XXVIII INTERNATIONAL BILE ACID MEETING: BILE ACIDS IN HEALTH AND DISEASE 2026

July, 3-4, 2026

Symposium 244
VIENNA, AUSTRIA

11,5 credit hours (CME) have been awarded by the European Union of Medical Specialists (UEMS).

PREFACE

Since the last International Bile Acid Meeting in Edinburgh in 2024, the field of bile acid research has continued to flourish. New insights have been gained into the role of bile acid signaling in the liver and intestine, the role of bile acids and their nuclear or membrane receptors in the gut-liver axis, bile acid microbiome interactions, bile acid control of immune reactions and malignant tumor development. Therapeutic bile acids like ursodeoxycholic acid (UDCA) and norucholic acid (NCA) as well as agonists for the nuclear farnesoid-X receptor (FXR) and the peroxisome proliferator-associated receptors (PPARs)α, β/δ and γ continue to be discussed as potential therapeutics not only for cholestatic liver diseases but more recently also for metabolic dysfunction-associated steatohepatitis (MASH) and other hepatobiliary disorders. Furthermore, availability of inhibitors of the ileal bile acid transporter ASBT for treatment of paediatric progressive familial intrahepatic cholestasis (PFIC) and Alagille's syndrome as well as inhibitors of the hepatic bile acid transporter NTCP for chronic HBV/HDV coinfection have opened potential therapeutic opportunities also for evaluation in other hepatopathies.

The XXVIII International Bile Acid Meeting will be dedicated to both, basic and clinical aspects of bile acid research with a focus on the role of bile acid transport and signaling in health and disease, the interaction of bile acids with the microbiome and the immune system, and the role of bile acids in tumor development. Novel aspects of therapeutic strategies using bile acid derivatives, bile acid receptor agonists or bile acid transporter inhibitors represent a focus of this conference. The latest findings will be presented by leading scientists and clinicians in these fields.

During the symposium, poster sessions will take place which will receive additional attention. In line with the tradition of the International Bile Acid Meetings several poster abstracts will be selected by the scientific committee and the authors will be invited for oral presentations.

The organizers of the XXVIII International Bile Acid Meeting look forward to welcoming you to Vienna.

Ulrich Beuers *Verena Keitel-Anselmino* *Michael Trauner*

XXVIII INTERNATIONAL BILE ACID MEETING: BILE ACIDS IN HEALTH AND DISEASE 2026

July, 3-4, 2026

Scientific Organization:
Ulrich Beuers, Amsterdam
Verena Keitel-Anselmino, Magdeburg
Michael Trauner, Vienna

Congress Venue:
Hyatt Regency Vienna
(formerly Andaz Vienna am Belvedere)
Arsenalstrasse 10
1100 Vienna
Austria

For admission to scientific events your name badge should be clearly visible.

Accompanying persons are not permitted during the conference at any time.

Start of Registration:
Thursday, July 2, 2026
16:00 – 20:00 h
at the congress office

FRIDAY, JULY 3, 2026

8:30	Welcome *Michael Trauner, Vienna*

SESSION I

Bile acid transport in health and disease

Chairs	*Paul A. Dawson, Atlanta; Stan F. J. van de Graaf, Amsterdam*
8:40	Molecular insights into the key bile acid uptake transporters NTCP and ASBT *Stan F. J. van de Graaf, Amsterdam*
9:00	East Asian "intrahepatic cholestasis of pregnancy" may be linked to a historic HBV epidemic *Siyang Liu, Shenzhen*
9:20	Repurposing reactions: How fatty acids use bile acid pathways to make and move bioactive molecules *Trisha J. Grevengoed, Copenhagen*
9:40	Bulevirtide-induced elevation of plasma bile salt levels alleviates DSS-induced colitis and LPS-induced inflammation in mice *Coen C. Paulusma, Amsterdam*
10:00	Novel insights to the multiorgan effects of systemic ASBT inhibition *Paul A. Dawson, Atlanta*
10:20	**Coffee break with ePoster session**

SESSION II

Bile acids in the intestinal microenvironment

Chairs	*Bernd Schnabl, La Jolla; Mark S. Sundrud, Lebanon*
11:00	Bile salt hydrolases catalyse formation of amine-conjugated bile acids *Andrew D. Patterson, University Park*
11:20	Effects of secondary and tertiary intestinal bile acid derivatives on host immune function *Jason M. Ridlon, Urbana*

11:40	Low dietary fiber alleviates bile acid–induced cholangiopathy via microbiota-derived hepatoprotective UDCA *Wenqiang Shen, Groningen*
12:00	Multi-modal analysis defines bile acid–induced stress as a rewiring mechanism of epithelial–immune communication in Crohn's disease *Claudia Günther, Erlangen*
12:20	**Lunch break with ePoster session** *Please see page 10 for further details*

SESSION III

Bile acids and immunity

Chairs	*Clarissa Campbell, Vienna ; Alexander G. Miethke, Cincinnati*
14:00	Intestinal bile acid metabolism/signaling and immunity *Mark S. Sundrud, Lebanon*
14:20	Bile acids engage the SIPR-STAT3 signaling axis to modulate regulatory T cell responses in fibrosing cholangiopathies *Alexander G. Miethke, Cincinnati*
14:40	Carbonic anhydrase autoantibodies in PSC and IgG4-related cholangitis: Breaking cholangiocellular bile acid defence? *David Trampert, Amsterdam*
15:00	Mitochondrial dynamics and autophagy are disturbed in patients' livers and experimental models of PBC, contributing to disease pathogenesis *Irune Lasa Elosegi, Milan*
15:20	Modifying bile acid synthesis (or UDCA) can improve tumor-specific T cell responses in liver cancer models *Siva K. Varanasi, Worcester*
15:40	**Coffee break with ePoster session**

FRIDAY, JULY 3, 2026

INTERNATIONAL ADOLF WINDAUS AWARD

16:30	Presentation of the International Adolf Windaus Award *Ulrich Beuers, Amsterdam*
16:40	International Adolf Windaus Award lecture

SESSION IV

"Old" and "new" bile acid receptors

Chairs	*Grace L. Guo, Piscataway; Ronald J. P. Oude Elferink, Amsterdam*
17:00	FXR agonist-induced pruritus: Is MRGPRX4 the answer? *Xiaoguang Lei, Beijing*
17:20	Discovery of novel MRGPRX4 agonists and antagonists with implications for hepatobiliary pruritus *Silvia M. Pickering, Zurich*
17:40	Insights into FXR function from mutagenesis studies *Holger Gohlke, Düsseldorf*
18:00	Hepatic FXR deficiency in mice: Lessons for MASH *Grace L. Guo, Piscataway*

SATURDAY, JULY 4, 2026

8:30	Welcome - In memoriam *Ulrich Beuers, Amsterdam*

SESSION V

Bile acids in metabolism and MASLD

Chairs	*Peter Fickert, Graz; Kristina Schoonjans, Lausanne*
8:40	Promoting metabolic health through bile acid-transforming bacteria *Kristina Schoonjans, Lausanne*
9:00	Microbial bile acid amidates are transported by ASBT, NTCP, and OATP1B1 *Frank Schaap, Maastricht*
9:20	Inhibition of bile acid conjugation improves cholestasis in humanized mice *Claudia Fuchs, Vienna*
9:40	Panel discussion: Bile acids in MASLD *P. Fickert, G. L. Guo, S. Karpen, K. Schoonjans, B. Schnabl*
10:00	**Coffee break with ePoster session** *Please see page 12 for further details*

SESSION VI

Bile acids as potential toxins

Chairs	*Jan G. Hengstler, Dortmund; Michael Trauner, Vienna*
11:00	Bile acid excretion by renal ASBT inhibition: Tissue protection and reduction of hepatic cholesterol *Jan G. Hengstler, Dortmund*
11:20	Silencing of the autophagy inhibitor Rubicon ameliorates liver injury in a PSC mouse model *Lukas M. Gulden, Graz*
11:40	Molecular to systems level interactions of the bile-acid derivate NorUDCA improve mood under high fat diet *Wulf Haubensak, Vienna*

SATURDAY, JULY 4, 2026

12:00	Role of pathobionts in liver disease *Bernd Schnabl, La Jolla*
12:20	**Presentation of Poster Awards**
12:40	**Lunch break with ePoster session** *Please see page 13 for further details*

SESSION VII

Bile acids and fibrosis

Chairs	*Ulrich Beuers, Amsterdam; Gideon Hirschfield, Toronto*
14:00	Bezafibrate plus UDCA: Advocating the "Old" pan-PPAR agonist as 2nd line treatment for PBC and PSC *Atsushi Tanaka, Tokyo*
14:20	Novel PPAR agonists as 2nd line treatment for PBC and PSC: Strengths and weaknesses *Gideon Hirschfield, Toronto*
14:40	Norucholic acid (NCA) for PSC and beyond *Michael Trauner, Vienna*
15:00	Panel discussion: Present and future therapeutic strategies in fibro-obliterative cholangiopathies *U. Beuers, G. Hirschfield, A. Tanaka, M. Trauner, C. Williamson*

SESSION VIII

Bile acids in genetic diseases

Chairs	*Saul Karpen, Richmond; Verena Keitel-Anselmino, Magdeburg*
15:30	Genetic abnormalities in LPAC (low phospholipid associated cholelithiasis) syndrome *Verena Keitel-Anselmino, Magdeburg*
15:50	Impaired biliary phospholipid secretion in PFIC3/ABCB4 deficiency: Molecular consequences and therapeutic priorities *Emmanuel Gonzales, Paris*
16:10	Enterohepatic circulation of bile acids as therapeutic target in genetic cholestatic diseases of children *Saul Karpen, Richmond*
16:30	ABC transporter variants in adult cholestatic liver diseases *Deepak Joshi, London*
16:50	**Closing Remarks** *Verena Keitel-Anselmino, Magdeburg*

MODERATED POSTER SESSIONS

LUNCH BREAK - FRIDAY, JULY 3, 13:00 - 13:50

Experimental

Moderators: Claudia Günther, Erlangen; Tarek Moustafa, Graz

13:00	Identifying solute carrier (SLC) transporters involved in cholangiocyte defense against toxic bile acids *Qinghong Li, Amsterdam*
13:08	FXR/LXRα coactivation suppresses Cyp8b1 and drives hepatic CDCA accumulation to promote MASH–HCC progression in high-fat/high-sucrose diet–fed mice with human-like bile acid composition *Hajime Ueda, Ami-machi*
13:16	Liver-specific somatic beta-klotho knockout dysregulates bile acid homeostasis and hepatic lipid metabolism *Alexandra Aaldijk, Groningen*
13:24	Novel epigenetic molecules selectively targeting class I and IV HDACs as a new therapeutic avenue for bile duct cancer *Noelia Pastor, San Sebastián*
13:32	Bile acid-S1PR2 signaling drives circAFF3 biogenesis and hepatic stellate cell activation in cholestatic liver fibrosis *Huiping Zhou, Richmond*
13:40	Methyldopa reduces plasma bile acid concentrations in a murine model of estrogen-induced cholestasis *Dina Manna, Hradec Králové*

LUNCH BREAK - FRIDAY, JULY 3, 13:00 - 13:50

Clinical

Moderators: Carola Droege, Magdeburg; Martin Wagner, Graz

13:00	Event-free survival of maralixibat-treated patients with progressive familial intrahepatic cholestasis compared with aligned real-world data of untreated patients from NAPPED *Henkjan J. Verkade, Groningen*
13:08	Missense variants in the ABCB11 gene and disease severity in patients with bile salt export pump deficiency *Henkjan J. Verkade, Groningen*
13:16	Epimerization of host-derived bile acids is a hallmark of IBD-associated dysbiosis *Maximilian Baumgartner, Vienna*
13:24	Patient-derived PSC organoids retain an overreactive memory to bile acid insult *Fabian Heinreichsberger, Vienna*
13:32	Investigation of ABCC6 genetic variants in a cohort of cholestatic liver disease patients using whole exome sequencing *Somayeh Alinaghi Arjas, Magdeburg*
13:40	Non-invasive assessment of progression to primary biliary cholangitis in patients with positive anti-mitochondrial antibodies *Marta Alonso Peña, Santander*

COFFEE BREAK - SATURDAY, JULY 4, 10:15 - 11:00

Experimental

Moderators: Clarissa Campbell, Vienna; Coen C. Paulusma, Amsterdam

10:15	Cyp2c70 silencing rewires hypothalamic synaptic plasticity by shifting bile acid composition *Emmanuel Dixon, Vienna*
10:23	High dietary cholesterol drives MASLD-to-MASH progression by induction of bile macropinocytosis *Ahmed Ghallab, Dortmund*
10:31	A human-like bile acid composition attenuates atherogenesis in female Ldlr-/- mice by reducing cholesterol absorption *Brecht Attema, Groningen*
10:39	Molecular characterisation of the trafficking rescue of defective ABCB4 variants by roscovitine analogues *Thomas Falguières, Orsay*

Clinical

Moderators: Rudi de Waart, Amsterdam; Kenneth Setchell, Cincinnati

10:15	Inhibition of hepatic bile salt uptake using Bulevirtide attenuates pre-existing diet-induced metabolic dysfunction-associated steatohepatitis in mice *Rudi de Waart, Amsterdam*
10:23	10 cases of ileal bile acid transporter (IBAT) inhibitor therapy in adults with hereditary bile salt export pump (BSEP)-deficiency *Carola Droege, Magdeburg*
10:31	Bile acid mediated protection from diet-induced adiposity and hepatic steatosis depends on amino acid conjugation metabolism. *Tarek Moustafa, Graz*
10:39	Individual participant data meta-analysis of perinatal adverse outcomes in cholestatic twin pregnancies: The IMPACT study *Agata Majewska, London*

LUNCH BREAK - SATURDAY, JULY 4, 13:10 - 14:00

Experimental

Moderators: Claudia Fuchs, Vienna; Thomas Vallim, Los Angeles

13:10	Circadian oscillations of microbial taxa with potential for bile acid modifications: Correlating diurnal shifts in bile acids with microbiota, microbial bsh and bai *Cormac Gahan, Cork*
13:18	Bile acid detergency as determinant of liver pathology in a humanized mouse model of progressive familial intrahepatic cholestasis type 3 *Lixin Ke, Groningen*
13:26	Mice expressing FXR with a humanized ligand-binding domain display distinct metabolic responses upon pharmacological FXR stimulation *Jinxiao Li, Groningen*
13:34	A cholestatic-autophagy signature defines a distinct phenotype in metabolic dysfunction-associated steatotic liver disease *Francesca Molinaro, Rome*
13:42	Activation of the bile acid receptor FXR changes the cellular microenvironment of human adipose tissue in obesity *Lena Vosko, Graz*
13:50	Synthesis of 3-NBD bile acid conjugates as probe substrates for hepatic and intestinal SLC10 and SLCO transporters *Celine Drossel, Giessen*

LUNCH BREAK - SATURDAY, JULY 4, 13:10 - 14:00

Clinical

Moderators: Marta Alonso Peña, Santander; Henkjan J. Verkade, Groningen

13:10	Ursodeoxycholic acid may reduce the risk of major postpartum haemorrhage for individuals with intrahepatic cholestasis of pregnancy: Secondary analysis of individual participant data meta-analyses *Caroline Ovadia, Edinburgh*
13:18	Rethinking the role of bile acids in primary biliary cholangitis. Nicolas Chignard, Neuilly-sur-Seine
13:26	The farnesoid X receptor (FXR) antagonist 7β-isopropylchenodeoxycholic acid improves glucose metabolism in mice on a Western diet *Alzbeta Stefela, Hradec Králové*
13:34	Genetic variability in LPAC syndrome: Is the name still correct? *Waqar Umer, Magdeburg*
13:42	Porphyran from discolored nori prevents metabolic syndrome through microbiota-bile acid-ceramide pathway *Mitsuhiro Watanabe, Fujisawa*
13:50	Impact of hypothermic oxygenated perfusion on bile acid composition and injury markers after extended cold preservation of porcine liver *Umran Ay, Aachen*

ADOLF WINDAUS (1876-1959)

Adolf Windaus was born on Christmas Day in 1876 in Berlin, where his father owned a factory. Even as a young student in the Berlin gymnasium, he was fascinated by the epochal discoveries of Koch and Pasteur, and by his 18th birthday he had decided on a scientific career. He entered medical school, taking his pre-clinical year at the University of Freiburg and his clinical years in Berlin. However, he soon realized, especially during the lectures of Emil Fischer, that biological processes could be understood only when the chemical structure of organisms was known. Therefore, as soon as he had finished medical school, he returned to Freiburg to study chemistry under the supervision of Heinrich Kiliani. In 1899, he completed his first research project which dealt with the chemical composition of digitalis. He then spent two years in compulsory military service in Berlin. During this time he also worked in the laboratory of Emil Fischer, carrying out studies on derivatives of aniline. On completing his military service, Windaus returned to the University of Freiburg where he began his life-long work on the structure of cholesterol. His thesis, which qualified him for the position of docent, had the simple title „Über Cholesterin“. The choice of this research topic originated from Windaus‘ logical belief that any substance which was so widely distributed in animal and plant tissues must have an important biological function, and that understanding of its structure and function might lead to unifying concepts, a hypothesis he would subsequently prove so brilliantly. In addition to initiating studies on cholesterol, he and his colleague Knoop soon discovered that an amino acid containing the imidazole ring, histidine, was present in proteins, and could be decarboxylated to give histamine. The discovery of histamine opened a vast area of pharmacological research.

In 1913, Adolf Windaus accepted a call to direct the prestigious Institute of Medical Chemistry in Innsbruck, Austria, where earlier Pregl had founded microanalytical chemistry. Two years later, in 1915, he was called to be Director of the Chemical Laboratories of the University of Göttingen, laboratories rich in tradition since the time of Wöhler. Here, he could pursue his work on elucidating the structure of cholesterol in a series of integrated investigations that were truly Herculean in scope. In the year 1919 a most significant discovery was made. Windaus found that coprostane could be oxidized to cholanic acid. With the knowledge of this transformation, came the realization of the close structural similarity of cholesterol and bile acids; one could now apply the existing knowledge of cholesterol structure to that of bile acids and that of bile acids to cholesterol. The work of elucidating the exact structure of the condensed steroid rings of steroids was extraordinarily difficult. To understand

the structural isomerism of the A / B ring juncture, it was necessary to study the simplest model compounds, cis and trans decalin. This was done with Hueckel, who later became one of the world's greatest physical chemists.

In the twenties, Adolf Windaus, with his pupils, established the relationships between cholesterol and other important steroids such as sitosterol, the saponins, and the various classes of cardiac steroids. He showed that all shared the cyclopentanophenanthrene nucleus. Inspired by Windaus, his pupil Butenandt isolated and determined the structure of the adrenal steroids whose origins from cholesterol had not been suspected by anyone. Butenandt was able to rapidly determine the structure of estrone, androsterone, and progesterone, for which he received the Nobel Prize in 1939.

Probably the climax in the extraordinary research output of Adolf Windaus was his elucidation of the structure and biosynthesis of vitamin D. Hess in New York had made the observation that ultraviolet radiation of a lipid extract induced the formation of active vitamin D. In the next 8 years, Adolf Windaus and his students succeeded in identifying the provitamin as ergosterol and 7-dehydrocholesterol and also in clarifying the structure of vitamin D2 and vitamin D3. The complex steps in photoactivation of the vitamin were clarified, and each intermediate was crystallized and its structure determined.

Thus, the research area of the chemical structure of cholesterol, which Adolf Windaus had selected when still a young docent in Freiburg led to studies spanning over 30 years – studies which opened up a vast – almost limitless field that continues to be active today. His work has been of inestimable significance for the practice of medicine. Adolf Windaus, however, insisted that his research was not aimed at applications, but only at understanding the mysteries of nature.

Adolf Windaus had a legendary reputation among his colleagues and students. He was a man of infinite energy and extraordinary insight, who could reduce scientific problems to their essence. He had the art to ask the right question and do the definitive experiment. Nature disclosed her secrets quickly to a man of such talent. His former associates had continuous admiration for his clarity of speech, both in conversation and scientific discussion. He was a man of modesty and dignity who combined the highest scientific standards with great personal generosity.

For his many discoveries, Adolf Windaus received many honors and awards. Under his leadership, the Chemical Institute in Göttingen became known throughout the world. He was honored by being chosen to receive the Nobel Prize for chemistry in 1928, and his lecture is a masterpiece of erudition, clarity and modesty.

W. Gerok (†)

INTERNATIONAL ADOLF WINDAUS AWARD

The "International Adolf Windaus Award" was founded by the Falk Foundation e.V. and will, for the twenty-fourth time, be presented on the occasion of the XXVIII International Bile Acid Meeting on July 3, 2026. The Award amounts to €15,000 and is awarded for outstanding contributions in the field of bile acid research.

Members of the Award Committee:

U. Beuers (Amsterdam)
P. A. Dawson (Atlanta)
V. Keitel-Anselmino (Magdeburg)
M. Trauner (Vienna)

Windaus Award Winners:

1980 - C. Einarsson (Stockholm) & K. Hellstrom (Stockholm)
1982 - E. H. Mosbach (New York) & H. Danielsson (Uppsala)
1984 - M. C. Carey (Boston)
1986 - I. Bjorkhem (Huddinge)
1988 - J. L. Boyer (New Haven)
1990 - P. B. Hylemon (Richmond) & P. J. Meier-Abt (Zurich)
1992 - K. Okuda (Hiroshima)
1994 - Z. R. Vlahcevic (Richmond)
1996 - W. Kramer (Frankfurt)
1998 - P. A. Dawson (Winston-Salem)
2000 - D. J. Mangelsdorf (Dallas)
2002 - D. W. Russell (Dallas)
2004 - K. D. R. Setchell (Cincinnati)
2006 - R. Poupon (Paris)
2008 - N. Ballatori (Rochester)
2010 - J. Auwerx & K. Schoonjans (Lausanne)
2012 - G. Paumgartner (Munich)
2014 - S. Kliewer (Dallas)
2016 - D. Keppler (Heidelberg)
2018 - B.B. Stieger (Zurich)
2020 - D. D. Moore (Berkeley)
2022 - R.J.P. Oude Elferink (Amsterdam)
2024 - D. Häussinger (Dusseldorf)

Coordinator of the Award Committee:

Prof. Dr. Ulrich Beuers

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REGISTRATION

You can register for the event via our homepage:
www.falkfoundation.org
Registration is only possible online.

