p < 0.05) and 53% (p < 0.05), respectively. Additionally, norUDCA strongly reduced mean fluorescence intensity of mTOR dependent GranzymeB and IFNγ by 73% and 56%. Moreover, similar to rapamycin, glycolysis but not oxidative phosphorylation in activated CD8 T-cells was inhibited by norUDCA as shown by a reduction in extracellular acidification rate without a change in oxygen consumption rate. In vivo, norUDCA ameliorated hepatic injury and inflammation induced by LCMV as shown by lower ALT (norUDCA+LCMV 88 U/l vs. LCMV 209.6 U/l, p < 0.005) and decreased liver expression of GranzymeB by 41% (p < 0.005) and Cxcl10 by 40% (p < 0.005). Supporting our in vitro finding, norUDCA remarkably reduced CD8 effector T-cell frequency in the liver of LCMV infected mice. Notably, this reduction led to improved control of inflammation without compromising quality of viral-specific CD8 T-cell immunity. Additionally, norUDCA (but again not UDCA) had similar impact on reducing liver CD8 T-cell number in Mdr2 KO mice.
**Discussion/Conclusion:** Overall, we unraveled novel anti-inflammatory mechanisms of *norUDCA* impacting mTOR signaling and distinct from UDCA, indicating that *norUDCA* may represent a promising immunometabolic drug for treatment of T-cell based inflammatory liver diseases including PSC.
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