You will receive an automatic confirmation of registration by e-mail.

CONGRESS FEES

Scientific Program of Symposium 244	EUR 300
Students (copy of student ID required)	EUR 150

The congress fees include:
- Pre-Opening and Welcome on July 2, 2026
- Refreshments during coffee breaks
- Lunch on July 3 and 4, 2026
- A copy of the final program

CONGRESS OFFICE AND REGISTRATION

Opening Hours:

Thursday, July 2	16:00 - 20:00 h
Friday, July 3	7:30 - 18:30 h
Saturday, July 4	8:00 - 17:00 h

The Falk Foundation will take pictures during the meeting. Additionally, parts of the meeting might be recorded. By participating all attendees consent and agree with the recording and the photo shoots.

ARRIVAL

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(formerly Andaz Vienna am Belvedere)
Arsenalstrasse 10
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Austria

By car

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By train

Vienna main station is a 4 minutes walk to the Hotel.

CONFLICTS OF INTEREST

Members of the scientific committee declare the following potential conflicts of interest:

Verena Keitel Anselmino: Astra Zeneca, Gilead, Dr. Falk Pharma, Mirum, Ipsen, Lilly, Madrigal, MSD

POSTER ABSTRACTS

1. Liver-specific somatic beta-klotho knockout dysregulates bile acid homeostasis and hepatic lipid metabolism
   A. Aaldijk, C. Verzijl, M. Hovingh, N. Huijkman, M. Smit, N. Kloosterhuis, N. Mulder, R. Havinga, A. Gerding, T. Bos, M. Koster, M. De Graaf, J. Wolters, B. Van de Sluis, D. Struik, J. Jonker (Groningen, NL)
2. Assessing the role of semaphorin 7A (SEMA7A) in intrahepatic cholestasis in vitro
   M. Abu Omar, A. Bastianelli, A. Noetzold, C. Droege, V. Keitel, T. Cantz, M. Sgodda (Magdeburg, Hannover, DE)
3. Investigation of ABCC6 genetic variants in a cohort of cholestatic liver disease patients using whole exome sequencing
   S. Alinaghi, L. Knopp, K. Stein, D. Schanze, M. Zenker, M. Rak, A. Noetzold, V. Keitel, C. Droege (Magdeburg, DE)
4. Non-invasive assessment of progression to primary biliary cholangitis in patients with positive anti-mitochondrial antibodies
   M. Alonso Pena, A. Diaz Gonzalez, M. Monte, C. Perello, M. Del Barrio, P. Iruzubieta, J. Irure, M. Lopez Hoyos, J. Calleja, J. Marin, J. Crespo (Santander, Salamanca, Madrid, ES)
5. A human-like bile acid composition attenuates atherogenesis in female Ldlr-/- mice by reducing cholesterol absorption
   B. Attema, T. Yntema, T. Eijgenraam, N. Kloosterhuis, M. Koster, R. Havinga, E. Weersing, D. Lubbinge, E. Kluter, N. Mulder, M. Hovingh, A. Pranger, L. Chen, J. Fu, N. Riksen, B. Van de Sluis, J. De Boer, D. Koonen, F. Kuipers (Groningen, Nijmegen, NL; Nanjing, CN)
6. Impact of hypothermic oxygenated perfusion on bile acid composition and injury markers after extended cold preservation of porcine liver
   U. Ay, F. Schaap, C. Weigle, O. Beetz, M. Kong, R. Bulow, S. Olde Damink, W. Ramackers, F. Vondran, F. Oldhafer (Aachen, DE; Maastricht, NL)
7. Discovery of novel bile acid-modifying enzymes in disease-associated gut microbiota
   Y. Bai, L. Zou, Y. Liu, J. Yang, H. Zou, H. Qi, X. Lei (Beijing, Chongqing, CN)
8. Constitutive androstane receptor activation modulates bile acid homeostasis during estrogen-induced cholestasis in mice
   M. Bajnokova, H. Lastuvkova, M. Ambroz, M. Hroch, A. Stefela, O. Kucera, R. Nencka, J. Hricova, S. Micuda, P. Pavek (Hradec Kralove, Prague, CZ)
9. Frequencies of polymorphic variants in the bile acid synthesis gene CYP7A1 differ in bile acid diarrhoea and chronic diarrhoea control subjects from the SINBAD trial
   S. Balesaria, J. Walters, C. Borup, L. Munck (London, GB; Copenhagen, DK)
10. The risk of Parkinson's disease following cholecystectomy: A UK Biobank prospective cohort study
    O. Bandmann, T. Payne, W. Fan, C. Chen, S. Zhou, V. Osmani, H. Lu (Sheffield, London, GB)
11. Epimerization of host-derived bile acids is a hallmark of IBD-associated dysbiosis
    M. Baumgartner, F. Schnaufer, M. Duquesnoy, T. Asatsuma, T. Khaing Nyein, C. Fernandes, A. Charkrabarty, D. Dollack, F. Pinter, F. Groessing, A. Frick, C. Fuchs, M. Gerstorfer, T. Koecher, P. Schimmel, M. Lichtenstein, G. Winter, S. Leistl, C. Hoegenauer, M. Trauner, G. Busslinger, G. Gorkiewicz, C. Guenther, B. Chassaing, C. Campbell; TRR241 IBDome Consortium (Vienna, Graz, AT; Paris, FR; Erlangen, DE)

12. Bile acids and FGF15/19 decouple hepatic injury and lean mass loss after vertical sleeve gastrectomy
N. Bozadjieva-Kramer, K. Wu, P. Henry, Y. Shao, J. Wean, A. Chan, J. Shin, A. Myronovych, R. Orourke, T. Vallim, N. Lanthier, R. Kohli, A. Schafer, R. Seeley (Ann Arbor, San Francisco, Los Angeles, US; Gosselies, Brussels, BE; Shanghai, CN)

13. Assessment of ABCB4 missense variant prediction accuracy using ClinVar classified variants as a functional benchmark
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15. Bile acids regulate lipid metabolism through selective actions on fatty acid absorption
A. Chan, K. Jarrett, R. Lai, M. Brearley-Sholto, E. Tarling, T. De Aguiar Vallim (Los Angeles, US)

16. Plasma sulfated lithocholic acid predicts mortality while conjugated primary bile acids associate with myocardial relaxation in cirrhosis patients undergoing TIPS
O. Chavez-Talavera, L. Voelkel, C. Angendohr, D. Herebian, J. Kielb, J. Weber, A. Polzin, V. Keitel, C. Engelmann, F. Tacke, A. Tailleux, B. Staels, A. Kunstein, J. Bode, T. Luedde, M. Kelm, L. Dannenberg (Düsseldorf, Magdeburg, Berlin, DE; Lille, FR)

17. Rifampicin, but not ursodeoxycholic acid, alters the taxonomic, but not the functional, composition of the gut microbiota in women with intrahepatic cholestasis of pregnancy
S. Coat, M. Dekker Nitert, X. Luo, S. Leech, B. Hague (Adelaide, St. Lucia, AU)

18. Clinical significance of serum total bile acid in the diagnosis and management of intrahepatic cholestasis of pregnancy
G. Davaadorj, S. Tserenchimed, O. Sahiya, G. Sambuu (Ulaanbaatar, MN)

19. Knockout of claudin-3 leads to altered bile metabolism and gut microbiota
T. De Enrique Schmidt, F. Baier, A. Keogh, D. Spari, B. Yilmaz, C. Krummenacher, D. Sanchez Taltavull, T. Yarahmadov, C. Gomez Castella, A. Odermatt, D. Candinas, D. Stroka (Bern, Basel, CH)

20. Inhibition of hepatic bile salt uptake using bulevirtide attenuates pre-existing diet-induced metabolic dysfunction-associated steatohepatitis in mice
R. De Waart (Amsterdam, NL)

21. ICP is associated with changes in 54 peptides in the metaproteome of women in late pregnancy
M. Dekker Nitert, W. Hague, S. Coat; Turrific Trial collaborators (St. Lucia, Adelaide, AU)

22. Cyp2c70 silencing rewires hypothalamic synaptic plasticity by shifting bile acid composition
E. Dixon, C. Barbosa, V. Morenoviedma, V. Mlitz, C. Fuchs, T. Claudel, W. Haubensak, M. Trauner (Vienna, AT)

23. 10 cases of ileal bile acid transporter (IBAT) inhibitor therapy in adults with hereditary bile salt export pump (BSEP)-deficiency
C. Droege, E. Pfister, P. Strnad, G. Rusignuolo, U. Baumann, S. Schulz-Juergensen, M. Sterneck, J. O Beirne, T. Goetze, M. Zenker, D. Schanze, K. Hippe, R. Thimme, V. Keitel (Magdeburg, Hannover, Aachen, Freiburg, Hamburg, DE; Sunshine Coast, AU)

24. Synthesis of 3-NBD bile acid conjugates as probe substrates for hepatic and intestinal SLC10 and SLCO transporters
C. Drossel, R. Goettlich (Giessen, DE)

25. Altered serum bile acid concentration correlate with lipid profile and cardiovascular disease risk in patients with liver cirrhosis
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26. Serum bile acid levels in non-diabetic patients with liver cirrhosis: Relation to insulin resistance and central obesity
H. El Aggan, S. Mahmoud, S. Sharaf (Alexandria, EG)

27. Molecular characterisation of the trafficking rescue of defective ABCB4 variants by roscovitine analogues
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28. Inhibition of bile acid conjugation improves cholestasis in humanized mice
C. Fuchs, O. Peterenko, V. Mlitz, H. Scharnagl, T. Reiberger, B. Garfinkel, M. Trauner (Vienna, Graz, AT; Cambridge, US)

29. Circadian oscillations of microbial taxa with potential for bile acid modifications: Correlating diurnal shifts in bile acids with microbiota, microbial bsh and bai
C. Gahan, K. Kaka Bra, M. Claesson, J. Cryan, G. Tofani, D. Flegar, M. Panchal, J. Swann (Cork, IE; Southampton, UK)

30. High dietary cholesterol drives MASLD-to-MASH progression by induction of bile macropinocytosis
A. Ghallab, M. Myllys, Z. Hobloss, D. Gonzalez, R. Hassan, J. Reinders, S. Neef, M. Schwab, A. Friebel, Q. Siddiqui, D. Zhipeng, S. Hoehme, T. Buschmann, B. Levkau, E. Halilbasic, M. Mandorfer, G. Stirnimann, E. Lindstrom, M. Krawczyk, H. Schmidt, M. Trauner, S. Van der Merwe, P. Dawson, J. Hengstler (Dortmund, Stuttgart, Leipzig, Düsseldorf, Essen, DE; Vienna, AT; Bern, CH; Boston, Atlanta, US; Leuven, BE)

31. Multi-modal analysis defines bile acid-induced stress as a rewiring mechanism of epithelial-immune communication in Crohn's disease
C. Guenther, K. Hu, S. Leistl, M. Rauh, M. Baumgartner, C. Plattner, T. Rath, T. Adolph, Z. Trajanoski, C. Campbell, A. Kremer, M. Neurath (Erlangen, D; Vienna, Innsbruck, AT; Zurich, CH)

32. Silencing of the autophagy inhibitor Rubicon ameliorates liver injury in a PSC mouse model
L. Gulden, L. Vosko, L. Katz, D. Silber-Wagner, J. Sommer, P. Fickert, K. Panzitt, M. Wagner (Graz, AT)

33. Prognostic impact of alkaline phosphatase normalization after one year of UDCA therapy in primary biliary cholangitis
H. Hassine, H. Kchir, H. Dabbebi, D. Cherif, H. Yacoub, N. Maamouri (Tunis, TN)

34. Xi'an criteria: An early tool for predicting UDCA response and hepatic decompensation in primary biliary cholangitis
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35. Molecular to systems level interactions of the bile-acid derivate NorUDCA improve mood under high fat diet
W. Haubensak, J. Griessner, D. Steinacher, F. Groessl, C. Fuchs-Steiner, M. Baumgartner, K. Kashofer, A. Hess, M. Trauner (Wien, Vienna, Graz, AT; Erlangen, DE)

36. Patient-derived PSC organoids retain an overreactive memory to bile acid insult
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37. Patterns of fecal and blood biomarkers in well-characterized cohorts with bile acid diarrhea or idiopathic chronic diarrhea
E. Hillman, J. Hawley, C. Bannon, H. Hussain, K. Chappel, M. Gomez, J. Walters (London, Manchester, Cambridge, GB)

38. Farnesoid X receptor activation upregulates expression of the transcription elongation factor, TCEA2, in intestinal epithelial cells
S. Keely, J. Smyth, J. Foulke-Abel, R. Lin, L. Adorini, M. Donowitz (Dublin, IE)

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40. Single-nucleotide polymorphism of NR1H4 and the bile acid profile in children with metabolic dysfunction-associated steatotic liver disease
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41. Genetic susceptibility as a risk factor for gallstones in PSC: A prospective single-center analysis
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43. Mice expressing FXR with a humanized ligand-binding domain display distinct metabolic responses upon pharmacological FXR stimulation
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44. Identifying solute carrier (SLC) transporters involved in cholangiocyte defense against toxic bile acids
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45. Gestational diabetes mellitus and intrahepatic cholestasis of pregnancy – Does metformin treatment affect the course of ICP?
A. Majewska, N. Williamson, J. Chambers, P. Dixon, M. Raczka, C. Ovadia, C. Williamson (London, Edinburgh, GB; Warsaw, PL)

46. Individual participant data meta-analysis of perinatal adverse outcomes in pregnancies: The IMPACT study
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47. Methyldopa reduces plasma bile acid concentrations in a murine model of estrogen-induced cholestasis
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48. Hydrophobic bile acids orchestrate gut-liver axis disruption in primary sclerosing cholangitis
T. Miyazaki, H. Ueda, T. Ikegami, A. Honda (Ibaraki, JP)

49. A cholestatic-autophagy signature defines a distinct phenotype in metabolic dysfunction-associated steatotic liver disease
F. Molinaro, L. Nevi, F. Terracciani, A. Baiocchini, T. Musarra, F. Zalfa, U. Vespasiani-Gentilucci, S. Carotti (Rome, IT)

50. Bile acid mediated protection from diet-induced adiposity and hepatic steatosis depends on amino acid conjugation metabolism
T. Moustafa, A. Zaufel, J. Sommer, C. Diwoky, D. Silbert-Wagner, J. Heeren, F. Fickert (Graz, AT; Hamburg, DE)

51. Identification of a novel ABCB4 variant causing low phospholipid-associated cholelithiasis
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52. Ursodeoxycholic acid may reduce the risk of major postpartum haemorrhage for individuals with intrahepatic cholestasis of pregnancy: Secondary analysis of individual participant data meta-analyses
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59. Cholic acid-cisplatin conjugates as selective drugs
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60. Monitoring atypical metabolite biomarkers in patients with bile acid synthesis disorders by a novel targeted tandem mass spectrometry assay
K. Setchell, W. Zhang, X. Zhao (Cincinnati, US)

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65. FXR/LXRα coactivation suppresses Cyp8b1 and drives hepatic CDCA accumulation to promote MASH-HCC progression in high-fat/high-sucrose diet-fed mice with human-like bile acid composition
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67. Event-free survival of maralixibat-treated patients with progressive familial intrahepatic cholestasis compared with aligned real-world data of untreated patients from NAPPED
H. Verkade, P. Miranda Afonso, A. De Groot, M. Nomden, P. Mian, B. Shneider, E. Gonzales, I. Jankowska, E. Sokal, R. Thompson, A. Goldstein, D. Mogul, S. Vandriel, B. Hansen; NAPPED registry (Groningen, Rotterdam, NL; Houston, Foster City, US; Paris, FR; Warsaw, PL; Brussels, BE; London, GB)

68. Missense variants in the ABCB11 gene and disease severity in patients with bile salt export pump deficiency
H. Verkade, M. Nomden, A. De Groot, A. Felzen, R. Thompson, E. Gonzales, I. Jankowska, E. Sokal, B. Shneider, B. Hansen, P. Mian; NAPPED and TreatFIC registries (Groningen, Rotterdam, NL; London, GB; Paris, FR; Warsaw, PL, Brussels, BE; Houston, US)

69. Activation of the bile acid receptor FXR changes the cellular microenvironment of human adipose tissue in obesity
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70. Porphyran from discolored nori prevents metabolic syndrome through microbiota-bile acid-ceramide pathway
M. Watanabe, Y. Yokoyama, J. Morel, Y. Takashina, S. Fukuda, N. Kitamura, T. Taworntawat, T. Miyazaki, A. Honda, M. Arita, J. Auwerx, K. Tsubota (Fujisawa, Ibaraki, Tokyo, JP; Lausanne, CH)

71. Long-term follow-up in women with severe intrahepatic cholestasis of pregnancy
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72. Chemical conjugation of Myrcludex B and triiodothyronine for liver-specific targeting
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73. Genetic architecture of intrahepatic cholestasis of pregnancy in a British South Asian population
X. Yang, A. Bracanovic, F. Pajuste, T. Laisk, C. Williamson, P. Dixon, J. Zollner (London, GB; Tartu, EE)

74. Gallstones associated with nonalcoholic steatohepatitis (NASH) and metabolic syndrome
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75. Auxiliary role of bile acids in emergency hematopoiesis in children
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77. A human ileal organoid platform defines bile acid transport and signaling defects in Crohn's disease
Y. Zhang, B. Vallance, S. Crowley (Vancouver, CA)

78. Intestinal TGR5-targeted carrier enables bile acid-mediated glycemic control with reduced toxicity in mice and pigs
Y. Zhang, G. Yong, Y. Wang, H. Huang, H. Zhou (Vancouver, CA; Shanghai, CN)

79. Chemoproteomic profiling reveals a novel covalent target of deoxycholic acid in tumor suppression
W. Zhao (Beijing, CN)

80. Bile acid-S1PR2 signaling drives circAFF3 biogenesis and hepatic stellate cell activation in cholestatic liver fibrosis
H. Zhou, H. Li, M. Song, G. Way, D. Zhao, N. Wu, Y. Huang, S. Bayatpour, H. Lu, L. Su, P. Hylemon (Richmond, US)

81. Alterations in bile acid biosynthesis in liver cirrhosis
K. Zizalova, M. Baronova, B. Novakova, J. Ramos Pittol, V. Smid, R. Bruha, L. Vitek (Prague, CZ; Innsbruck, AT)

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1. Liver-specific somatic beta-klotho knockout dysregulates bile acid homeostasis and hepatic lipid metabolism

Alexandra Aaldijk (Groningen, NL), Cristy Verzijl (Groningen, NL), Milaine Hovingh (Groningen, NL), Nicolette Huijkman (Groningen, NL), Marieke Smit (Groningen, NL), Niels Kloosterhuis (Groningen, NL), Niels Mulder (Groningen, NL), Rick Havinga (Groningen, NL), Albert Gerding (Groningen, NL), Trijnie Bos (Groningen, NL), Mirjam Koster (Groningen, NL), Mariska De Graaf (Groningen, NL), Justina Wolters (Groningen, NL), Bart Van de Sluis (Groningen, NL), Dicky Struik (Groningen, NL), Johan Jonker (Groningen, NL)

Introduction: Beta-klotho (KLB), a coreceptor required for fibroblast growth factor (FGF)19 and FGF21 signaling, has been identified as a drug target for metabolic and cholestatic liver diseases. Understanding of its function in bile acid homeostasis and metabolism in vivo is mainly based on germline mouse models exhibiting developmental abnormalities, potentially confounding the interpretation of KLB in adult physiology and FGF19/21 pharmacodynamics. In this study, we applied liver-specific somatic gene editing to establish the role of hepatic KLB in adult physiology and validate its therapeutic potential as a drug target in liver disease.

Methods: Liver-specific Cas9-transgenic mice on a C57BL/6J background were retro-orbitally injected with adeno-associated virus particles containing guide RNAs targeting exon 1 of the mouse Klb gene to generate a liver-specific Klb knockout (KlbHepKO). Control mice received a matched viral dose of empty vector. Mice were fed a chow diet for 4 weeks or a high-fat diet for 12 weeks post-virus injection, followed by phenotypic characterization.

Results: Under chow- and HFD-fed conditions, liver-specific knockout of Klb resulted in increased bile acid synthesis as shown by 2.6-fold ($p = 0.008$) and 5.8-fold ($p = 0.004$) elevated Cyp7a1 expression, respectively. Fecal bile acid excretion was increased 2.9-fold in chow-fed ($p = 0.008$) and 7.9-fold ($p = 0.000$) in HFD-fed KlbHepKO mice, and bile acid composition was altered as shown by a more hydrophobic bile acid composition under chow ($p = 0.008$) and HFD ($p = 0.004$) conditions. Hepatic Klb knockout also affected lipid metabolism, indicated by 41.4% lower plasma triglyceride levels ($p = 0.008$) and 37.0% higher hepatic triglyceride levels ($p = 0.016$), but only under chow conditions. The fraction of VLDL was 42.7% reduced ($p = 0.008$) in KlbHepKO mice, suggesting impaired VLDL secretion, which could potentially explain the increased hepatic levels of triglycerides. Upon a HFD, effects on hepatic triglyceride levels were less pronounced ($p = 0.052$), showing only a trend towards altered lipid metabolism.

Discussion/Conclusion: Liver-specific Cas9-mediated gene editing reveals hepatic KLB as a key regulator of bile acid homeostasis and hepatic lipid metab-

olism. These findings validate hepatic KLB as a target for FGF19/FGF21-based therapies in various liver diseases.

2. Assessing the role of semaphorin 7A (SEMA7A) in intrahepatic cholestasis in vitro

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Introduction: Semaphorins are signalling proteins, classified into eight subgroups, that contribute to maintaining tissue homeostasis and play essential roles in cell activation and migration in diverse organ systems. Semaphorin 7A (SEMA7A) is a glycosylphosphatidyl-inositol-anchored semaphorin expressed in hepatic tissue. It is well established that mutations in genes encoding hepatic bile acid transporters disrupt bile flow and cause intrahepatic cholestasis. Emerging evidence suggests that mutations in SEMA7A can potentially impair bile flow and lead to clinical presentations of cholestasis. A patient of the HiChol registry presenting with Intrahepatic Cholestasis of Pregnancy (ICP) and recurrent cholelithiasis carried a heterozygous in frame duplication p.(Arg32_Leu33dup) in the SEMA7A gene, warranting further in vitro investigation of SEMA7A's potential role in cholestasis.

Methods: Human hepatocellular carcinoma cell lines (Huh-7, Hep3B and HepG2) were stimulated with estradiol and progesterone. SEMA7A mRNA expression was assessed using RT-PCR. Additionally, the aforementioned cells were transfected with a plasmid encoding a wild-type SEMA7A construct fused to GFP to evaluate protein localization using immunofluorescence. Moreover, the patient's duplication variant was introduced into the SEMA7A construct to further assess the potential impact.

Results: We identified 2 women carrying potentially cholestasis associated SEMA7A variants. In vitro hormonal stimulation did not result in significant changes in SEMA7A mRNA expression. SEMA7A cDNA was generated and variants were successfully introduced and verified by sequencing. Both the wildtype SEMA7A-GFP as well as the mutated SEMA7A proteins were detected intracellularly in transfected cells, which was unexpected. iPSC of the patient were generated for further functional analysis.

Discussion/Conclusion: Initial findings do not support a direct regulation of SEMA7A expression by pregnancy-related hormones in vitro. Moreover, in vitro analysis of protein localization did not show any difference, which may be due to the C-terminal GFP-tag, demonstrating the need for an optimized approach to determine the functional impact of SEMA7A mutations on bile formation.

3. Investigation of ABCC6 genetic variants in a cohort of cholestatic liver disease patients using whole exome sequencing

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Introduction: Dysfunction of ABCC6 has been associated with overexpression of ABCB11 and ABCG5/G8, leading to altered bile composition, gallstone formation, and cholestasis. ABCC6 variants have also been reported in low phospholipid-associated cholelithiasis (LPAC) and intrahepatic cholestasis of pregnancy (ICP). ABCC6 is also a well-established causative gene for pseudoxanthoma elasticum (PXE), a multisystem disorder characterized by ectopic mineralization of connective tissues. We subsequently investigated rare ABCC6 variants in whole-exome sequencing (WES) data from patients with suspected cholestatic liver disease.

Methods: Variant evaluation, annotation and prioritization analysis was carried out on WES data, using Exomiser software. A comprehensive customization setting has been applied on loss of function and missense variants, with focus on genes relevant to the patients' clinical phenotype. In parallel, an ABCC6 expression construct was generated and introduced into Huh7 cells via transfection.

Results: Twenty-one rare heterozygous ABCC6 variants were identified in 28 samples among 356 WES datasets. Despite one case with two heterozygous ABCC6 variants, all other were single heterozygous variants. Four splicing variants were detected in patients with cholangiocarcinoma (CCA), LPAC, and cholestasis, and one nonsense variant was identified in a patient with unclear cholestatic liver disease. 33% had LPAC or gallstone disease; and 66% of ABCC6 variants carriers were female. All variants were classified as variants of uncertain significance (VUS). Expression of the construct was confirmed by Western blot and will be used for subsequent mutagenesis analysis.

Discussion/Conclusion: In-depth analysis of WES data, identified several potentially disease-relevant ABCC6 variants. These findings suggest a broader role for ABCC6 in hepatic phenotypes beyond PXE and highlight the importance of integrating genetic and functional data to refine genotype–phenotype correlations. Future investigations will include detailed immunofluorescence analyses to characterize ABCC6 subcellular localization, as well as the introduction of patient-derived variants into our expression system.

4. Non-invasive assessment of progression to primary biliary cholangitis in patients with positive anti-mitochondrial antibodies

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Introduction: Only a low percentage of people with positive antimitochondrial antibodies (AMA+) develop primary biliary cholangitis (PBC). However, there is

no tool to predict who will and what their management should be. Here, we aim to evaluate the usefulness of the serum bile acid (BA) profile as a non-invasive follow-up tool in AMA+ subjects.

Methods: Longitudinal study in AMA+ individuals identified in the ETHON cohort (multicenter population-based study) between 2015–2017 in Spain, including transient elastography (TE) data. Twenty BA species and 7alpha-hydroxy-4-cholesten-3-one (C4) were analyzed in serum samples by mass spectrometry (HPLC-MS/MS), both at inclusion and follow-up. Patients with a diagnosis of PBC during recruitment were excluded.

Results: Fifty-four AMA+ individuals were identified, of whom 38 (73%) were women with a median age of 49.5 years. None had alterations in liver biology. Baseline liver stiffness was normal (4.5 kPA). Of the initial cohort, 47 (87%) continued follow-up and 6 (12.7%) developed PBC over a median of 5.3 years. The BA profile of non-progressors compared with those who progressed to PBC during follow-up was assessed at both baseline and follow-up visit (median time 4.8 years, IQR, 4.7–5). At baseline, progressors had a higher concentration of non-12-alpha-hydroxylated BAs (non-12aOH) (progressors 2.49 vs. non-progressors 1.45 μM; p = 0.04), glycoconjugated BAs (1.75 vs. 1.03 μM; p = 0.06), as well as lower percentage distribution of cholic acid (CA) species with respect to non-progressors (9.9 vs. 17.1% p = 0.02). At follow-up, progressors presented higher values in 12aOH (1.75 vs. 0.69 μM; p = 0.01), non-12aOH (progressors 2.00 vs. non-progressors 0.84 μM; p = 0.04) and glycoconjugated (progressors 1.86 vs. non-progressors 0.69; p = 0.05) BAs concentrations. In paired analysis, progressors had increased concentration and percentage of CA species (p = 0.02 and p = 0.04, respectively), an increase that was also reflected in the concentration of 12aOH BAs. Moreover, the concentration of non-12aOH BAs in progressors remained elevated and stable (p = 0.91). On the other hand, the profile of all BAs remained stable during follow-up in non-progressors, showing no alterations in serum BA concentrations or in their percentage distribution.

Discussion/Conclusion: AMA+ individuals without progression to PBC show a stable and non-pathological serum BA profile, whereas those who progress show significant changes in the BA profile. Patients who progress show significantly higher non-12aOH BAs values at baseline than those who do not progress, which may be a useful early biomarker for the development of PBC.

5. A human-like bile acid composition attenuates atherogenesis in female Ldlr-/- mice by reducing cholesterol absorption

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Introduction: Emerging evidence links bile acid (BA) metabolism to cardiovascular disease, however, interspecies differences in BA profiles limit the translational value of murine models. In humans, chenodeoxycholic acid (CDCA) is a major BA, whereas mice express hepatic CYP2C70, which converts CDCA into hydrophilic muricholic acids (MCAs). Mice also express CYP2A12, which rehydroxylates secondary BAs into primary forms.

Methods: To investigate the impact of varying degrees of BA humanization on atherogenesis and cardiac function, we generated male and female low-density lipoprotein receptor knockout (Ldlr-/-) mice with liver-specific knockdown of Cyp2a12, Cyp2c70 or both using CRISPR-Cas9. Mice were a fed low-fat, cholesterol-enriched diet for 12 weeks to induce atherogenesis.

Results: Knockdown of Cyp2a12 (C12), Cyp2c70 (C70), or both (double knockdown, DKD) resulted in distinct alterations in BA composition in plasma and bile. This was characterized by elevated deoxycholic acid (DCA) in C12, reduced MCA and increased CDCA in C70, while DKD mice exhibited all of these changes, displaying a progressive increase in BA pool hydrophobicity and humanization. Female DKD mice developed significantly smaller atherosclerotic lesions and necrotic cores compared to wild-type mice, whereas no effect was observed in C12 and C70 mice. In males, BA humanization did not affect atherogenesis. Cardiac function remained unchanged across all groups and sexes. Mechanistically, female DKD mice showed reduced total and very-low-density-lipoprotein (VLDL) cholesterol in plasma, while hepatic cholesterol was significantly lower in both sexes. Serum plant sterols were reduced in all knockdown groups, most notably in DKD mice, indicating decreased intestinal cholesterol absorption. BA humanization had minimal effects on plaque macrophage content, circulating immune cells, or plasma cytokines.

Discussion/Conclusion: These findings demonstrate that a humanized BA profile confers atheroprotection in female DKD mice, likely due to suppressed Cyp8b1-mediated 12α-hydroxylation of taurocholic acid, and primarily through improved lipid metabolism rather than immune modulation.

6. Impact of hypothermic oxygenated perfusion on bile acid composition and injury markers after extended cold preservation of porcine liver

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Introduction: Prolonged cold storage induces substantial ischemia reperfusion injury that impairs hepatocellular and biliary function during subsequent normothermic machine perfusion (NMP).

Methods: In this porcine study, livers preserved for 20 h were assigned to NMP only (control; n = 5), hypothermic oxygenated perfusion (HOPE) with portal perfusion (mHOPE, n = 5), or dual-HOPE with portal and arterial perfusion (dHOPE, n = 6). After 2 h of m/dHOPE at 4–11°C and 6 h of NMP at 37°C, bile acid (BA) compo-

sition, metabolic performance, injury markers and histopathology were evaluated. Histopathology included scoring of ischemia reperfusion injury, bile duct injury, endothelial integrity, and cholestasis.

Results/Discussion: Across all groups, glycohyocholic acid (GHCA) was the most abundant BA, reflecting the naturally hydrophilic porcine BA pool. In the NMP group, BA composition was stable but became slightly more hydrophobic over time, while total perfusate BA strongly correlated with ALT/AST, indicating that BA spillover tracks hepatocellular injury. Total BAs increased in all groups, peaking in NMP. mHOPE produced the most hydrophilic BA profile with rising GHCA and reduced glycochenodeoxycholic acid, while dHOPE induced a taurine-enriched pattern. Both HOPE strategies lowered C4 versus NMP, with dHOPE lowest, suggesting decreased CYP7A1 mediated BA synthesis and preserved FXR feedback. In mHOPE, hydrophobic BAs remained significantly associated with biliary pH, bicarbonate and GGT, reflecting persistent ductal sensitivity. Both HOPE strategies attenuated progression of ALT or AST and reduced endothelial injury compared with controls. dHOPE further decreased cholestasis severity. ALT and AST plateaued in both HOPE groups but continued to rise in NMP.

Conclusion: Collectively, these findings indicate that HOPE modulates both the quantity and quality of the BA pool and uncouples BA-driven toxicity from progressive parenchymal and ductal injury. Dynamic monitoring of total BA, C4 and BA composition during perfusion provides mechanistically informative readouts of graft injury and HOPE efficacy.

7. Discovery of novel bile acid-modifying enzymes in disease-associated gut microbiota

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Introduction: Bile acids play pivotal roles in mediating host physiology and modulating host-microbiota interactions. Secondary bile acids are generated through the co-metabolism of the host and commensal microorganisms. However, only a few bile acid metabolizing bacteria and modifying enzymes have been reported so far.

Methods: In this study, we characterized plasma and fecal bile acid profiles alongside fecal metagenomic data from 67 patients stratified by the severity of intrahepatic cholestasis of pregnancy (ICP). Based on these metagenomic datasets, candidate bile acid-metabolizing enzymes were cloned, heterologous expressed, and assayed for their catalytic activities against a panel of bile acids. Moreover, the underlying chemical mechanisms were elucidated through site-directed mutagenesis and co-crystallization analyses.

Results: We identified novel bacteria and enzymes capable of catalyzing bile acid modifications, specifically 7-hydroxylation and 3-sulfation. We further characterized the structure, catalytic mechanism, and substrate spectra of these

bacterial enzymes. Notably, we also discovered novel endogenous bile acid nuclear receptors associated with disease pathogenesis.

Discussion/Conclusion: We have elucidated the enzymatic pathways responsible for generating these unique bile acid modifications and profiled their associated gut microbiota. These findings highlight the potential of targeting these bile acid-modifying enzymes as therapeutic strategies for related diseases.

8. Constitutive androstane receptor activation modulates bile acid homeostasis during estrogen-induced cholestasis in mice

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Introduction: Estrogens impair bile acid (BA) homeostasis, leading to intrahepatic cholestasis of pregnancy (ICP). ICP is characterized by elevated serum bilirubin and BAs, severe pruritus, and an increased risk of adverse perinatal outcomes. The constitutive androstane receptor (CAR) regulates genes involved in xenobiotic and BA detoxification. Previous studies suggest that CAR activation is protective in bile duct ligation-induced cholestasis and that major genes involved in BA elimination are downregulated in ethinylestradiol (EE)-induced cholestasis in mice.

Methods: We investigated whether CAR ligands ameliorate cholestasis and prevent hepatic injury in EE-induced wild-type and humanized mouse models using biochemical, transcriptomic, and bile acid metabolomic analyses.

Results: We demonstrate that estradiol inhibits and significantly downregulates CAR gene expression in the liver. Consistently, EE significantly suppresses hepatic CAR target gene expression. The murine CAR agonist TCPOBOP partially restores the expression of selected detoxification genes, improves plasma alkaline phosphatase levels, and decreases hepatic BA accumulation. In humanized PXR-CAR-CYP3A mice with EE-induced cholestasis, MI763F, a novel and potent human CAR agonist, recapitulates the effects of TCPOBOP on key genes involved in BA metabolism and the BA metabolome, and significantly reduces hepatic BA accumulation.

Discussion/Conclusion: Our results demonstrate that CAR ligands counteract EE-mediated effects on genes involved in BA homeostasis and may be beneficial for alleviating estradiol-induced cholestasis.

9. Frequencies of polymorphic variants in the bile acid synthesis gene CYP7A1 differ in bile acid diarrhoea and chronic diarrhoea control subjects from the SINBAD trial

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Introduction: Chronic diarrhoea, with frequent watery motions and urgency, is commonly a result of excessive bile acids (BA) in the colon, the condition known as bile acid diarrhoea (BAD). This may be secondary to BA malabsorption, or associated with BA overproduction. BA synthesis in the liver is a result of activity of the enzyme CYP7A1. Previously, we found certain polymorphisms in CYP7A1 gene were associated with severe BAD, as defined by the SeHCAT retention test. We aimed to confirm these findings in a larger, distinct cohort. Other polymorphisms in the genes of both DIET1 and KLB was also assessed.

Methods: Subjects who were screened for the Sequestrant colesevelam IN Bile Acid Diarrhoea trial (SINBAD) had undergone SeHCAT testing and had given blood for measurement of 7α-OH-4-cholestenone (C4) and DNA preparation. The previously studied polymorphisms in CYP7A1, rs3808607, rs8192877 and rs9297994, were genotyped along with rs12256835 (DIET1) and rs17618244 (KLB) by Taqman allelic discrimination assays (Thermofisher Scientific) in 199 subjects.

Results: The allelic frequencies for the two polymorphisms around the promoter of CYP7A1, rs3808607 and rs9297994, were both significantly different in patients with SeHCAT-diagnosed BAD. Comparing BAD patients with SeHCAT < 10% with those > 10% resulted in odds ratios (OR) of 1.88 (p = 0.004) for rs3808607 and 1.90 (p = 0.005) for rs9297994. Other cut-offs were also significant: for instance, < 5% vs. > 15% had OR of 2.12 (p = 0.008) and 2.13 (p = 0.010) for the two sites respectively. When 39 patients with previous cholecystectomy were excluded from the analyses, the effects were larger: for < 10% vs. > 10%, the OR were 2.13 and 2.42. The SeHCAT medians differed between genotypes, with some indication of dominant allele effects.

No differences were found overall in allelic frequencies using C4 diagnostic cutoffs of 46 or 31 ng/mL. However, there were significant differences in the median SeHCAT values between the different genotypes of rs9297994 for those with C4 values > 31 ng/mL (4.6%, 6.0% and 12%; p = 0.046).

The other polymorphic variant site rs8192877 found previously did not show any significant associations in this population. Differences in frequency of DIET1 and KLB polymorphisms also did not reach significance in this population.

Discussion/Conclusion: This study has confirmed in a separate population that specific variants in the promoter of the CYP7A1 gene are associated with low SeHCAT values diagnostic of bile acid diarrhoea. Further studies are needed to define how the genetic findings relate to differences in bile acid synthesis and metabolism.

10. The risk of Parkinson's disease following cholecystectomy: A UK Biobank prospective cohort study

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Introduction: There is increasing evidence for a prominent role of the gut-brain axis in Parkinson's disease (PD), but the precise underlying mechanisms remain poorly understood. Bile acid biosynthesis is intimately related to the gut microbiome and altered in PD. Cholecystectomy induces changes in the bile acid pool similar to those recently identified in PD. A previous retrospective South Korean population study suggested an increased risk of PD after cholecystectomy. Cholecystectomy is a common surgical procedure performed worldwide. A confirmed increased risk of PD after cholecystectomy would consequently have considerable public health implications.

Methods: We therefore interrogated the UK Biobank dataset in a prospective cohort study of 31,994 participants with a history of cholecystectomy, applying Cox proportional hazards modelling to assess the effect of cholecystectomy on the risk of developing PD. We used nearest-neighbour logistic regression propensity scores to match 2:1 a further 63,988 participants without a history of cholecystectomy. This survival analysis was complemented by machine-learning approaches, using Random Forest classifier and regression models to identify whether cholecystectomy is associated with either the development of PD or modifies the age at onset. Average Treatment Effect and Conditional Average Treatment Effect were further applied to investigate causal relationships between cholecystectomy and PD.

Results: Overall, Cox proportional hazards modeling, causality effect estimation and both classifier and regression random forest models did not demonstrate a causal relationship between cholecystectomy and PD.

Discussion/Conclusion: Modulation of the bile acid pool through cholecystectomy alone does therefore not appear to be sufficient to contribute to the pathogenesis of PD.

11. Epimerization of host-derived bile acids is a hallmark of IBD-associated dysbiosis

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Introduction: Inflammatory bowel diseases (IBD) impose a significant global health burden. Bile acids (BA) are central regulators of gastrointestinal homeostasis and are consistently altered in IBD, suggesting a role in disease pathophys-

iology. Host-derived BA (HBA) are primarily converted into microbe-derived BA through C7-dehydroxylation mediated by bai-operon carrying strictly anaerobic commensals such as CAG-103 (Firmicutes) and Clostridium scindens. BA hydroxyl groups can also be epimerized by hydroxysteroid dehydrogenases (HSDH), which are encoded by a limited set of bacteria including Ruminococcus gnavus (3α/β-HSDH, 7β-HSDH) and Escherichia coli (7α-HSDH).

Methods: To investigate microbial BA metabolism in IBD, we analyzed five large publicly available datasets containing paired fecal shotgun metagenomics and untargeted metabolomics. We curated a custom database of microbial BA metabolism genes (> 11,000 genes; github.com/maximilianBaumgartner/MetaBile) to map metagenomic reads. Neural networks were then trained to identify gene modules associated with different BA clusters. Findings were validated in more than 5,000 individuals across 14 cohorts, including two new cohorts from Austria and Germany. To test effects of HBA on gut microbiota, continuous anaerobic cultures inoculated with stool from healthy donors were exposed to HBA in bioreactors. To assess whether BA epimerization promotes survival under high-HBA conditions, wild-type and HSDH knockout strains were grown anaerobically in the presence of cholic acid (CA) and chenodeoxycholic acid (CDCA). Effects on host signaling were studied using public and newly generated transcriptomics datasets and intestinal organoids.

Results: Beyond the previously reported reduction in C7-dehydroxylated BA and increase in HBA, we identified a strong positive association between epimerized HBA clusters (i.e. (iso)UDCA, isoCDCA, isoCA) and IBD. Across ethnically diverse cohorts, gene modules involved in epimerized HBA synthesis were consistently associated with ulcerative colitis, Crohn's disease, and dysbiosis. Elevated HBA are also a hallmark of non-inflammatory idiopathic bile acid diarrhea (BAD). Notably, gene clusters linked to epimerized HBA were similarly enriched in BAD, and microbial alterations overlapped substantially between BAD and IBD, indicating that elevated HBA alone can drive microbiota changes independently of inflammation.

Using in vitro human microbiota bioreactors, we observed that HBA exposure reduced microbial diversity, depleted BA-dehydroxylating bacteria, and enriched taxa commonly associated with IBD and BAD, especially E. coli and R. gnavus. Pure-culture experiments confirmed that these species are intrinsically HBA-tolerant, unlike BA-dehydroxylating commensals, which were more sensitive. Mechanistically, E. coli and R. gnavus encode stereoselective HSDH that epimerize HBA, contributing both to BA transformation and bacterial resistance.

Finally, we investigated BA-mediated host feedback regulation. In homeostasis, HBA activate intestinal FXR to induce FGF19 and suppress hepatic BA synthesis. In IBD, however, CDCA levels no longer correlated with FGF19, indicating disruption of this feedback loop. We found that epimerized HBA inhibit FXR activity, and the ileal abundance of E. coli and R. gnavus inversely correlated with FGF19 levels.

Discussion/Conclusion: Our results implicate elevated HBA as key ecological driver of dysbiosis and microbial diversity loss in IBD. BA-resistant HSDH-carrying bacteria such as E. coli and R. gnavus generate epimerized HBA that further

disrupt the gut-liver BA feedback axis. These results have immediate translational relevance, as BA-sequestering agents could be repurposed to precondition patients prior to microbiome restoration therapies.

12. Bile acids and FGF15/19 decouple hepatic injury and lean mass loss after vertical sleeve gastrectomy

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Introduction: Bariatric surgery, including vertical sleeve gastrectomy (VSG), produces weight-independent metabolic benefits. However, a subset of patients experiences post-bariatric liver decompensation, and the mechanisms remain unclear. VSG alters enterohepatic circulation, increasing systemic plasma bile acid levels and shifting bile acid composition. Our prior work showed that loss of intestinal FGF15 exaggerates the post-VSG rise in plasma bile acids and is associated with liver injury and loss of lean mass. Here, we tested whether these harmful outcomes are directly driven by increased bile acids.

Methods: We combined analyses from a clinical cohort of 38 patients undergoing VSG with complementary genetic and pharmacologic mouse models. In mice, we evaluated the effects of FGF15 deficiency and post-surgical bile acid surges, and tested bile acid modulation via pharmacologic inhibition of the apical sodium-bile acid transporter (ASBT) using maralixibat.

Results: In humans, plasma FGF19 increased after VSG and correlated with higher systemic bile acid levels. Changes in specific bile acids were associated with postoperative improvements in markers of liver injury (ALT, AST) and lipid metabolism (triglycerides). In mice that have undergone surgery, hepatic injury caused by FGF15 deficiency and the resultant post-surgical bile acid surge was rescued by ASBT inhibition with maralixibat. However, lean mass loss persisted in both FGF15-deficient and control mice treated with maralixibat after undergoing VSG. Microbiome analyses showed that FGF15 is required for surgery-induced enrichment of beneficial taxa, whereas FGF15 loss produced a distinct, less favorable microbial profile. ASBT inhibition shifted the microbiome toward increased abundance of beneficial species.

Discussion/Conclusion: These findings suggest that bile acid excess is a key driver of post-VSG hepatic injury and that enhancing bile acid clearance can restore hepatic recovery. In contrast, maintenance of lean mass appears to require adequate FGF15/19 signaling and is not fully corrected by bile acid lowering alone. Overall, the data mechanistically decouple hepatic recovery from lean mass preservation after VSG and suggest that improving long-term outcomes may require combined strategies that enhance bile acid clearance while maintaining FGF15/19 signaling.

13. Assessment of ABCB4 missense variant prediction accuracy using ClinVar classified variants as a functional benchmark

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Introduction: Interpretation of rare ABCB4 missense variants remains a major challenge in cholestatic liver disease, including intrahepatic cholestasis of pregnancy (ICP), because computational tools often provide discordant results and functional data are limited. We assessed the accuracy of commonly used and gene-specific in silico predictors for ABCB4 missense variant classification, using ClinVar-labelled variants as a functional benchmark.

Methods: ABCB4 missense variants with available ClinVar evaluations were curated, excluding conflicting or uncertain labels and ensuring that all classifications had been evaluated post-2015 after the release of the American College of Medical Genetics (ACMG) variant interpretation framework. All variants were then evaluated using SIFT, PolyPhen-2, CADD, VASOR and AlphaMissense. Tool performance was assessed by concordance with ClinVar using Matthews Correlation Coefficient (MCC) and F1 score. An ICP cohort in the Genomics England Research Environment was curated and rare missense variants in this cohort were assessed to evaluate overlap with in silico tool prediction and ClinVar benchmark.

Results: ClinVar had a total of 41 missense ABCB4 variants which had been evaluated after the release of the ACMG in 2015. Benchmarking against ClinVar-labelled variants demonstrated that CADD and AlphaMissense performed best, with MCC's of 0.781 and 0.731 and F1 scores of 0.955 and 0.923, respectively. SIFT showed intermediate performance, while PolyPhen-2 and the ABCB4-specific tool VASOR performed least well in this dataset. The Genomics England ICP cohort was made up of 253 women in whom 4 rare missense variants overlapped with the ClinVar ABCB4 missense variant list.

Discussion/Conclusion: Prediction accuracy for ABCB4 missense variants varies substantially between tools, and even the best-performing algorithms do not consistently reflect published functional evidence. ClinVar-based benchmarking suggests that CADD and AlphaMissense currently provide the strongest computational support, but in silico data should be used cautiously and only as one component of variant interpretation. These findings emphasise the need for gene-specific functional validation and better curated benchmark datasets for ABCB4.

14. Efficacy and tolerability of maralixibat, an ileal bile acid transporter inhibitor, for the treatment of intrahepatic cholestasis of pregnancy

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Introduction: Intrahepatic cholestasis of pregnancy (ICP) is characterized by elevated maternal total serum bile acids (TBA) and liver transaminases. Maralixibat (MRX) is an ileal bile acid transporter (IBAT) inhibitor that effectively reduces pruritus and total serum bile acid (TBA) levels in non-gestational cholestasis. This case series reviewed the effectiveness and tolerability of MRX in ICP.

Methods: Retrospective analysis of four cases of recurrent, severe ICP treated with MRX through a compassionate use program was conducted. Key clinical, biochemical, and safety data were collected.

Results: Case 1 was a 38-years-old White woman with elevated TBA (148 µmol/L). MRX treatment (70 µg/kg/d, uptitrated to 300 µg/kg/d) reduced TBA (52–93 µmol/L), but it rose (125 µmol/L) and delivery was expedited. Case 2 was a 42-years-old South Indian woman with elevated TBA (237 µmol/L). MRX treatment (300 µg/kg BID) improved TBA (< 40 µmol/L). Case 3 was a 36-years-old Black woman with pruritus and elevated TBA (219 µmol/L). MRX treatment (70 µg/kg/d, uptitrated to 210 µg/kg/d) improved pruritus and TBA (< 40 µmol/L). Case 4 was a 37-years-old White woman with elevated TBA (118 µmol/L). MRX treatment (70 µg/kg/d, uptitrated to 175 µg/kg/d) reduced TBA (< 40 µmol/L). All patients delivered live babies. Mild, but manageable, GI events occurred in three patients during MRX treatment.

Discussion/Conclusion: These findings suggest that MRX may effectively manage ICP by lowering TBA and improving pruritus.

15. Bile acids regulate lipid metabolism through selective actions on fatty acid absorption

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Introduction: Intestinal lipid absorption, the entry point for fats into the body, requires the coordinated actions of bile acids and lipases. Here, we uncover distinct yet cooperative roles of bile acids in driving the differential uptake of dietary fatty acids.

Methods: To determine the role of bile acids, we first decreased the bile acid pool size by disrupting the rate-limiting enzyme in bile acid synthesis, Cyp7a1, using liver-directed AAV-CRISPR in mice. To contrast the effects of bile acid pool modulation, we used the lipase inhibitor tetrahydrolipstatin (orlistat) as an alternative strategy to modify dietary fat absorption.

Results: Compared with lipase inhibition, reducing bile acids prevented diet-induced obesity, increased anorectic hormones (GLP-1, PYY), suppressed excessive

eating, and improved systemic lipid metabolism. Remarkably, decreasing bile acids selectively reduced the absorption of saturated fatty acids but preserved uptake of polyunsaturated fatty acids. By targeting additional bile acid enzymes (Cyp8b1, Cyp2c70, Cyp2a12) with AAV-CRISPR, we identified specific functions of individual bile acid species in the bile acid pool. Using in vitro solubility assays and an intestinal organoid system, we show that cholic acid preferentially solubilizes polyunsaturated fatty acids into mixed micelles for intestinal uptake, thus providing a mechanism for the enhanced uptake of these fatty acids.

Discussion/Conclusion: Taken together, our data unveils multifaceted aspects of dietary fat absorption and highlights the natural selectivity in the absorption of fatty acids by bile acids. These findings further advance our basic understanding of dietary fat absorption and suggest bile acid pool modulation as a potential therapeutic strategy to regulate differential absorption in health and disease.

16. Plasma sulfated lithocholic acid predicts mortality while conjugated primary bile acids associate with myocardial relaxation in cirrhosis patients undergoing TIPS

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Introduction: Bile acids exert systemic signaling effects, including regulation of cardiovascular function. Their role in the pathogenesis of cirrhotic cardiomyopathy and clinical outcomes of cirrhosis remains incompletely understood. We investigated associations between bile acid composition, cardiac function, and survival in patients with cirrhosis undergoing transjugular intrahepatic portosystemic shunt (TIPS).

Methods: In this observational study, 28 patients with cirrhosis undergoing TIPS were included (median age 63 years; Child-Pugh class A/B/C: 28.6%/50.0%/21.4%; main TIPS indications: ascites and variceal bleeding). Fasting bile acids were quantified in peripheral plasma by UHPLC-MS/MS. Transthoracic echocardiography was performed before TIPS. Associations between bile acids and echocardiographic parameters were assessed using Spearman correlation. Patients were stratified according to left ventricular relaxation (e′ septal < 7 cm/s). Survival was analyzed using Kaplan-Meier curves and log-rank testing during follow-up after TIPS (median follow-up approximately 25 months; 13 deaths/28 patients).

Results: Primary conjugated bile acids were positively associated with myocardial relaxation and were reduced in patients with relaxation impairment, whereas secondary unconjugated bile acids showed no relevant associations. Sulfated lithocholic acid (LCA-S) was inversely associated with cardiac structural param-

eters, including end-systolic and left atrial volumes. Patients who died (n = 13) had higher LCA-S levels than survivors (n = 15), and Kaplan–Meier analysis demonstrated reduced survival in patients with elevated LCA-S.

Discussion/Conclusion: Peripheral fasting plasma bile acid composition is differentially associated with cardiac function and survival in cirrhosis. Primary conjugated bile acids are linked to preserved myocardial relaxation, whereas LCA-S reflects disease severity and predicts mortality.

17. Rifampicin, but not ursodeoxycholic acid, alters the taxonomic, but not the functional, composition of the gut microbiota in women with intrahepatic cholestasis of pregnancy

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Introduction: Increased maternal total bile acid (TBA) concentrations and symptoms, especially pruritus, characterise Intrahepatic Cholestasis of Pregnancy (ICP) in women without known active liver disease. Severe ICP (TBA ≥ 100 µmol/L) is associated with increased risk of stillbirth. The gut microbiome converts primary BA into secondary BA, which can signal through the TGR5 receptor. Ursodeoxycholic acid (UCDA) is the standard treatment for ICP with variable effectiveness. The TURRIFIC RCT compares use of UCDA with the antibiotic rifampicin (RIF).

Methods: We aimed to compare the effect of UCDA and RIF on the taxonomic and functional composition of the gut microbiome in stool samples collected throughout pregnancy in 5 women treated with UCDA and in 8 treated with RIF. Metagenomic sequencing at 3 GB depth was performed and analysed using the MetaPhlAn pipeline for taxonomy and HuManN3 for function. Differential abundance was determined using MaAsLin2 and adjusted for multiple testing.

Results: Interindividual variability was extensive. Treatment with RIF significantly reduced alpha diversity in both taxonomic and functional analyses, whereas beta diversity was altered only at the taxonomic level. RIF treatment decreased the abundance of Ruminococcus bromii, Romboutsia and Candidatus Cibionibacter compared with that seen in UCDA-treated or untreated ICP. No significant changes to the functional pathways or to antibiotic-resistance gene abundance in the microbiomes were observed between the groups.

Discussion/Conclusion: In this small cohort of women with ICP, treatment with UCDA or RIF did not have a major effect on the composition of the gut microbiome or on antibiotic-resistance gene carriage. This may reflect the small sample size or a lack of impact of the treatments on the microbiome in women with ICP.

18. Clinical significance of serum total bile acid in the diagnosis and management of intrahepatic cholestasis of pregnancy

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Introduction: Intrahepatic cholestasis of pregnancy (ICP) represents a significant risk factor for perinatal mortality and stillbirth. Research conducted at the Amgalan Maternity Hospital (2020–2022) underscores the critical role of serum total bile acid (TBA) as the primary biochemical marker for diagnosing and monitoring fetal risk. The primary objective of the research was to evaluate serum bile acid levels in pregnant women to determine their clinical importance in the Mongolian healthcare context.

Methods: Study Design: A cross-sectional analytic study. Participant Cohort: 96 women receiving prenatal care at Amgalan Maternity Hospital between 2020 and 2023.

Groups: Healthy Group: 38 women. ICP Group: 37 women diagnosed with Intrahepatic Cholestasis of Pregnancy. CVH Group: 21 women with Chronic Viral Hepatitis. Data Collection: Demographic data and obstetric risk factors were gathered via questionnaires, supplemented by physical examinations, laboratory tests, imaging, and retrospective analysis of birth outcomes.

Results: 1. Biochemical Analysis of Serum Total Bile Acid (TBA)

The median and quartile range of serum total bile acid levels of pregnant women were 3.36 (2.2–5.4) μmol/L in the healthy group, 18.68 (13.4–23.9) μmol/L in the ICP group, and 19.1 (10.5–33.6) μmol/L in the CVH group. Normal level of serum total bile acid noted 94.7 percent of healthy pregnant women in the healthy group, only 13.5 and 19.0 percent in the ICP and CVH groups respectively. Moreover an elevation of serum total bile acid detected in 86.2 percent (n = 32) of ICP group, among them 75.7 percent of women had an elevation of serum total bile acid from 10 to 39 μmol/L and 10.8 percent of women had an severe elevation, higher than 40 μmol/L. In the CVH group, patients with elevation of serum total bile acid were 81.0 percent (n = 17), among them 66.7 percent of women had an elevation of serum total 6 bile acid from 10 to 39 μmol/L and 14.3 percent of women had an severe elevation, higher than 40 μmol/L.

2. Clinical Manifestations and Symptoms of ICP

Pruritus (Itching): 100% of women in the ICP group experienced pruritus without a primary skin rash. Onset: Most commonly occurred between 26 and 32 weeks of pregnancy (43.2%). Intensity: 51.4% reported moderate to severe itching. Dermatological Impact: 62.1% of patients presented with moderate to severe or multiple scabs and scars resulting from skin changes. Liver Function Markers: In the ICP group, the Alkaline Phosphatase (ALP) median value increased to 195.0 units/L.

3. Obstetric and Perinatal Outcomes

Gestational Period: The ICP group had a statistically significant shorter gestational period ($p = 0.04$) compared to the healthy group, with an average gestational age at birth of 37.1 ± 1.4 weeks. Preterm Delivery: 56.6% of births in the ICP group were preterm, primarily driven by “fetus health indication” ($p = 0.006$).

Infant Maturity: Mature Infants: 66.6%, Premature Infants: 33.3% (statistically higher than the healthy group, $p = 0.028$).

Parity: ICP was more predominant in first-time pregnancies (p = 0.001) within this study cohort.

4. Differential Diagnosis: ICP vs. Chronic Viral Hepatitis (CVH)

While both ICP and CVH patients show elevated TBA levels, the study identified distinct biochemical differences to assist in differential diagnosis: ASAT Levels: The CVH group showed a significant increase in ASAT (median 45.4 µ/L) compared to the ICP group (p = 0.035). Direct Bilirubin: The CVH group had higher mean values of direct bilirubin (8.2 ± 3.1 mmol/L) than the ICP group (p = 0.041).

Discussion/Conclusion: The analysis confirms that serum TBA is an essential test for evaluating liver function and bile metabolism during pregnancy.

1. Diagnostic Threshold: Elevated TBA levels (> 10 µmol/l) combined with pruritus in the third trimester are definitive clinical features of ICP.
2. Risk Correlation: Severe elevation of TBA is directly correlated with preterm delivery necessitated by fetal health indications.
3. Health Service Integration: There is a demonstrated necessity to introduce routine serum TBA testing into Mongolian health services to improve the detection and management of ICP, thereby mitigating the risk of stillbirth and perinatal mortality.

19. Knockout of claudin-3 leads to altered bile metabolism and gut microbiota

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Introduction: The tight junction protein claudin-3 acts as a paracellular barrier component separating bile and blood flow in liver hepatocytes. Here, we investigated how loss of claudin-3 expression alters the composition the bile and the gut microbiota.

Methods: Bile was collected from Cldn3+/+ and Cldn3-/- mice and bile acid concentration and composition were measured. The microbiota from feces were analyzed using Ion Torrent 16S rRNA sequencing. Intestinal morphology was evaluated by hematoxylin and eosin (H&E) staining.

Results: Total bile acid concentration was lower in Cldn3-/- mice; however, the percentual composition of the bile acid pool was similar in Cldn3-/- and Cldn3+/+ mice. We found an altered microbial composition, with an increased abundance of Bacteroidia in Cldn3-/- mice.

Discussion/Conclusion: We found that claudin-3 knockout led to an overall dilution of bile, without changes in bile acid composition. We hypothesize that the dilution of bile contributed to the observed alteration in gut microbiota. Future

studies will focus on the functional consequences of these microbiome changes, including effects on intestinal gene expression and proteomic profiles, as well as mucus layer structure and composition and epithelial cell populations such as goblet and paneth cells.

20. Inhibition of hepatic bile salt uptake using bulevirtide attenuates pre-existing diet-induced metabolic dysfunction-associated steatohepatitis in mice

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Introduction: Metabolic dysfunction-associated steatotic liver disease (MASLD) affects over 30% of the global population and is characterized by hepatic fat accumulation. MASLD can progress into steatohepatitis (MASH), which involves inflammation that can lead to fibrosis and eventually cirrhosis and liver failure.

Inhibition of the Sodium-dependent Taurocholate Cotransporting Polypeptide (NTCP) represents a potential therapeutic strategy, as this increases circulating bile salts, thereby improving glucose and lipid metabolism and reducing inflammation. We investigated whether NTCP inhibition with bulevirtide, a safe and approved drug for treatment of hepatitis B and D, could attenuate liver steatosis, inflammation, and fibrosis in a MASH mouse model.

Methods: Hyperlipidemic C57BL/6Joatp1a1/1a4/1b2/ldlr-/- male and female mice were used to mimic human hepatic bile salt uptake dynamics. Mice were fed a high-fat/high-cholesterol diet (40% and 0.15% w/w) and 15% fructose water (HFHCF) for 22 weeks to induce MASH. Liver biopsies confirmed features of MASH at baseline week 16. Subsequently, mice received daily subcutaneous injections of bulevirtide (2.5 mg/kg) or vehicle for 6 weeks while continuing the HFHCF diet.

Results: HFHCF diet significantly increased liver-to-body weight ratios, plasma triglyceride, insulin levels, and induced hepatic steatosis, lobular inflammation and F2 perisinusoidal and periportal fibrosis. Bulevirtide increased plasma bile salt levels 20-fold. Bulevirtide treatment significantly decreased liver-to-body weight ratios (-19%) and tended to reduce Oil Red O-positive area percentage (-19%), liver triglyceride (-15%) and cholesterol content (-19%). Bulevirtide significantly lowered plasma triglyceride levels (-84%) and reduced plasma insulin levels (-45%). Bulevirtide treatment lowered abundance of monocytes in plasma (-35%) and liver (-22%) and induced a shift towards a less inflammatory (Ly6ChighTREML4low) and more regulatory (Ly6ClowTREML4high) profile. Bulevirtide treatment tended to lower F2 perisinusoidal and portal/periportal fibrosis scores, and significantly reduced liver COL1A1 abundance (3.5-fold) compared to vehicle in males only.

Discussion/Conclusion: Bulevirtide improved MASH, but also metabolic parameters in a mouse model with pre-existing MASH. These findings suggest that NTCP inhibition may be a promising treatment for MASH, addressing both hepatic pathology and metabolic dysfunction.

21. ICP is associated with changes in 54 peptides in the metaproteome of women in late pregnancy

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Introduction: In Intrahepatic Cholestasis of Pregnancy (ICP), women without known active liver disease develop increased circulating total bile acids (BAs) often in conjunction with pruritus. The gut microbiome plays an important role in BA metabolism, and variable changes to its composition and function have been reported in ICP using metagenomic analysis. The proteins secreted by both microbiome and host can be studied using metaproteomics of the faeces.

Methods: The aim of this analysis was to compare metaproteomic profiles between women at risk of ICP and those with, or who developed, ICP. Faecal samples (n = 64) were collected throughout pregnancy from the TURRIFIC (Trial of URsodeoxycholic acid vs. RIFampicin in ICp) and Pre-TURRIFIC (at-risk women) cohorts. Protein was isolated and digested with trypsin prior to analysis with LC-MS using DIA-NN software. Peptides were mapped back to the MetaPep database. The groups were compared using t-tests, retaining significantly different peptides at a false discovery rate of 0.01.

Results: A median of 4811 peptides (IQR, 2772–8130) per sample were retrieved. 335 peptides were present in > 80% samples and these were compared. Principal Component Analysis did not show significant separation between the groups. 54 peptides, 51 from bacteria mapping to 32 proteins and 3 human peptides, were found to be differentially expressed between the groups. 27 bacterial proteins were downregulated in women with ICP; 5 were upregulated. The downregulated proteins had functions in catabolic metabolism, protein synthesis and defence to reactive oxygen species. The upregulated bacterial proteins were involved in anabolic and iron metabolism. The human proteins were chymotrypsin (upregulated), GAPDH and heat shock protein 70 (downregulated).

Discussion/Conclusion: These results suggest that there are functional changes to the microbiome in women with ICP, indicating shifts in nutrient availability and oxidative stress response. This could result in changes in gut permeability and host immune response.

22. Cyp2c70 silencing rewires hypothalamic synaptic plasticity by shifting bile acid composition

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Introduction: Bile acid (BA), a digestive surfactant, is also considered a signalling molecule. Through receptors like TGR5 and FXR, BA can modulate neuronal activity and synaptic function. However, the extent to which alterations in BA

composition (humanized BA pool) impact hypothalamic synaptic plasticity and signalling remains poorly understood.

Methods: Eight-week-old male mice received siRNA treatment for 2 weeks to silence the Cyp2c70 (siCyp2c70) gene, thereby humanising the BA pool. The hypothalamus punches were subjected to bulk RNAseq.

Results: Principal component analysis and hierarchical clustering heatmap revealed that the majority of the siCyp2c70 group clustered distinctly from the WT, indicating a genotype-driven transcriptional shift. As an initial, hypothesis-driven entry point into synaptic plasticity, we focused on postsynaptic long-term depression (LTD) and potentiation (LTP) pathways, which link mGluR signalling, calcium dynamics, and AMPAR trafficking. Inositol phospholipase C isoforms (Plcb1, $p < 0.001$; Plcb4, $p < 0.01$) are significantly downregulated in siCyp2c70 mice compared with WT. Calcium release channels, Cacna1d and Ryr2, show significantly lower expression in siCyp2c70 mice, suggesting a broad weakening of mGluR-dependent LTD signalling. The postsynaptic AMPAR content and trafficking capacity were diminished in siCyp2c70, since Gria1, Gria2, and Grip1 were significantly downregulated. Moreover, the protein phosphatases, Ppp1cb ($p < 0.0001$), and the phosphatase regulatory gene Pten ($p < 0.0001$), were robustly reduced in the iCyp2c70 mice, suggesting a compromised phosphatase-dependent dephosphorylation. To contextualise these transcriptional changes within a broader synaptic framework, we projected the genes onto the KEGG LTD/LTP pathways, which highlighted coordinated modulation of both pre- and postsynaptic elements. Next, we examined whether these changes were accompanied by alterations in neurotransmitter handling and BA transport. The BA transporters (Slc10a3 and Slc10a4), vesicular GABA/glycine transporter, Slc32a1, and catecholamine synthesis enzyme Th were upregulated in siCyp2c70.

Discussion/Conclusion: Loss of Cyp2c70 enhances hypothalamic BA and monoaminergic signalling capacity, but reduces glutamatergic plasticity machinery, potentially shifting network plasticity towards rigid, neuromodulator-dominated control of energy balance circuits. This preliminary study warrants single-cell and spatial transcriptomics, proteomics, and functional follow-up work in Cyp2c70-based humanised BA models.

23. 10 cases of ileal bile acid transporter (IBAT) inhibitor therapy in adults with hereditary bile salt export pump (BSEP)-deficiency

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Introduction: Variants in the bile salt export pump BSEP predispose to various cholestatic liver diseases. While patients carrying biallelic protein truncating

variants present in infancy with rapidly progressive familial intrahepatic cholestasis (PFIC2), patients with missense mutations and residual BSEP function tend to present with slower disease progression. Therapeutic approaches in BSEP deficiency aim to decrease serum bile acids (SBA) to prolong native liver survival. Ileal bile acid transporter inhibitors (IBATi) reduce SBA and pruritus in PFIC2 patients. So far, little is known about the use of IBATi in adults. This case series summarizes the IBATi treatment of 10 adults with BSEP deficiency.

Methods: Patients presented at German and Australian tertiary centers. Disease onset, symptoms, interventions, medication, lab values, personal and family medical history, and pruritus assessment were collected prospectively. Genetic variants were determined by whole exome sequencing (WES).

Results: 6 patients reported disease onset within infancy while 4 patients had reached adolescence or adulthood. From 6 with early onset 5 had biallelic BSEP mutations and in 3 of 4 with late onset only one BSEP mutation was found. Despite missense mutations, one splice and one nonsense mutation were detected. Without treatment, all patients suffered from severe itch affecting quality of life. IBATi therapy improved itch 2 points on a VAS in all patients with clinically significant itch (5/10). SBA reached values < 102 µmol/l in all patients with adequate compliance. IBATi therapy was stopped after 7 months in 1 cirrhotic patient due to an increase in transaminases, bilirubin, SBA, and liver stiffness, interpreted as disease progression and not as IBATi-related adverse event.

Discussion/Conclusion: Adults with BSEP deficiency showed similar reduction in itch and SBA in response to IBATi therapy as reported in pediatric phase III trials. Weight adjusted IBATi dosing as used in pediatric trials was safe and well tolerated in our cohort.

24. Synthesis of 3-NBD bile acid conjugates as probe substrates for hepatic and intestinal SLC10 and SLCO transporters

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Introduction: Enterohepatic circulation of bile acids (BA) is pivotal for the absorption of hydrophobic nutrients and the maintenance of cholesterol homeostasis. Thereby, several carrier proteins, including the hepatic Na+/taurocholate cotransporting polypeptide (NTCP) and the intestinal apical sodium-dependent BA transporter (ASBT), are involved in the active transport of BA. Commonly, transport studies are performed using radio-labelled BA (e.g. [3H]-TCA), entailing limitations due to radioisotope handling. In contrast, fluorescent probe substrates enable broader applications to visualize and elucidate BA transport in vitro and in vivo.

Methods: We synthesized an extensive series of fluorophore-labelled BA derivatives, differing in the BA monomer (CA, CDCA, DCA, LCA), the stereochemistry of the fluorescence label in 3-position (3α, 3β), and the conjugation (unconjugated, glycine conjugated, taurine conjugated). As fluorophore, we utilized 4-nitrobenzo-2-oxa-1,3-diazole (NBD), exploiting its low sterical impact. For all derivatives, in vitro transport studies on transfected HEK293 cells were perfomed.

Results: In vitro transport studies could demonstrate that all glycine and taurine conjugated BA were actively transported by human NTCP, mouse mNtcp, and mouse mAsbt, with 3β-NBD-TCA demonstrating superior transport efficacy. For human ASBT, exclusively 3α-NBD-GCA was reliably transported in a sodium-dependent manner.

Discussion/Conclusion: 3β-NBD-TCA proved applicable for comparative transport studies involving the carrier proteins human NTCP, mouse mNtcp, and mouse mAsbt. Due to a reliable in vitro transport activity for all four carrier proteins, 3α-NBD-GCA emerged as promising probe substrate for fluorescence-based NTCP and ASBT inhibitor screenings.

25. Altered serum bile acid concentration correlate with lipid profile and cardiovascular disease risk in patients with liver cirrhosis

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Introduction: Liver cirrhosis is increasingly recognized as a significant cardiovascular risk factor. Bile acids (BAs) are crucial in regulating lipid metabolism and cardiovascular function. Elevated BA levels are associated with dyslipidemia and risk of adverse cardiovascular events. The present study was designed to evaluate serum levels of BAs in patients with liver cirrhosis and their relation to lipid profile and cardiovascular disease (CVD) risk

Methods: This cross-sectional study included 30 patients with liver cirrhosis without CVD and 15 age- and sex-matched healthy subjects. Serum levels of total BAs were measured using fluorometric enzyme method and levels of free cholic acid (CA), and its glycine and taurine conjugates [glycocholic acid (GCA) and taurocholic acid (TCA)], and sulphate ester [thioglycocholic acid (TGCA)] were assayed using thin layer chromatography. Lipid profile [total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), and triglycerides (TG)], fasting plasma glucose, serum creatinine, urinary albumin-creatinine ratio, and estimated glomerular filtration rate were assayed. Cardiovascular disease (CVD) 10-year risk was assessed by calculating the CVD Risk Estimator Plus (CVD-REP) score proposed by the American College of Cardiology based on age, sex, TC, HDL-C, systolic blood pressure, renal function, body mass index, diabetes, and smoking status. Also, the atherogenic index of plasma (AIP) TC/HDL-C were estimated as measures of CVD risk.

Results: Patients with liver cirrhosis showed significant increases in serum levels of total BAs, free CA, GCA, TCA, and TGCA, and GCA/TCA ratio compared with healthy subjects ($p < 0.01$). According to CVD-REP, patients were categorized as low risk (< 5%) in 11 (36.7%) patients, moderate risk (5–19.9%) in 14 (46.7%) patients, and high risk (> 20%) in 5 (16.7) patients. Serum levels of total and conjugated BAs were positively correlated with serum levels of TC, LDL-C, TG and TG, CVD-REP score, AIP and TG/HDL-C and were negatively correlated with serum HDL-C levels while serum free CA levels were inversely correlated with CVD risk scores ($p < 0.05$).

Discussion/Conclusion: Altered serum BA profile with high conjugated forms, was associated with disturbed lipid metabolism and CVD risk in patients with liver cirrhosis.

26. Serum bile acid levels in non-diabetic patients with liver cirrhosis: Relation to insulin resistance and central obesity

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Introduction: Liver cirrhosis is often associated with insulin resistance (IR), hyperinsulinemia and impaired glucose tolerance. Bile acids (BAs) play a major role in glucose metabolism and energy expenditure. Alterations in BA profile may be linked to IR type 2 diabetes mellitus, and obesity. The present work was designed to study serum levels of BAs in non-diabetic patients with liver cirrhosis and their relation to IR and central obesity.

Methods: Thirty non-diabetic patients with liver cirrhosis (body mass index (BMI) < 30 kg/m2) and 15 age- and sex-matched healthy subjects were included in the present study. Serum total BAs were measured using fluorometric enzyme method and serum free cholic acid (CA), glycocholic acid (GCA) and taurocholic acid (TCA), and thioglycocholic acid (TGCA) were assayed using thinlayer chromatography. Liver function tests, lipid profile, fasting plasma glucose (FPG), and fasting serum insulin (FSI) were assayed. IR was assessed by measuring the homeostasis model assessment of insulin resistance (HOMA-IR), triglyceride glucose index (TyG) and quantitative insulin-sensitivity check index (QUICKI). Central obesity was assessed by measuring waist circumference (WC), waist-to-hip ratio (WHR), and visceral adiposity index (VAI).

Results: Serum levels of total and individual BAs, FBG, FSI, HOMA-IR, TyG were significantly higher while QUICKI was significantly lower in patients with liver cirrhosis than in healthy subjects and in patients with IR than in patients without IR ($p < 0.01$). Serum levels of total and conjugated BAs (GCA and TCA) showed positive correlations with FPG, FSI, HOMA-IR, TyG, WC, WHR, and VAI and a negative correlation with QUICKI in patients with liver cirrhosis ($p < 0.05$).

Discussion/Conclusion: High serum BA concentration, particularly conjugated BAs, may play an important role in the development of IR and central obesity in non-diabetic patients with liver cirrhosis.

27. Molecular characterisation of the trafficking rescue of defective ABCB4 variants by roscovitine analogues

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Introduction: ABCB4 is expressed at the canalicular membrane of hepatocytes and is responsible for the secretion of phosphatidylcholine into bile. Genetic variations of ABCB4 are associated with rare cholestatic liver diseases, the most severe being progressive familial intrahepatic cholestasis type 3, for which most of patients require liver transplantation before adulthood. Therefore, the development of alternative pharmacotherapies is urgently needed. We have previously shown that structural analogues of roscovitine rescue the maturation, localisation and indirectly the function of class II intracellularly-retained ABCB4 variants. Nevertheless, in order to develop molecules with better benefit/toxicity ratios, new structural analogues of roscovitine were tested in cell models as potential correctors of three class II ABCB4 variants.

Methods: We used cell models (HEK293 and HepG2) expressing wild type and mutated forms of ABCB4 to test the effect of roscovitine analogues (10 µM) on the maturation, localisation and function of the transporter, using immunoanalyses (immunoblots and immunofluorescence) and functional assays. In addition, the potential interaction of roscovitine analogues with ABCB4 was investigated by ensemble docking calculations.

Results: We show here that nine roscovitine analogues are able to significantly rescue the maturation and canalicular localisation of these ABCB4 variants. Three of these analogues were also able to partially restore the transporter-mediated activity of ABCB4 variants, while being less inhibitory of wild type ABCB4 function than roscovitine itself. In addition, ensemble docking calculations suggest that the selected roscovitine analogues may directly interact with wild type or mutated ABCB4.

Discussion/Conclusion: Our results represent a new step towards the identification of pharmacological correctors for ER-retained variants of the ABCB4 transporter.

28. Inhibition of bile acid conjugation improves cholestasis in humanized mice

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Introduction: In cholestasis, intrahepatic accumulation of bile acids (BAs) contributes to liver and bile duct injury. We hypothesized that inhibiting BA conjugation—thereby increasing non-amidated BAs and altering BA composition—may enhance bile flow and ameliorate cholestatic injury.

Methods: FVB/N wild-type mice were fed 3,5-diethoxycarbonyl-1,4-dihydrocollidine (DDC) for 4 weeks to induce sclerosing cholangitis and cholestasis. To humanize the BA pool, mice received siRNA targeting Cyp2c70 (siCyp2c70). BA conjugation was inhibited using siRNA against BACL (siBACL). Liver injury was evaluated using biochemical, histological, and immunohistochemical analyses. Bile flow, BA profiles (serum and bile), biliary glutathione levels, and hepatic hydroxy-

proline content were assessed. RNA sequencing, RT-PCR, and immunoblotting were performed to analyze gene and protein expression.

Results: In DDC-fed siCyp2c70 mice, BACL silencing reduced conjugated BAs and increased unconjugated BAs, with cholic acid predominating. This shift was associated with a 2-fold increase in bile flow and a 3-fold increase in biliary glutathione output. siBACL treatment improved biochemical and histological markers of liver and bile duct injury. While inflammation was only partially reduced, fibrosis significantly improved, evidenced by decreased fibrogenic gene expression (e.g., Col12a1, Col14a1, Col4a2, Fbn1) and a ~50% reduction in hepatic hydroxyproline levels. Markers of bile duct proliferation (CK19) and reactive cholangiocyte phenotype (VCAM) were also reduced. RNA sequencing revealed upregulation of genes involved in ROS detoxification (e.g., Gsta1, Gsta3, Gsta5, Gstm2, Gstm3).

Discussion/Conclusion: Inhibition of BA conjugation alleviates cholestatic liver and bile duct injury, likely through increased bile flow, enhanced biliary glutathione secretion, and improved oxidative stress defense.

29. Circadian oscillations of microbial taxa with potential for bile acid modifications: Correlating diurnal shifts in bile acids with microbiota, microbial bsh and bai

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Introduction: Disruption of circadian rhythms by modern lifestyle, unhealthy dietary habits and stress has been linked to impaired metabolic health. Bile acid (BA) synthesis is subject to strict diurnal regulation suggesting that circadian orchestration of bile acid signatures is important for homeostasis. Indeed, we have previously demonstrated that elevation of microbial bile salt hydrolase (BSH) activity in the murine gut influences transcriptional control of host genes responsible for adiposity/lipid metabolism, and also networks responsible for entraining local circadian rhythm (Joyce et al, PNAS. 2014). In this study we focused upon the potential for microbial alterations of bile acid signatures over the 24-hour cycle in normal SPF mice under strict light/dark cycles.

Methods: Mouse groups were sampled at Zeitgeber Times (ZT) ZT0, ZT6, ZT12 and ZT18. Bile acid signatures were established using UPLC-mass spectrometry. Gut microbiota was analyzed by 16s rDNA and shotgun sequencing. Host gene expression from ileum, cecum, colon and liver was measured using qRT-PCR.

Results: We verified diurnal oscillations of BA species levels in the contents of the large intestine and blood of mice measured at 6-hour intervals. Analyses revealed an elevation of unconjugated bile acids at ZT6 concomitant with increased abundance of taxa with potent BSH activity (including Lactobacillus species) and elevated circadian host Rev-erbα, Fxr and Fgf15 expression (and decreased expression of Per2). Microbial taxa with known bile acid inducible (bai)

genes (responsible for secondary BA synthesis) were subject to elevation at ZT18. Follow-up with shotgun metagenomic analysis of the caecal microbiota revealed nuances in the abundance of particular bsh and bai orthologues which may provide insights into taxa/genes of importance for regulating microbial diurnal alterations in BA signals.

Discussion/Conclusion: Our study describes the complex time-dependent environment in which BA species are produced in mice and can be used to guide further investigations in this area.

30. High dietary cholesterol drives MASLD-to-MASH progression by induction of bile macropinocytosis

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Introduction: Metabolic Dysfunction-Associated Steatotic Liver Disease (MASLD) affects nearly 38% of adults worldwide, yet the mechanisms underlying its progression to Metabolic Dysfunctiona-Associated Steatohepatitis (MASH) remain unclear.

Methods: Using a mouse model fed a Western-style diet (WD) with 40 kcal% fat, 20 kcal% fructose and 2% cholesterol, we show that elevated cholesterol induces cholestasis and MASLD progression

Results: Intravital imaging of WD fed mice revealed vesicular regurgitation of bile from canaliculi into hepatocytes, a process we identify as bile macropinocytosis. Crucially, similar bile vesicles were detected in liver biopsies from MASH patients. Cholesterol dependency for vesicular bile regurgitation was confirmed: bile macropinocytosis was absent in WD fed mice without cholesterol but triggered by supplementing a standard diet with 2% cholesterol. Therapeutically, systemic inhibition of the apical sodium-dependent bile acid transporter (ASBT) for 12 weeks significantly reduced hepatic free as well as total cholesterol, blocked bile macropinocytosis, lowered systemic bile acids, and alleviated hepatic inflammation and fibrosis in WD-fed mice with MASH.

Discussion/Conclusion: These findings establish high cholesterol-driven bile macro-pinocytosis as a component in MASLD-to-MASH progression and identify systemic ASBT inhibition as a promising therapeutic strategy.

31. Multi-modal analysis defines bile acid-induced stress as a rewiring mechanism of epithelial-immune communication in Crohn's disease

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Introduction: Crohn's disease (CD) is increasingly recognized as a disorder involving metabolic disturbances, in which epithelial stress emerges from disrupted host-microbiome interactions, yet the mechanisms linking these perturbations to chronic inflammation remain unclear.

Methods: Patient-derived small and large intestinal organoids were established to model Crohn's disease-specific epithelial responses. Bile acid profiles from patients were integrated with fecal metagenomic and ileal transcriptomic datasets from two independent Crohn's disease cohorts. This combined approach allowed us to investigate the impact of bile acid imbalances on epithelial-immune circuit remodeling.

Results: Here we report that elevated chenodeoxycholic acid (CDCA) drives OPA1 suppression, leading to mitochondrial destabilization and oxidative stress that activates interferon signaling, thereby triggering epithelial necroptosis. By integrating patient-specific bile acid profiles with fecal metagenomic and mucosal transcriptomic of ileal tissue, we disentangle two independent host-microbiome communication hubs and uncover that elevated CDCA coincides with loss of SCFA-producing microbial taxa, alongside reduction in butyrate and epithelial FXR expression. Butyrate restores FXR expression, mechanistically linking microbial metabolism to host bile acid sensing. Finally, CDCA-driven epithelial stress promotes neutrophil recruitment and NETosis, amplifying mucosal inflammation.

Discussion/Conclusion: In summary, this study uncovers a metabolic stress axis governed by CDCA/SCFA imbalance and mitochondrial perturbation that controls epithelial resilience and immune homeostasis, highlighting bile acids as a promising target and stratification criterion for therapeutic intervention in CD.

32. Silencing of the autophagy inhibitor Rubicon ameliorates liver injury in a PSC mouse model

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Introduction: Autophagy is essential for cellular homeostasis. We previously reported that hepatic autophagy is impaired in chronic cholestasis, including primary sclerosing cholangitis (PSC). In addition, we identified Rubicon, an inhibitor of autophagy, to be significantly upregulated in these patients, possibly worsening

liver injury due to impaired autophagy. We hypothesized that Rubicon inhibition enhances autophagy and reduces cholestatic liver injury in the Mdr2-knockout (KO) mouse model of PSC.

Methods: Full-body Rubicon-Knockout (Rubcn-KO) mice were crossbred with Mdr2-KO mice to generate wildtype (WT), Mdr2-KO, and Rubcn/Mdr2-double-KO (DKO) mice. Male and female animals were analyzed at 8–10 and 25–27 weeks. Liver injury, inflammation, fibrosis, and ductular reaction were assessed on serum, histology, mRNA and protein levels. Bile flow was measured in vivo under basal and choleretic conditions, while autophagy was further examined in primary hepatocytes from Rubcn-KO mice.

Results: Rubcn-KO mice showed increased autophagic activity, indicated by reduced p62 protein levels, which can be further stimulated in vitro via mTOR inhibition with torin. Male DKO mice exhibited reduced liver injury (AST, ALT, AP), fibrosis (Sirius Red, Tgf-β, Desmin), and inflammation (Icam, Nlrp3, Ccl2, Cd11b), with moderate effects in young and significant improvement in older animals. This age-dependent protection paralleled increased age-dependent Rubicon expression in WT and Mdr2-KO mice. Female mice showed less pronounced effects. Bile flow and ductular reaction were unchanged across genotypes.

Discussion/Conclusion: Rubicon knockout alleviates liver injury and fibrosis in the Mdr2-mouse model for PSC age-dependently, which reflects age-dependent Rubicon expression levels. Older Mdr2 KO mice with highest Rubicon expression, profit most from Rubicon silencing. Thus, deficiency of Rubicon is able to ameliorate the detrimental hepatocellular consequences of the increasing pathophysiology of cholestatic liver diseases. We therefore think that inhibition of Rubicon may be a future distinct molecular target for chronic cholangiopathies including PSC.

33. Prognostic impact of alkaline phosphatase normalization after one year of UDCA therapy in primary biliary cholangitis

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Introduction: Biochemical response to ursodeoxycholic acid (UDCA) is a major prognostic factor in primary biliary cholangitis (PBC). It is primarily assessed based on alkaline phosphatase (ALP) and bilirubin levels. Most validated response criteria consider mildly elevated ALP levels as acceptable to define a good response. The aim of this study was to evaluate the prognosis of patients with strictly normal ALP levels compared to those with mildly elevated levels.

Methods: This was a retrospective single-center study including all patients followed for PBC and treated with UDCA. Clinical and biochemical data at diagnosis and after one year of treatment were collected. Patients were classified into three groups according to ALP and bilirubin levels after one year of therapy: Group 1 [normal ALP and bilirubin levels], Group 2 [ALP levels between 1.1 and 1.5 times the upper limit of normal (ULN) with normal bilirubin], and Group 3 [ALP

> 1.5 × ULN and/or bilirubin > ULN]. Hepatic decompensation during follow-up (ascites, variceal bleeding, and hepatic encephalopathy) was recorded. Survival analysis was performed using the Kaplan-Meier method with the log-rank test.

Results: Sixty patients with a mean age of 50 ± 12 years, predominantly female (96.7%), were included. Cirrhosis was present at baseline in 17 patients (28.3%). At one year of treatment, median [interquartile range] values were 160 IU/L [100–386] for ALP, 46 IU/L [35–125] for gamma-glutamyl transferase, 9.5 µmol/L [6.3–13.9] for bilirubin, 33 IU/L [23–45] for aspartate aminotransferase, and 28.5 IU/L [21–41.5] for alanine aminotransferase.

Thirty-two patients (53.3%) were classified in Group 1, 21 (35%) in Group 2, and 7 (11.7%) in Group 3. Over a median follow-up of 72 months [37.5–117], hepatic decompensation occurred in 10 patients (16.7%). The mean decompensation-free survival was shortest in Group 3 at 24 months (95% confidence interval [CI]: 6–42 months), whereas it reached 178 months (95% CI: 137–219 months) in Group 2. No patient in Group 1 experienced hepatic decompensation during follow-up. Accordingly, 5-year event-free survival rates were 100% in Group 1, 92% in Group 2, and 0% in Group 3, with p-values < 0.001 for comparisons between Groups 1 and 3 and between Groups 2 and 3. Importantly, statistical analysis also demonstrated a significantly better survival in Group 1 compared with Group 2 (p = 0.014).

Discussion/Conclusion: Normalization of ALP and bilirubin levels after 12 months of UDCA therapy appears to confer a more favorable prognosis compared to patients with ALP levels remaining below 1.5 × ULN but not normalized. These findings support the need to optimize therapeutic targets in PBC in order to improve patient outcomes.

34. Xi'an criteria: An early tool for predicting UDCA response and hepatic decompensation in primary biliary cholangitis

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Introduction: Ursodeoxycholic acid (UDCA) is the first-line treatment for primary biliary cholangitis (PBC). However, up to 40% of patients exhibit an inadequate response, making the standard 12-month response assessment relatively delayed. The Xi'an criteria have recently been proposed to identify responders early, as soon as one month after treatment initiation. This study aimed to evaluate the correlation between the Xi'an criteria and established 12-month response criteria, as well as to compare their predictive performance for hepatic decompensation.

Methods: A retrospective study spanning 20 years was conducted, including patients with PBC followed in our department and treated with UDCA. The Xi'an criteria at one month were defined as: alkaline phosphatase ≤ 2.5 × upper limit of normal (ULN), aspartate aminotransferase ≤ 2 × ULN, and total bilirubin ≤ 1 × ULN. Spearman correlation analysis was used to assess the association with established 12-month response criteria (Paris I, Paris II, Rotterdam, and Toronto). Hepatic decompensation was defined as the occurrence of ascites, variceal gastrointes-

tinal bleeding, or hepatic encephalopathy. The predictive performance of the Xi'an criteria for decompensation was evaluated using receiver operating characteristic (ROC) curve analysis with calculation of the area under the curve (AUROC), as well as Kaplan-Meier survival analysis and Cox proportional hazards models.

Results: Seventy-eight patients were included, with a mean age of 51 ± 13 years and a female predominance (93.6%). Cirrhosis was present in 24 patients (30.8%). The proportion of UDCA responders according to each criterion was as follows: Xi'an (n = 47; 60.3%), Paris I (n = 47; 60.3%), Paris II (n = 40; 51.3%), Rotterdam (n = 33; 42.3%), and Toronto (n = 40; 51.3%). The Xi'an criteria identified responders in 77% of cases according to Paris I ($r = 0.58$; $p < 0.001$), 80% according to Paris II ($r = 0.54$; $p < 0.001$), 85% according to Rotterdam ($r = 0.52$; $p < 0.001$), and 80% according to Toronto ($r = 0.54$; $p < 0.001$).

During a median follow-up of 60 months (IQR, 24–108 months), 16 patients (20.5%) developed hepatic decompensation. The Xi'an criteria were predictive of 5-year decompensation risk (AUROC = 0.77; $p = 0.002$), comparable to Paris I (AUROC = 0.86; $p = 0.001$), Paris II (AUROC = 0.86; $p = 0.001$), Rotterdam (AUROC = 0.79; $p = 0.005$), and Toronto (AUROC = 0.86; $p = 0.001$). Non-responders according to the Xi'an criteria.

Discussion/Conclusion: The Xi'an criteria provide an early and reliable tool for assessing response to UDCA and predicting hepatic decompensation. Their use may help avoid prolonged ineffective treatment in non-responders and support earlier initiation of more intensive therapeutic strategies.

35. Molecular to systems level interactions of the bile-acid derivate NorUDCA improve mood under high fat diet

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Introduction: Diet-related obesity and mood disorders are frequently comorbid conditions that currently lack effective treatment options. While bile-acid (BA) metabolism is emerging as a promising target for novel therapeutics, a comprehensive molecular-to-systems level survey of how BA interactions influence brain function is still lacking.

Methods: Here, we related multi-modal data from a mouse model of high-fat diet (HFD) and NorUDCA treatment to next-generation sequencing of cortico-limbic brain regions, brain metabolomics, functional magnetic resonance imaging (fMRI), behavioral test batteries, and gut microbiome profiling.

Results: We found that NorUDCA remodeled aversive brain states from molecular to network levels to mitigate anhedonia associated with HFD and established a multi-level resource that can be mined for mechanistic insight. Our data show that NorUDCA suppresses the onset of intestinal dysbiosis and attenuates mood-dis-

order-related gene expression signatures induced by HFD in the brain. Further, NorUDCA modulated brain neurotransmitters, limbic functional connectivity, and consequently depression-associated behaviors in HFD.

Discussion/Conclusion: Based on these data, we propose that bile acid-mediated signaling in the gut links metabolic states to limbic networks in the brain and that NorUDCA may exert beneficial therapeutic effects in diet-related mood disorders.

36. Patient-derived PSC organoids retain an overreactive memory to bile acid insult

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Introduction: Primary sclerosing cholangitis (PSC) is a chronic fibro-inflammatory cholangiopathy of unknown etiology. Cholangiocytes are both culprits and victims of biliary injury in PSC by actively contributing to disease perpetuation through pro-inflammatory signaling. This study aims to characterize cholangiocyte organoids originating from patients suffering from PSC and if their pathologic phenotype is retained when cultured in vitro

Methods: Cholangiocytes from the common bile duct of PSC (PSC) and non-PSC (nPSC) patients were cultured as organoids (CO) and stimulated with chenodeoxycholic acid (CDCA; 200 µM) or ursodeoxycholic acid (UDCA; 100 µM) for 24 hours. Gene expression profiles were analyzed via real-time PCR (RT-qPCR). Statistical significance was determined by 2way-ANOVA. * = $p < 0.05$; ** = $p < 0.005$; *** = $p < 0.0005$; ****$p < 0.0001$ ns = not significant

Results: Stimulation with CDCA significantly upregulated the tight junction components in PSC-COs and nPSC-COs with nPSC-COs exhibiting a higher baseline expression (BE) and an aggravated response: ZO-1 (nPSC by 72% [**], PSC by 218% [****], 1.73-fold BE [**]), ZO-2 (nPSC 129% [*], PSC 207% [****], 1.55-fold BE [ns]), Claudin-1 (nPSC 243% [****], PSC 293% [****], 2.32-fold BE [**]), b-Cathenin (nPSC 86% [ns], PSC 141% [****], 4.30-fold BE [***]), and Occludin (nPSC 598% [****], PSC 572% [****], 2.50-fold BE [ns]).

CDCA failed to induce significant expression of pro-inflammatory-genes in nPSC-COs, while PSC-COs displayed a drastically amplified response upon CDCA challenge, inducing CXCL2 28-fold (****), IL-8 29-fold (***), ORM2 8-fold (****) and SCL22A17 3.5-fold (**). AE2's BE was twice in PSC-COs (**) and upregulated by 47% upon CDCA challenge (****).

In response to CDCA, PSC organoids further demonstrated exaggerated upregulation of the genes related to lipid metabolism PPARa (nPSC 287% [****], PSC 481% [****]), PPARd (nPSC 193% [****], PSC 392% [****]), CPT1a (nPSC -25% [ns], PSC 55% [****]), and PGC1a (nPSC 30% [ns], PSC 87% [*]).

Notably, mRNA levels following UDCA treatment remained mostly comparable to control.

Conclusion: PSC-COs retain a pathologically overreactive transcriptional phenotype when challenged with potentially toxic CDCA in vitro.

37. Patterns of fecal and blood biomarkers in well-characterized cohorts with bile acid diarrhea or idiopathic chronic diarrhea

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Introduction: Bile acids are a common cause of chronic diarrhea, with symptoms including frequency, soft/watery stools, urgency, incontinence and associated anxiety. This condition, bile acid diarrhea (BAD), usually responds to bile acid sequestrants, but is frequently undiagnosed, as the gold-standard SeHCAT test is unavailable in many countries, including the U.S. Different fecal and blood biomarkers have been proposed for diagnosis and we recently showed a high prevalence of Ruminococcus gnavus (R. gnavus, Rg) in feces from BAD subjects compared with healthy controls. We aimed to define the relative roles of established and potential markers in two cohorts initially: SeHCAT-diagnosed severe or moderate BAD, and SeHCAT-negative idiopathic chronic diarrhea.

Methods: Patients with chronic diarrhea referred for diagnostic SeHCAT testing, were prospectively recruited in 2024-2025. They provided a single stool sample, and in most cases, a fasting blood sample. Fecal bile acids were measured by liquid-chromatography-mass-spectrometry, and microbiota taxa including R. gnavus by shotgun metagenomics. 7α-OH-4-cholestenone (C4) and FGF19 were determined in blood. A pilot analysis of primary BAD patients (SeHCAT < 10%, n = 25), and diarrhea-controls (SeHCAT > 20%, n = 29) was undertaken.

Results: The two groups were similar in age and gender ratio. The BAD group had a slightly higher median BMI and average stool type, with 87% having at least a partial response to bile acid sequestrants. Over both groups, SeHCAT was correlated with stool type (rs = -0.43) and C4 (rs = -0.78).

The median total BA (TBA) was 2.3-fold higher in the BAD group ($p < 0.0003$) but with wide and overlapping ranges. 40% of the BAD group were below the 75% quartile value of controls; 34% of the controls were above the 25% quartile value of BAD. TBA was associated with SeHCAT (rs = -0.49) and C4 (rs = 0.46).

Percentages of primary BA (%PBA), ursodeoxycholic acid (%UDCA) and R.gnavus (%Rg) were all significantly higher in the BAD group. %PBA > 4% was found in 60% of the BAD group, and in 31% of controls. An association between %PBA and TBA was not found (rs = -0.02) but %PBA was correlated with SeHCAT (rs = -0.29), %UDCA (rs = 0.72) and %Rg (rs = 0.48). %Rg was associated with SeHCAT (rs = -0.46), C4 (rs = 0.43) and %UDCA (rs = 0.49). FGF19 correlated with SeHCAT but not significantly with the other biomarkers.

Discussion/Conclusion: In these cohorts of SeHCAT-defined BAD and idiopathic diarrhea patients, there is significant overlap of several markers. Although there

are clear differences between the groups, findings in individual patients are variable. Metabolic disparities in the proportions of bile acids, including UDCA, reflect differences in microbiota such as R. gnavus, and their roles in the pathophysiology and diagnosis merit further study.

38. Farnesoid X receptor activation upregulates expression of the transcription elongation factor, TCEA2, in intestinal epithelial cells

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Introduction: Oxidative DNA damage occurs during mucosal inflammation, with such lesions stalling RNA polymerase II and impairing transcription fidelity, thereby driving genomic instability, a hallmark of cancer. TCEA2 (TFIIS) is a transcription elongation factor that rescues stalled polymerases by stimulating transcript cleavage, enabling lesion bypass and transcription-coupled repair. Dysregulated TCEA2 may worsen transcription stress and mutagenesis under inflammatory conditions. Farnesoid X receptor (FXR), a bile acid–activated nuclear receptor, preserves intestinal barrier integrity, prevents mucosal inflammation and colitis-associated colorectal cancer by regulating immune signaling and epithelial homeostasis. This suggests FXR may influence transcriptional stress pathways, including TCEA2 regulation, linking bile acid signaling to genome stability.

AIM: To determine whether FXR activation modulates TCEA2 expression in intestinal epithelial cells.

Methods: Differentiated and undifferentiated ileal and colonic enteroids were grown as monolayers and treated with the FXR agonist, obeticholic acid (OCA; 10 μM) for 6–24 hrs. Changes in gene expression were analysed by bulk RNASeq and were expressed as fold change in Reads Per Kilobase of Transcript (RPKM) compared to untreated controls (n = 3 throughout). TCEA2 expression in T84 cell monolayers was analyzed by qPCR.

Results: Treatment with OCA increased TCEA2 expression in UD colonic enteroids by 17.7 ± 10.9-fold after 6 hrs and 53.0 ± 33.1-fold after 24 hrs compared to unstimulated cells (n = 9; $p < 0.001$). Similarly, in DF colonic enteroids OCA treatment increased TCEA2 expression by 24.8 ± 5.4 and 33.4 ± 7.3-fold over controls at 6 and 24 hrs, respectively. OCA also stimulated TCEA2 expression in ileal enteroids with maximal responses occurring after 24 hrs of 127.5 ± 41.5 and 782.2 ± 303.2-fold of control in UD and DF cells, respectively (n = 3; $p < 0.05$). The effects of OCA were specific to TCEA2 as the agonist did not increase expression of the related elongation factors, TCEA1 or TCEA3. In cultured monolayers of T84 colonic epithelial cells the synthetic FXR agonist, GW4064 (5 μM; 3 - 48 hrs), also increased TCEA2 mRNA expression with a maximal effect of 390.3 ± 75.4-fold over controls occurring after 24 hrs of treatment (n = 4; $p < 0.05$). The effects of GW4064 on TCEA2 expression were significantly inhibited by pretreatment with the NFkB inhibitor, BMS 345541, from 405.3 ± 60.7 to 96.4 ± 34.1-fold of controls (n = 5; $p < 0.01$).

Discussion/Conclusion: These data demonstrate FXR activation to strongly upregulate TCEA2 mRNA expression in colonic epithelial cells, with effects confirmed across enteroid and T84 cell models. The response is specific to TCEA2 and, at least partially, NF-κB-dependent, suggesting a novel FXR–NF-κB–TCEA2 regulatory axis. These findings indicate FXR may enhance transcriptional resilience under conditions of stress, an effect that may contribute to its anti-inflammatory and anti-cancer effects in vivo.

39. Bile acid detergency as determinant of liver pathology in a humanized mouse model of progressive familial intrahepatic cholestasis type 3

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Introduction: Progressive familial intrahepatic cholestasis type 3 (PFIC3) is caused by impaired activity of ABCB4 that transports phosphatidylcholines (PC) from hepatocytes into bile. Abcb4-knockout (KO) mice have been generated, but hydrophilic muricholic acids in their bile acid (BA) pool may limit pathology and thereby hamper translation to human PFIC3. Therefore, we addressed whether Abcb4 knockdown (Abcb4-KD) in the livers of Cyp2c70-KO mice with a human-like BA composition leads to liver pathology that more closely resembles human PFIC3.

Methods: Expression of Abcb4 gene was suppressed in livers of Cyp2c70-KO/L-Cas9tg mice and control-Cas9tg mice using CRISPR/Cas9-technology to assess short- and long-term consequences. The BA sequestrant colesevelam was mixed into the chow diet (2% w/w) of Cyp2c70-KO mice with or without Abcb4-KD and the effects were evaluated after 6 weeks.

Results: Abcb4-KD strongly reduced biliary PL:BA ratios in both Cyp2c70-KO and control mice. However, plasma transaminases elevations, liver fibrosis and ductular reactions were markedly aggravated by Abcb4-KD in the context of a human-like BA composition. Importantly, the biliary lipid compositions differed markedly between the two Abcb4-KD models. Reduced biliary PC and increased concentrations of lysoPC, sphingomyelin, and ceramide were associated with the severity of liver disease only in Cyp2c70-KO/Abcb4-KD mice. Gene set enrichment analysis indicated that epithelial-mesenchymal transition was induced in the livers of Cyp2c70-KO/Abcb4-KD mice compared to control Abcb4-KD mice. BA sequestration reduced biliary BA secretion rates and mitigated liver pathology in Cyp2c70-KO/Abcb4-KD mice.

Discussion/Conclusion: The presence of a human-like BA composition profoundly aggravates liver pathology upon Abcb4 reduction in mice, conceivably due to

the augmented extrusion of non-PC phospholipids by hydrophobic BAs from membranes into bile. This humanized PFIC3 model is anticipated to accelerate the development of novel therapies for PFIC3 and potentially other cholestatic liver diseases.

40. Single-nucleotide polymorphism of NR1H4 and the bile acid profile in children with metabolic dysfunction-associated steatotic liver disease

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Introduction: The farnesoid X receptor (FXR), encoded by the NR1H4 gene, is a key regulator of bile acid synthesis, transport, and signaling. Polymorphic variants of NR1H4 may alter FXR-dependent regulation of enterohepatic bile acid metabolism and determine the individual risk of developing metabolic dysfunction-associated steatotic liver disease (MASLD) in children. Aim. To evaluate the association of the NR1H4 rs11110390 polymorphism with the bile acid profile and metabolic parameters in children with MASLD.

Methods: . A total of 55 children with obesity aged 10–17 years were examined. Obesity was confirmed by bioimpedance analysis (TANITA MC-780MA, Japan). The presence of hepatic steatosis was assessed by transient elastography using FibroScan® 502 Touch (Echosens, France) with controlled attenuation parameter (CAP) measurement. Depending on the presence of steatosis, the children were divided into two groups: 40 patients with MASLD and 15 children in the comparison group. Genotyping of NR1H4 rs11110390 was performed using allele-specific real-time PCR. Bile acid fractions in bile portions B and C were determined by thin-layer chromatography. Serum lipid profile, insulin, and glucose levels were assessed, and HOMA-IR was calculated.

Results: In children with MASLD, the CC, CT, and TT genotypes were detected in 35.0%, 47.5%, and 17.5% of cases, respectively; the frequency of the minor T allele was 41.2%. In portion B, total bile acid levels increased 1.6-fold in patients with the CC genotype ($p < 0.001$) and 1.9-fold in those with the TT genotype ($p < 0.01$). In portion C, they decreased 1.7-fold in patients with the CT genotype and 1.9-fold in those with the TT genotype ($p < 0.05$). In hepatic bile, taurocholic acid also increased 1.8-fold in patients with the CC and TT genotypes ($p < 0.001$), taurodeoxycholic acid increased 2.0-fold in patients with the CT genotype ($p < 0.05$) and 1.8-fold in those with the TT genotype ($p < 0.001$), while glycodeoxycholic acid increased 2.1-fold in patients with the CT genotype ($p < 0.001$) and 2.3-fold in those with the TT genotype ($p < 0.05$). In carriers of the TT genotype, whereas HOMA-IR increased 2.3–2.8-fold across all genotype groups ($p < 0.05$).

Discussion/Conclusion: The NR1H4 rs11110390 polymorphism, particularly the TT genotype, is associated with MASLD in children and is linked to an imbalance in bile acid fractions. Disruption of FXR-dependent bile acid homeostasis may represent one of the early mechanisms underlying the development of hepatic steatosis, in combination with dyslipidemia and insulin resistance.

41. Genetic susceptibility as a risk factor for gallstones in PSC: A prospective single-center analysis

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Introduction: Gallstone disease (GD) is common in patients with primary sclerosing cholangitis (PSC) (Said et al. J Hepatol 2008). Its development is linked to impaired gallbladder motility, disturbances in bile acid homeostasis, cholestasis, which are all frequent in PSC. We investigated the prevalence of GD in adults with PSC and assessed both clinical and genetic risk factors, including the p.D19H polymorphism in the cholesterol transporter ABCG8.

Methods: A total of 663 adults with PSC (218 women, age 18–74 years) were prospectively recruited. Data on GD, cholecystectomy and gallbladder cancer were obtained from clinical records, abdominal sonography, and MRI. The ABCG8 p.D19H polymorphism was genotyped using TaqMan assays.

Results: Among 663 patients, a total of 93 (14%) had history of GD: sixty-six had gallstones and gallbladder in situ, whereas 27 had undergone cholecystectomy before inclusion. Six patients had diagnosis of gallbladder cancer (three before inclusion and three during follow-up). The minor allele frequency ABCG8 p.D19H was higher in those with GD than in those without (8.6% vs. 4.5%). The variant was associated with an increased risk of GD (OR = 2.06; 95% CI: 1.13–3.75; $p = 0.018$). Among the non-genetic risk factors, GD was associated with age ($p < 0.001$), but not with BMI, sex, or ulcerative colitis (all $p > 0.05$). In multivariate analysis, both the ABCG8 p.D19H polymorphism ($p = 0.015$) and age ($p < 0.001$) remained independently associated with GD. One of six patients with gallbladder cancer was heterozygous for the transporter variant.

Discussion/Conclusion: GD in PSC is a multifactorial condition, more common in older patients and those with genetic predisposition. These results further underscore the use of ursodeoxycholic acid in PSC to mitigate the risk of developing gallstones and their complications in predisposed patients.

42. Mitochondrial dynamics and autophagy are disturbed in patients livers and experimental models of primary biliary cholangitis, contributing to disease pathogenesis

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Introduction: Primary biliary cholangitis (PBC) is a chronic immune-mediated cholestatic liver disease of unknown origin. We previously reported that miR-506 is upregulated in PBC cholangiocytes, impairing AE2, disrupting intracellular pH, and inducing PBC-like features including PDC-E2 overexpression and immune activation. Because mitophagy is a pH-dependent, anti-inflammatory mitochondrial quality-control process that eliminates dysfunctional mitochondria, we aimed to define mitochondrial dynamics and auto(mito)phagy alterations in PBC and their role in disease pathogenesis.

Methods: Mitochondrial mass, dynamics, network and bioenergics, as well as lysosomal function and autophagy/mitophagy were assessed in PBC livers and cholangiocytes by single cell RNA-sequencing (scRNA-seq) and in miR-506-overexpressing cholangiocytes (in vitro PBC model [Banales JM et al. Hepatology. 2012; Erice O et al. Hepatology. 2018]) using molecular and functional assays.

Results: Single-cell RNA-seq of PBC livers revealed dysregulation of mitochondrial homeostasis, autophagy, and mitophagy pathways, particularly in cholangiocytes. Isolated PBC cholangiocytes showed impaired mitophagy initiation and flux, evidenced by the downregulation of PRKN and RAB7A genes. In an experimental miR-506-overexpressing PBC model, mitochondrial network and energetic abnormalities were confirmed as well as defective lysosomal function and autophagosome accumulation, analyzed by proteomic analysis, flow cytometry, confocal imaging and western blotting. Pharmacological activation of autophagy and mitophagy with dactolisib (DACT; BEZ235) or ursodeoxycholic acid (UDCA) restored mitochondrial function, improved lysosomal activity, reduced PDC-E2 levels, and attenuated immune activation, supporting impaired auto(mito)phagy as a driver of PBC pathogenesis and a potential therapeutic target.

Discussion/Conclusion: Impaired auto(mito)phagy is a driver of cholangiocyte dysfunction and immune activation in PBC. Importantly, therapeutic restoration of auto(mito)phagic flux by UDCA or DACT effectively reverses key pathological features in vitro, supporting mitophagy activation as promising therapeutic target for PBC.

43. Mice expressing FXR with a humanized ligand-binding domain display distinct metabolic responses upon pharmacological FXR stimulation

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Introduction: Farnesoid-X-receptor (FXR), a bile acid (BA)-activated nuclear receptor, is a therapeutic target for cholestatic and metabolic liver diseases.

However, species differences in BA metabolism and FXR signaling hamper translation from mice to humans. The human FXR-ligand binding domain (LBD) structurally differs from the murine LBD, potentially impacting pharmacological responses. Therefore, we generated mice with "humanized" FXR by replacing the murine LBD by the human LBD (FXR-hLBD) and assessed its impact on BA and cholesterol metabolism.

Methods: Male and female FXR-hLBD mice on wild-type (WT) or Cyp2c70-/- backgrounds were compared with FXR-mLBD controls under non-stimulated conditions. Additionally, WT mice expressing FXR-mLBD or FXR-hLBD received either vehicle or obeticholic acid (OCA; 40 mg/kg/day, p.o.) for 7 days.

Results: FXR humanization did not alter hepatic or intestinal FXR expression levels. Under basal conditions, physiological parameters, liver pathology markers, and hepatic transcriptomes were similar between FXR-hLBD and FXR-mLBD mice on a WT C57BL/6J background and in mice with a human-like BA composition (Cyp2c70-/-). OCA did, however, elicit markedly stronger transcriptional responses in FXR-hLBD mice, including more pronounced suppression of hepatic BA synthesis genes and differential regulation of BA transporters. Intriguingly, pathways involved in cell proliferation and fibrogenesis were induced in FXR-hLBD mice. Furthermore, OCA lowered plasma cholesterol to a greater extent in FXR-hLBD than FXR-mLBD mice, primarily due to a reduction of HDL-cholesterol.

Discussion/Conclusion: FXR-hLBD mice resemble FXR-mLBD controls under basal conditions but exhibit enhanced responses to FXR agonism by OCA. This model may improve preclinical evaluation of FXR-targeting drugs in a translation-relevant context.

44. Identifying solute carrier (SLC) transporters involved in cholangiocyte defense against toxic bile acids

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Introduction: Human cholangiocytes are exposed to high millimolar concentrations of hydrophobic bile salts. Although micellization with cholesterol and phospholipids lowers free biliary bile salts to low millimolar concentrations, other human cell types would not resist these conditions. Cholangiocytes secrete 25–40% of bile fluid in humans, mainly bicarbonate and water. The 'biliary bicarbonate umbrella' hypothesis proposes that bicarbonate maintains an alkaline pH at the apical membrane, thereby limiting entry of protonated, apolar glycine-conjugated bile acids that otherwise induce apoptosis and subsequent immune attack. Defects in this potentially protective mechanism have been reported in primary biliary cholangitis (PBC), primary sclerosing cholangitis (PSC), and IgG4-related cholangitis (IRC). In PBC, SLC4A2, the main apical chloride-bicarbonate exchanger in cholangiocytes, is downregulated. This study aims to identify additional SLC transporters that may function as 'umbrella genes' in pH homeo-

stasis and protection against hydrophobic bile acid–induced injury in fibrosing cholangiopathies.

Methods: This study utilized immortalized human H69 cholangiocytes with short hairpin RNA (shRNA) knockdown of 8 SLC genes involved in pH regulation. Fluorometric measurement of intracellular pHi, radiochemical determination of 3H-glycochenodeoxycholic acid uptake, and quantification of apoptosis by caspase-3/7 assays were performed. Publicly available RNA sequencing data were analyzed using R2 Platform (https://r2.amc.nl,GSE206364,GSE159676).

Results: Knockdown of SLC transporters SLC9A1 and SLC26A2 resulted in decreased cholangiocellular pHi; knockdown of SLC9A1, SLC26A2 and SLC26A6 enhanced uptake of GCDC, and increased GCDC-induced apoptosis in H69 cells. Analysis of published data revealed that mRNA of SLC9A1, SLC26A2, and SLC26A6 were differentially expressed in blood transcriptome of PBC or PSC patients compared to healthy individuals; mRNA of SLC9A1 was upregulated in liver tissue of PBC and PSC patients compared to healthy individuals.

Discussion/Conclusion: SLC transporters SLC9A1, SLC26A2, and SLC26A6 may be involved in human cholangiocyte defense against toxic bile acids and deserve further studies as potential therapeutic targets in fibrosing cholangiopathies.

45. Gestational diabetes mellitus and intrahepatic cholestasis of pregnancy – Does metformin treatment affect the course of ICP?

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Introduction: Intrahepatic cholestasis of pregnancy (ICP) is the commonest gestational liver disease, with emerging links to glucose metabolism. Metformin is widely used in gestational diabetes mellitus (GDM), yet its impact on ICP severity remains unclear. We aimed to evaluate the association between metformin treatment in GDM and the severity of ICP.

Methods: This retrospective multicentre study included cases collected between 1995 and 2025. Women with ICP and GDM treated with metformin were identified and stratified into two subgroups based on the timing of metformin initiation (before or after ICP diagnosis). ICP severity was classified based on peak total serum bile acid (TSBA) concentrations. TSBA trajectories were analysed using linear regression, and segmented regression was used to assess changes in bile acid trajectories in relation to metformin exposure. Comparisons were performed with a cohort of ICP cases without GDM.

Results: Twenty-eight cases met inclusion criteria. ICP severity was mild (TSBA < 40 μmol/L) in 32.1%, moderate (TSBA 40-99 μmol/L) in 39.3% and severe (TSBA ≥ 100 μmol/L) in 28.6%. Preterm birth occurred in 35.7% with no stillbirths observed. Metformin was initiated before ICP diagnosis in 56% and after diagnosis in 44% of cases. No severe ICP cases occurred in the subgroup in which metformin

was commenced before ICP, whereas 72.7% were severe when metformin was initiated after ICP diagnosis. Earlier ICP onset (p = 0.0038) and higher peak TSBA concentrations (p = 0.0001) were observed in in the subgroup with ICP diagnosed prior to metformin commencement. Metformin exposure was not associated with significant differences in TSBA trajectories compared with ICP without GDM.

Discussion/Conclusion: Metformin was not associated with a more severe course of ICP. While no beneficial effect was demonstrated, differences in disease severity appear to reflect underlying heterogeneity rather than treatment effect.

46. Individual participant data meta-analysis of perinatal adverse outcomes in cholestatic twin pregnancies: The IMPACT study

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Introduction: Intrahepatic cholestasis of pregnancy (ICP) is a liver-specific disorder, that affects 0.3–5.6% of pregnant women. Multifetal pregnancy is a recognised risk factor for ICP; however, it is frequently excluded from studies. In our study, we aimed to evaluate the impact of ICP on adverse perinatal outcomes in multifetal pregnancies.

Methods: We performed a systematic review and meta-analysis and searched Medline, Embase, Web of Science, and Cochrane Library from database inception to 31 of March 2022. Aggregate data meta-analysis included studies with ICP and control groups. An individual participant data meta-analysis (IPD-MA) included published datasets and two unpublished cohorts of uncomplicated twin pregnancies. Summary data were compared with a random-effects meta-analysis; IPD were analysed by chorionicity using logistic regression in Stata 17.0. PROSPERO registration: CRD42020216725.

Results: Meta-analysis of eight studies (873 pregnancies with ICP, 4736 without) showed that twin pregnancies with ICP had a higher risk of early preterm birth (below < 34 weeks of gestation) (OR = 3.73 [95% CI: 1.30–10.73]); stillbirth risk did not differ (OR = 1.20 [0.35–4.10]). In contrast, IPD from 28 studies (895 pregnancies with ICP) and two unpublished cohorts (5068 pregnancies) showed that, for dichorionic twins (615 ICP pregnancies), there was no difference in spontaneous early preterm birth (aOR = 0.89 [0.76–1.11]) or stillbirth (aOR = 1.52 [0.68–3.39]). However, peak bile acid concentration was moderately associated with early spontaneous preterm birth (ROC AUC 0.63 [0.55–0.70]); for monochorionic twins (98 cases) no association was found (ROC AUC 0.49 [0.19–0.79]).

Discussion/Conclusion: Higher bile acid concentrations in ICP increase the risk of spontaneous early preterm birth in multifetal pregnancies; in contrast to singleton pregnancies, there is no evidence of increased stillbirth risk associated with ICP for twins; data for monochorionic pregnancies are limited.

47. Methyldopa reduces plasma bile acid concentrations in a murine model of estrogen-induced cholestasis

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Introduction: Intrahepatic cholestasis of pregnancy (ICP) is the most common gestational liver disorder and is characterized by elevated serum bile acid (BA) concentrations. This results in increased BA placental barrier penetration and adverse pregnancy outcomes. Epidemiological studies suggest that preeclampsia may precede and coexist with ICP, and methyldopa is often used early in antihypertensive management before ICP is diagnosed but is commonly reconsidered once cholestasis is recognized. However, its impact on BA homeostasis remains unclear.

Methods: We examined methyldopa in a murine model of estrogen-induced cholestasis. Female C57BL/6J mice received ethinylestradiol (10 mg/kg, s.c.) with or without methyldopa (250 mg/kg, p.o.) for 7 days. BA profiles were quantified by LC–MS across plasma, bile, liver, urine, intestine, and feces. Hepatic, ileal, and renal expression of BA-related enzymes/transporters was assessed by RT-qPCR and Western blotting.

Results: In estrogen-induced cholestatic mice, methyldopa decreased total plasma and liver BA concentrations without increasing fecal or urinary BA excretion. This reduction was associated with upregulation of phase I/II enzymes (Cyp2b10, Ugt1a1, Sult2a1) and transporters (Abcb11, Slc10a1, Abcc3/4), consistent with transcriptional changes indicative of indirect constitutive androstane receptor (CAR) activation via p-ERK downregulation. Methyldopa also lowered hepatic free cholesterol by protein downregulation of Hmgcr and upregulation of Acat2, suggesting altered cholesterol handling that limits substrate availability for BA synthesis.

Discussion/Conclusion: Methyldopa lowered plasma and liver BA concentrations under estrogen-induced stress by reducing BA synthesis and increasing detoxification. In the context of pregnancy-related BA dysregulation, these findings suggest that methyldopa, when used to treat hypertensive disorders of pregnancy, may attenuate BA accumulation and potentially delay the onset of cholestatic phenotypes—an observation that generates hypotheses warranting clinical validation.

48. Hydrophobic bile acids orchestrate gut-liver axis disruption in primary sclerosing cholangitis

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Introduction: Hydrophobic (Hbic) bile acids (BAs) circulating within the cholehepatic and enterohepatic axes been proposed as potential trigger of primary sclerosing cholangitis (PSC), although this remains unclear. One major obstacle to establishing a PSC mouse model that recapitulates both biliary and intestinal lesions is the species-specific differences in bile acid composition between humans and mice. PSC-like lesion was observed in double KO (HuBA) mice lacking Cyp2c70 and Cyp2a12, enzymes that convert the Hbic BA CDCA into the more hydrophilic mouse-specific MCA, and reconvert secondary BAs such as LCA and DCA to CDCA and CA, respectively. This study investigated the roles of Hbic BAs in the onset of PSC using HuBA mice and cultured human cholangiocytes (MMNK-1 cells).

Methods: A PSC mouse model was established by administering of 0.0075% LCA in drinking water to male HuBA mice for 15 weeks. BA profiles in the liver, serum, and feces, histological features and gene expression in the liver and intestine, and intestinal injury were compared between untreated HuBA mice and PSC model mice. In vitro, MMNK-1 cells were exposed to 100 μM CA, CDCA, DCA, LCA, or UDCA for 24 hours, with or without LPS pretreatment (5 μg/mL) for 24 hours.

Results: Onion-skin fibrosis was observed in all of the PSC mice, with a significantly greater fibrosis area than in untreated HuBA mice. No significant differences in BA composition of the liver, serum, or feces were detected between the two groups. In the PSC model mice, hepatic mRNA levels of Tgf-β1, Tlr4, and Integrins(Itg)-αv/β6 in the liver as well as ileal expression of E-cadherin (Cad), Itg-β6, and Zo-1 in the ileum were significantly increased. In addition, intestinal permeability and serum LPS levels were significantly elevated in the PSC model. In the MMNK1 cells pretreated with LPS, Hbic BAs (LCA, CDCA, and DCA) significantly decreased E-cadherin expression and increased TLR4 expression. CDCA further induced significant upregulation of Itg-β6 and Snail1 expression.

Discussion/Conclusion: The findings suggest that Hbic BAs, particularly CDCA and LCA, disrupt tight junctions in cholangiocytes and enterocytes, thereby contributing to the onset of PSC. Furthermore, CDCA-induced TLR-4 expression in the cholangiocytes may enhance endotoxin-driven inflammation, facilitated by LCA-induced increases in intestinal permeability. Collectively, Hbic BAs may serve as key triggers of bile duct and intestinal injury via the cholehepatic and enterohepatic axes.

49. A cholestatic-autophagy signature defines a distinct phenotype in metabolic dysfunction-associated steatotic liver disease

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Introduction: Cholestatic features in metabolic dysfunction-associated steatotic liver disease (MASLD) are increasingly recognized as markers of disease severity, yet their mechanistic basis remains poorly defined. Emerging evidence suggests

that bile acid dysregulation may impair autophagy, a key pathway in hepatic lipid homeostasis. We aimed to characterize the interplay between cholestatic injury, autophagy dysfunction, and biliary remodeling in MASLD through an integrated histological and molecular approach.

Methods: Eighty-four MASLD patients were stratified according to biochemical liver injury patterns. Liver tissue was analyzed by immunohistochemistry for impaired autophagy (p62) and biliary markers (CK7) of ductular reaction (DR), alongside targeted gene expression profiling of 58 genes involved in lipid metabolism, bile acid handling, and autophagy. Histological, molecular, and clinical data were integrated.

Results: Autophagy impairment, reflected by p62 tissue accumulation, was significantly associated with increasing DR severity (DR score 0 vs. 2, $p < 0.01$; DR score 1 vs. 2, $p < 0.001$). Transcriptional analysis revealed that genes involved in lipid metabolism (NPC1, SREBF2, IGFBP7) showed strong correlations with p62 immunohistochemical expression ($p<0.01$), while key autophagy regulators (ATG12, ATG5, BECN1, SQSTM1) and genes involved in extracellular matrix remodeling (COL1A2, FSTL1) were significantly associated with both autophagy impairment and cholestatic histological features ($p < 0.05$). Moreover, gene expression profiling refined patient stratification beyond conventional biochemical classification, better distinguishing hepatocellular from non-hepatocellular phenotypes ($p < 0.01$).

Discussion/Conclusion: These findings support the existence of a distinct cholestatic–autophagy phenotype in MASLD, characterized by biliary remodeling and coherent transcriptional signatures. This integrated framework may improve patient stratification, enabling the identification of individuals at higher risk of disease progression.

50. Bile acid mediated protection from diet-induced adiposity and hepatic steatosis depends on amino acid conjugation metabolism

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Introduction: The liver is a central regulator of lipid metabolism. Bile acids (BAs) modulate key metabolic pathways, including lipid absorption, fatty acid oxidation, and de novo lipogenesis. The effect of BAs in control of lipid, and energy metabolism as been attributed to activating Takeda G protein-coupled receptor 5 (TGR5) and farnesoid X receptor (FXR). Notably, cholic acid supplementation reduces adiposity and hepatic steatosis in mice, however, these effects cannot be fully explained by activation of FXR / TGR5, or increased energy expenditure, suggesting alternative mechanisms.

Methods: We combined MRI-based liver fat quantification, LC-MS/MS lipid profiling, radiotracer-based lipoprotein uptake assays, and molecular analyses (by qPCR, Western blotting) of lipogenesis to investigate the metabolic effects of BA supplementation.

Results: Cholic acid supplementation reduced adipose tissue mass and hepatic triglyceride levels across multiple knockout models ($Fxr^{-/-}$, $Tgr5^{-/-}$, $Fgf15^{-/-}$, $Fgf21^{-/-}$). Gene expression analyses identified suppression of hepatic lipogenesis, associated with downregulation of ChREBP and its target Acss2. Notably, these metabolic effects were not observed in hamsters, a model with BA metabolism more similar to humans. Consistently, glycine-conjugated BA administration (GCA or GCDCA), did neither reduce fat mass nor suppress hepatic de novo lipogenesis. However, GCA and GCDCA still induced Fgf15 in the intestine and repressed the expression of Cyp7a1 and Cyp8b1 in the liver.

Discussion/Conclusion: Our findings identify BA conjugation patterns as a critical determinant of metabolic responses. The taurine-dominant BA profile in mice limits translational relevance, underscoring the importance of selecting appropriate preclinical models for studying BA-mediated metabolic regulation.

51. Identification of a novel ABCB4 variant causing low phospholipid-associated cholelithiasis

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Introduction: Low phospholipid-associated cholelithiasis (LPAC) is a cholestatic genetic disorder caused by mutations in the ABCB4 gene, which encodes the multidrug resistance protein 3 (MDR3) which is crucial for bile phospholipid transport.

Methods: We report a male in late adolescence presenting with recurrent biliary symptoms post cholecystectomy, elevated cholestatic liver enzymes, and a family history of hepatobiliary and pancreatic disease

Results: Genetic testing revealed a novel heterozygous ABCB4 variant (c.833+5G>A). Functional studies demonstrated abnormal mRNA splicing, resulting in reduced MDR3 protein expression. The same variant was also identified in the patient's mother and sister, consistent with autosomal dominant inheritance. Following treatment with ursodeoxycholic acid, the patient achieved clinical and biochemical remission.

Discussion/Conclusion: This case highlights the importance of considering rarer genetic causes, including LPAC, in young patients with symptomatic cholelithiasis, particularly when there is a suggestive family history. Early recognition and targeted therapy can significantly improve clinical outcomes.

52. Ursodeoxycholic acid may reduce the risk of major postpartum haemorrhage for individuals with intrahepatic cholestasis of pregnancy: Secondary analysis of individual participant data meta-analyses

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Introduction: For patients with intrahepatic cholestasis of pregnancy (ICP), possible disrupted hepatic synthetic function and malabsorption of fat-soluble vitamins necessary for effective coagulation has led to concerns regarding an increased risk of postpartum haemorrhage (PPH). Ursodeoxycholic acid (UDCA) reduces disease-associated spontaneous preterm birth, likely by reducing taurocholate-induced enhanced uterine contractility, via oxytocin and prostaglandin-mediated pathways. Theoretically, this tocolytic action could impair postpartum uterine contraction, enhancing the risk of PPH. We aimed to determine whether UDCA treatment impacted the risk of major PPH.

Methods: Individual participant data (IPD) from two previous IPD meta-analyses were combined, and duplicates removed; data from individuals with estimated blood loss (EBL) volumes were kept for analysis. Major PPH was defined as EBL > 1000 ml. Binomial logistic regression was performed in R software, adjusting groups by peak maternal bile acid concentration and multifetal pregnancy.

Results: Data from 3542 individuals were available for analysis, of whom 2430 (68.6%) received UDCA. 58% had moderate-severe disease (defined by peak serum bile acids ≥ 40 μmol/L), and, overall, 6.3% (223) of participants had a major PPH. UDCA treatment was associated with a reduced risk of major PPH (adjusted odds ratio [aOR] = 0.72, 95% confidence interval [CI]: 0.54–0.97). Although numbers of included individuals were smaller (222), there was no difference in rates of major PPH with additional rifampicin treatment (aOR = 0.70, 95% CI: 0.38–1.21). Patients with higher bile acid concentrations had slightly higher rates of major PPH (0.3% increased odds with each μmol/L increase in bile acids), although maternal factors (such as use of assisted conception and multi-fetal pregnancies) had the greatest influence on the risk of major PPH.

Discussion/Conclusion: Reassuringly, we have not demonstrated that UDCA treatment is associated with an increased risk of major PPH, and a possible reduction in this risk gives an additional indication for the benefit of UDCA in ICP.

53. Novel epigenetic molecules selectively targeting class I and IV HDACs as a new therapeutic avenue for bile duct cancer

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Introduction: Cholangiocarcinoma (CCA) comprises a heterogeneous group of biliary tract malignancies with poor prognosis and limited treatment options. Dysregulation of epigenetic regulators, particularly histone deacetylases (HDACs), plays a key role in cancer development and progression, including CCA. However, selective inhibitors targeting specific HDAC classes remain scarce. Here, we aimed

to evaluate alterations in HDAC subtype expression in CCA and to develop novel selective HDAC inhibitors targeting dysregulated isoforms as an epigenetic therapeutic strategy using experimental models.

Methods: HDAC expression was analyzed in tumor samples from nine international CCA patient cohorts. Advanced computational approaches were used to design ursodeoxycholic acid (UDCA)-based conjugates targeting dysregulated HDACs, and their activity and selectivity were assessed using HDAC-specific enzymatic assays. Therapeutic efficacy was evaluated in experimental models of CCA.

Results: Class I HDACs 1, 2, 3 and 8, and class IV HDAC11 were consistently overexpressed in human CCA tumors compared to controls. Rational design identified nine UDCA conjugates with HDAC inhibitory activity. The lead compound, Epi-UDCA, demonstrated strong and selective inhibition of both class I and IV HDACs in enzymatic assays. Epi-UDCA exhibited potent cytostatic and anti-invasive effects at low concentrations (2 μM) and induced apoptosis at higher doses (10 μM) in CCA cells, without affecting normal human cholangiocytes. Moreover, Epi-UDCA suppressed growth in 3D CCA human spheroids and murine organoids, and enhanced the efficacy of cisplatin in both naïve and cisplatin-resistant CCA models. The critical role of UDCA in the compound's efficacy and safety was confirmed through comparison with other bile acid conjugates. Proteomic analysis revealed modulation of key cancer-related pathways, including cell cycle progression, angiogenesis, and p53-mediated transcription.

Discussion/Conclusion: Epi-UDCA, a novel UDCA-derived selective HDAC class I and IV inhibitor, demonstrates promising therapeutic potential for both naïve and cisplatin-resistant CCA.

54. Bulevirtide-induced elevation of plasma bile salt levels alleviates DSS-induced colitis and LPS-induced inflammation in mice

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Introduction: Bulevirtide, a viral entry inhibitor used to treat chronic hepatitis delta virus (HDV) infection, targets the hepatic bile salt transporter Na+-Taurocholate Co-transporting Polypeptide (NTCP). As Bulevirtide displays preclinical potential to mitigate cholestatic liver injury, NTCP inhibition is currently explored as treatment for primary sclerosing cholangitis (PSC), which is often associated with colitis. Here, we investigated the immunomodulatory effects of Bulevirtide in lipopolysaccharide (LPS)-induced inflammation and dextran sodium sulfate (DSS)-induced colitis in mice.

Methods: Mouse bone marrow-derived macrophages (BMDMs) and human BLaER1 macrophages were pre-incubated with taurochenodeoxycholic acid (TCDCA) prior to a 4-hour LPS challenge. For in vivo studies, wild-type and Slco1a/1b$^{-/-}$ FVB and C57BL/6J mice, models that better recapitulate human bile salt transport, were used. Mice received LPS (i.p.) one hour after Bulevirtide administration (i.v.) and were sacrificed 6 hours later. Colitis was induced by DSS in drinking water (6% FVB; 2.5% C57BL/6J) for 5 days, with daily i.v. treatment of Bulevirtide or

vehicle; mice were sacrificed on day 7. Plasma bile salt levels and composition, cytokines, and colitis scores were assessed.

Results: Taurochenodeoxycholic acid suppressed tumor necrosis factor alpha (TNF), increased interleukin (IL)-10, and suppressed inflammasome activation, as evidenced by reduced IL-1β, IL-18 and cleaved-IL-1β levels. Similar effects were observed in human macrophages. In both FVB and C57BL/6J Slco1a/1b-/- mice, Bulevirtide increased plasma bile salt levels at least 30-fold. This systemic elevation of bile salts was associated with reduced plasma TNF and increased IL-10 in LPS-treated mice. Moreover, Bulevirtide attenuated DSS-induced colitis, as evidenced by lower colitis scores and reduced intestinal Tnf expression.

Discussion/Conclusion: Our findings highlight the anti-inflammatory effects of bile salts in preclinical models of colitis and support NTCP inhibition as a potential therapeutic strategy to ameliorate cholestasis and colitis in PSC.

55. Secondary bile acids reveal bile acid-specific metabolic signaling responses in white adipose tissue of a teleost fish model

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Introduction: Bile acids (BAs) are recognized as signaling molecules that regulate metabolic homeostasis through specific transporters and receptors. While their roles in hepatic metabolism have been extensively studied, far less is known about their potential regulatory functions in white adipose tissue (WAT), particularly outside mammalian systems. Expanding our understanding of how distinct BA species influence metabolic tissues may provide new insights into the broader physiological roles of BA signaling.

Methods: In this study, we investigated the short-term effects of secondary bile acids (SBAs), including lithocholic acid (LCA), deoxycholic acid (DCA), and their taurine-conjugated forms using rainbow trout (Oncorhynchus mykiss) as a fish model species. We performed comprehensive transcriptomic (RNAseq) profiling of WAT, complemented by targeted analyses of BA transporters and receptors, together with key indicators of glucose and lipid metabolism.

esults: Our results suggest that SBAs may influence adipose metabolic pathways in a BA-dependent manner, with distinct transcriptional responses according to BA identity and conjugation state. Similar BA-dependent regulatory patterns have been observed in this species in hepatic metabolism and hypothalamic mechanisms involved in feeding control. In addition, the expression profiles of BA transporters and receptors in WAT differed from other tissues in this species, suggesting tissue-specific features of BA sensing and signaling in rainbow trout WAT.

Discussion/Conclusion: Together, these findings raise the possibility that BA function as metabolic signaling molecules beyond their classical digestive role across vertebrates. The apparent BA-specific nature of these responses supports

the idea that BA identity may represent a key but still underappreciated dimension of BA physiology. From a comparative perspective, the presence of these signaling elements and responses in teleost fish suggests a degree of evolutionary conservation of BA-mediated metabolic regulation, opening interesting physiological and phylogenetic questions regarding the diversification of BA signaling across vertebrates.

56. Discovery of novel MRGPRX4 agonists and antagonists with implications for hepatobiliary pruritus

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Background: The MAS-related G protein-coupled receptor X4 (MRGPRX4) is a bile acid and heme metabolite-sensing receptor predominantly expressed in small-diameter non-peptidergic sensory neurons of dorsal root and trigeminal ganglia. MRGPRX4 has emerged as a key mediator of hepatobiliary pruritus. Despite its pathophysiological relevance, the receptor remains insufficiently characterized, and selective pharmacological antagonists are lacking, hindering biological investigation and therapeutic advancement. Recently developed potent phosphate and phosphonate-bearing agonists challenge the proposed role of lipophilic steroid and heme-derived ligands as primary (patho)physiological agonists.

Methods: Natural product and pharmacological compound libraries were screened to identify MRGPRX4 ligands using β-arrestin recruitment assays (PathHunter, DiscoverX) at 10 µM. For selected β-arrestin recruitment compounds, concentration–response curves determined EC50 or IC50 values, complemented by calcium mobilization assays (Flexstation 3, Molecular Devices), yielding comparable results.

Results: Novel hMRGPRX4 agonists were identified, including sophoraflavanone G (EC50: 5.93 µM), and steroid-like and triterpenoid-like molecules dioscin (EC50: 3.83 µM), echinocystic acid (EC50: 4.17 µM) and 18-β-glycyrrhetinic acid (EC50: 11.2 µM). Additionally, FXR agonists cilofexor (EC50: 52.1 µM), tropifexor (EC50: 19.4 µM) and obeticholic acid (EC50: 23.1 µM) activated MRGPRX4. Identified antagonists exhibited diverse scaffolds, including psoralidin (IC50: 0.608 µM), disulfiram (IC50: 1.50 µM), flavonol (IC50: 2.82 µM) and berbamine (IC50: 5.16 µM). Intriguingly, clinically used PPAR agonists bezafibrate (IC50: 83.2 µM), elafibranor (IC50: 3.26 µM), fenofibrate (IC50: 2.89 µM) and seladelpar (IC50: 2.25 µM) also inhibited MRGPRX4.

Conclusions: This study identified structurally diverse MRGPRX4 agonists and antagonists. FXR agonist engagement of MRGPRX4 may contribute to hepatobiliary itch, while PPAR agonist-mediated MRGPRX4 inhibition could explain antipruritic effects and suggest repurposing opportunities. Functional interplay between MRGPRX4, FXR and PPAR pathways in hepatobiliary pruritus warrants further investigation. The identified compounds may serve as lead structures for developing targeted hepatobiliary pruritus therapies.

57. Rethinking the role of bile acids in primary biliary cholangitis

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Background/Aim: Bile acids (BA) appear to be major regulators of the gut microbiome and vice versa. In human liver diseases and experimentally, low secondary BA levels in bile and intestine have been shown to be linked to bacterial dysbiosis and to modulate immune response. Aim: To determine the extent of BA disrupted homeostasis that could potentially affect dysbiosis and immune response in primary biliary cholangitis (PBC).

Methods: We analyzed the fasting blood BA profile by an HPLC/MS-MS method. The serum fasting BA profile is known to accurately reflect the bile and intestinal BA profile. Serum BA levels were measured in 19 healthy controls and 90 PBC patients, according to whether they had early (I–II) or advanced (III–IV) histological stages or whether they were responders or non-responders (according to Paris 1 criteria) to 13–15 mg/kg/day of UDCA. Severe forms were defined by advanced stages or non-response to UDCA. The results were compared to those obtained in healthy subjects.

Results: As expected, UDCA was the major circulating BA in the treated PBC patients. However, the 3-sulfate-glycoUDCA (the putative inactive metabolite product) constituted 40 and 60% of blood levels (in non-severe vs. severe forms of PBC). Secondary BA formation as assessed by the deoxycholic acid (DCA)/cholic acid (CA) ratio was found to be significantly reduced in early-stage disease (DCA/CA = 1.65 ± 0.64) as compared to healthy controls (DCA/CA = 2.50 ± 0.66). The reduction was more pronounced in severe forms of PBC: histological-stage III–IV: 0.32 ± 0.11 vs. stage I–II:1.65 ± 0.64; non-responders to UDCA: 0.37 ± 0.22 vs. responders 2.5 ± 1.30, $p < 0.0001$. Most of the lithocholic acid (LCA) was under 3-sulfate LCA (80%) whatever the severity of PBC and the LCA/chenodeoxycholic acid (CDCA) ratio tended to be lower in advanced stages (0.18 ± 0.03 vs. 0.37 ± 0.29) but the difference remained at the limit of significance suggesting that UDCA epimerisation to CDCA counterbalanced the impact of reduced dehydroxylation rate.

Discussion/Conclusion: This work provides evidence for an altered DCA processing in particular in the severe forms of PBC. UDCA administration restores a normal processing in the patients with early stages or those who respond biochemically to UDCA but not in those with advanced disease or non-responders, thereby suggesting in these patients a loss of DCA-TGR5 signaling. We thus propose that the disrupted DCA formation (deconjugation and dehydroxylation) and the associated enteric dysbiosis might shape the immunity in the small bile ducts and the gut and thus the pathophysiology, clinical outcome and therapy of PBC.

58. The impact of intestinal schistosomiasis on bile acid metabolism

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Introduction: Intestinal schistosomiasis is a neglected parasitic disease characterized by the accumulation of parasite eggs and subsequent granuloma formation and fibrosis in the host liver. Viral liver diseases (hepatitis C virus and lymphocytic choriomeningitis virus) have been shown alter bile acid metabolism in a consistent manner, namely increasing taurine-conjugated bile acid levels. Bile acids are immuno-active metabolites, with their receptors including FXR, TGR5, VDR and S1PR2 being expressed on both adaptive and innate cell types. Bile acid receptor agonists have been shown to have a positive impact on hepatic fibrosis and inflammation. Here, we investigated whether bile acid metabolism is altered during schistosomiasis and examined how such changes influence the hepatic immune response.

Methods: Using a murine model of intestinal schistosomiasis, we performed both untargeted and targeted bile acid metabolomic analyses of stool, serum, and liver tissue. Expression of key proteins involved in bile acid metabolism was assessed by qPCR and Western blotting. To determine the immunological impact of altered bile acid metabolism independent of infection, we employed a taurine-supplementation model, as taurine is known to promote bile acid synthesis.

Results: Intestinal schistosomiasis led to changes in the expression of genes involved in bile acid metabolism including reduced BSEP and NTCP expression and increased OSTB expression. Consequently, taurine-conjugated bile acids were increased in serum and stool. Mice supplemented with taurine showed increases in hepatic Tregs.

Discussion/Conclusion: We identified changes in bile acid metabolism during schistosomiasis which may have important consequences for modulating the hepatic immuno-pathology via interaction with T cells. We will further investigate how these changes arise, and whether similar changes are seen in humans.

59. Cholic acid-cisplatin conjugates as selective drugs

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Introduction: Carrier-drug conjugates enable targeted delivery of therapeutic molecules to specific tissues, improving bioavailability, efficacy, and reducing side effects through receptor-mediated uptake (Paschke et al., 2003).

Cisplatin is a widely used anticancer agent, but its clinical effectiveness is often limited by resistance mechanisms linked to apoptosis. To overcome this, various platinum-based compounds have been developed to enhance cellular uptake and bioavailability. Cisplatin-bile acid conjugates have emerged as promising candidates, with Bamet/ChAPt–derivatives showing improved potency and pharmacokinetic properties (Paschke et al., 2003; Marin et al., 1998). Their rapid hepatic

clearance and biliary excretion indicate involvement in enterohepatic circulation. Importantly, these conjugates demonstrate the ability to overcome cisplatin resistance (Marin et al., 1998).

Recent studies highlight that bile acid conjugation with glycine and taurine promotes selective uptake into hepatocytes via NTCP (Na+/taurocholate cotransporting polypeptide) (Drossel, et al., 2025). Additionally, analogous systems such as chlorambucil-taurocholate conjugates confirm the 3-OH position of cholic acid as a favourable conjugation site, preserving the side chain for transporter interactions (Kramer, Wess et al. 1997).

Methods: Based on these insights, a series of cisplatin-cholic acid conjugates was synthesized, incorporating variations in linker length and chemical properties (alkyl-, aryl- and peptide-linker structures), as well as glycine and taurine conjugations. Additionally, selected compounds were modified with a NBD (4-nitrobenzo-2-oxa-1,3-diazole) fluorescent tag to facilitate cellular uptake studies.

Discussion/Conclusion: Overall, this project underscores the potential of cisplatin-cholic acid conjugates as a novel class of antitumor agents with enhanced and selective hepatic uptake due to the enterohepatic circle.

60. Monitoring atypical metabolite biomarkers in patients with bile acid synthesis disorders by a novel targeted tandem mass spectrometry assay

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Introduction: Bile acid synthesis disorders (BASD) represent a distinct category of progressive familiar cholestatic liver disease. A novel targeted mass spectrometry assay was developed for the accurate measurement of the major urinary atypical bile acids and bile alcohols that are biomarkers for the HSD3B7, AKR1D1, CYP7B1 and CYP27A1 deficiencies, the 4 most common BASD disorders.

Methods: Stable-isotope dilution UPLC tandem mass spectrometry was used for the simultaneous quantification of 12 key atypical bile acid biomarkers in urine from patients with BASD. Typical concentration ranges for these metabolites were established from the analysis of urine samples collected from a large cohort of patients with biochemically and/or genetically confirmed BASD and compared with non-cholestatic and cholestatic controls.

Results: Separation of major 3β-hydroxy-Δ5-bile acid sulfates, taurine and glycine conjugated 3-oxo-Δ4-bile acids, and bile alcohol glucuronides was achieved in a 20 min chromatographic run with the intra- and inter-batch imprecisions of < 15% for all metabolites. The mean ± SEM urinary concentration of the total 3β-sulfated-Δ5-cholenoic acids the HSD3B7 deficiency was 704 ± 204 μmol/L (n = 22), approximately 2000-fold higher than in cholestastic patients (n = 168) or non-cholestatic controls (n = 127). Similarly, the concentration 5β-cholestane-3α,7α,12α,24,25-pentol-glucuronide the major bile alcohol in CYP27A1

deficiency was 95 ± 17 µmol/L (n = 12). For CYP7B1 deficiency, two confirmed cases showed elevated levels (average, 7.5 µmol/L) for the glycine conjugate of 3β-sulfooxy-Δ5-bile acid. In AKR1D1 deficiency total 3-oxo-Δ4-bile acids in urine were elevated (81 ± 16 µmol/L, n = 48), but concentrations showed overlap with cholestatic and non-cholestatic controls making definitive diagnosis of a 'primary' AKR1D1 more challenging.

Discussion/Conclusion: A novel quantitative tandem mass spectrometry assay is described for the measurement of the major atypical metabolites and biomarkers in urine, and for the first time typical concentration ranges are established for these BASD applicable to diagnosis and accurate monitoring of treatment responses.

61. Low dietary fiber alleviates bile acid-induced cholangiopathy via microbiota-derived hepatoprotective ursodeoxycholic acid

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Introduction: Dietary fibers are recognized for their health-promoting effects through multiple mechanisms. Dietary fibers can bind bile acids (BAs), which act as metabolic integrators but also as potential hepatotoxins due to their detergent properties. Additionally, soluble fibers exhibit higher BA binding capacity (BABC) than insoluble fibers. However, whether different types of dietary fiber differentially modulate BA metabolism in vivo and interfere with BA-induced liver pathology remains unclear.

Methods: Male and female control mice and Cyp2c70-/- mice with a human-like, hydrophobic BA pool were fed either chow, high soluble fiber (HFi-h), high insoluble fiber (HFi-l) or low fiber (LFi) diets, followed by comprehensive analyses of BA metabolism and liver pathology. A human population cohort (n = 1073) was analyzed to determine associations between dietary fiber intake and circulating BA levels.

Results: HFi-h exhibited higher BABC compared to HFi-l and LFi , as evident from increased fecal BA excretion. Unexpectedly, both HFi-h and HFi-l aggravated liver pathology compared to LFi and chow-fed Cyp2c70-/- mice, with HFi-l induced more severe liver pathology than HFi-h. Interestingly, cholangiopathic liver injury in male and female Cyp2c70-/- mice was fully prevented by LFi feeding. Mechanistically, LFi markedly increased the production of hepatoprotective, hydrophilic ursodeoxycholic acid (UDCA, ~40% and 46% of total biliary BAs in males and females, respectively) via modulating gut microbiota composition. In contrast, the detrimental effects of HFi-l were associated with an increased proportion of hydrophobic chenodeoxycholic acid (CDCA, ~51% and 76% in males and females, respectively), whereas, HFi-h was associated with a markedly increased BA pool size. In the human cohort, dietary fiber intake was inversely associated with circulating UDCA species and positively associated with plasma ALT and AST levels. Microbial profiling further revealed that bacterial taxa enriched with low dietary fiber intake, including Ruminococcus gnavus

and Ruminococcus torques, were positively associated with circulating UDCA levels. Consistently, the abundance of key microbial gene involved in UDCA synthesis, 7β-Hsdh, was increased under low-fiber conditions.

Discussion/Conclusion: Low dietary fiber alleviates cholangiopathic liver disease in mice with a hydrophobic, human-like BA composition, most likely due to enrichment hepato-protective UDCA driven by altered gut microbiota. These findings challenge conventional perceptions on fiber supplementation benefits in BA-related liver diseases.

62. Optimized models for studying intra- and intercellular crosstalk in biliary diseases

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Introduction: Understanding the role of bile acid receptors Tgr5 and S1pr2 remains challenging in simplified systems that do not recapitulate the multicellular architecture of the liver. These receptors regulate bile acid signaling and influence inflammatory responses, extracellular matrix (ECM) remodeling, and intercellular communication within the hepatic microenvironment. Therefore, developing multicellular liver organoid models that incorporate major hepatic cell populations and diverse genetic backgrounds is essential for elucidating how Tgr5 and S1pr2 coordinate bile acid driven signaling networks and contribute to the pathogenesis of biliary diseases.

Methods: Multicellular liver organoids were generated from liver tissue isolated from wild-type and double-knockout mice that lack Tgr5 and S1pr2 receptors. After liver perfusion with different collagenases, distinct populations of hepatic cells were isolated. Hepatocytes and non-parenchymal cells (NPCs) were seeded at a 2:1 ratio and cultured for 15 to 20 days to promote organoid formation. Organoid morphology was evaluated using hematoxylin and eosin (H&E) staining, while cellular composition was confirmed through immunohistochemistry (IHC). Bile canaliculi-like structures were assessed using the CellTracker Green CMFDA assay, and inflammatory cytokine expression (TNF-α and IL-6) was quantified by qRT-PCR.

Results: Liver organoids derived from both wild-type and double-knockout mice developed organized, multicellular structures that resembled native liver tissue. IHC confirmed the presence of hepatocytes, liver sinusoidal endothelial cells, Kupffer cells, and hepatic stellate cells (HSCs). Functional analysis indicated the formation of bile canaliculi-like structures and active transporter function, confirming the physiological relevance of the organoids. Stimulation with LPS significantly increased IL-6 and TNF-α expression in both wild-type and homozygous multicellular organoids, confirming their inflammatory responsiveness.

Conclusion: Multicellular liver organoids were successfully established and characterized both structurally and functionally, providing a physiologically relevant platform for studying the mechanisms involved in biliary disease related to Tgr5 and S1pr2.

63. The farnesoid X receptor (FXR) antagonist 7β-isopropylchenodeoxycholic acid improves glucose metabolism in mice on a Western diet

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Introduction: The significant roles of the farnesoid X receptor (FXR) and Takeda G protein-coupled receptor 5 (TGR5) in regulating metabolic pathways have recently been demonstrated. However, the precise effects of FXR inhibitors on glucose metabolism remain to be elucidated. In this study, we examined the impact of 7β-isopropylchenodeoxycholic acid (7β-ipCDCA), a dual FXR antagonist and TGR5 agonist, on impaired glucose metabolism in mice fed a Western diet.

Methods: The dual FXR antagonistic/TGR5 agonistic activity of 7β-ipCDCA was confirmed through gene reporter assays. We evaluated its effects on glucose homeostasis using a glucose tolerance test in C57BL/6 mice fed a Western diet supplemented with sugar in drinking water for 24 weeks. The glucose-lowering mechanism was further investigated by measuring GLP-1 release, mRNA expression of glucose transporters, and relevant genes involved in glucose metabolism in intestinal, hepatic, white adipose, and kidney tissues, and in human NCI-H716 and murine GLUTag L cell lines. Additionally, bile acid metabolome and hepatic lipidome analyses were conducted.

Results: Results showed that 7β-ipCDCA improves glucose homeostasis altered by a Western diet in mice via enhanced GLP-1 secretion and decreased expression of glucose transporters in the ileum and kidneys. While its impact on liver function was marginal, 7β-ipCDCA increased plasma taurocholic acid levels. Furthermore, 7β-ipCDCA elevated hepatic triacylglycerols in mice on a chow diet, whereas mice on the Western diet were protected from triacylglycerol accumulation.

Discussion/Conclusion: This study highlights the role of FXR in regulating intestinal glucose transporters and GLP-1 secretion, emphasizing the therapeutic potential of combined FXR antagonists/TGR5 agonists in managing hyperglycemia.

64. Improvements in serum bile acids are associated with improvements in markers of liver health after maralixibat treatment in children with PFIC: Data from MARCH/MARCH-ON trials

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Introduction: Progressive Familial Intrahepatic Cholestasis (PFIC) refers to inherited disorders of abnormal bile secretion and/or formation causing pruritus, increased serum bile acid (sBA) levels, and chronic liver disease. Most patients with PFIC develop ESLD before adulthood and become candidates for liver transplantation, highlighting the importance of liver health in this population. Reductions in bile acids and bilirubin after surgical biliary diversion (SBD) are the only predictors of longer native liver survival (NLS) in patients with PFIC. We evaluated the impact of maralixibat (MRX), an ileal bile acid transporter (IBAT) inhibitor, on improvements in liver health through a sub-analysis on the correlation of sBA improvements and key liver parameters from the MARCH/MARCH-ON trials.

Methods: MARCH/MARCH-ON has been previously summarized. Spearman's (r) coefficients were determined to evaluate the relationship between sBA and key liver health parameters (AST, ALT, total bilirubin [TB], and direct bilirubin [DB]).

Results: This analysis included 64 patients from the AII-PFIC cohort (13 FIC1, 31 nt-BSEP, 9 MDR3, 7 TJP2, and 4 MYO5B). Mean baseline age, sBA, pruritus, and liver biochemistries were well balanced between MRX and placebo (PBO) groups. In MARCH among PBO participants, there was no significant correlation between changes in sBA and ALT (r 0.094), AST (r 0.25), DB (r 0.11), TB (r 0.13), and 7αC4 (r -0.15). When analyzed for all participants receiving 26 weeks of MRX treatment, correlations were stronger and statistically significant between reductions in sBA and in ALT (r 0.44, $p < 0.001$), AST (r 0.62, $p < 0.001$), DB (r 0.64, $p < 0.001$), TB (r 0.56, $p < 0.001$), and 7αC4 (r -0.79, $p < 0.001$).

Discussion/Conclusion: Reductions in sBA after MRX treatment, as anticipated by the drug's mechanism of action, are correlated with reductions in biomarkers of liver disease which have been associated with long-term clinical outcomes, such as bilirubin, in all PFIC types except for MDR3 deficiency. These results demonstrate the potential for MRX to have disease-modifying effects in the treatment of PFIC.

65. FXR/LXRα coactivation suppresses Cyp8b1 and drives hepatic CDCA accumulation to promote MASH-HCC progression in high-fat/high-sucrose diet-fed mice with human-like bile acid composition

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Introduction: Wild-type (WT) mice fed a conventional high-fat/high-sucrose diet (HFHSD) rarely develop metabolic dysfunction-associated steatohepatitis (MASH) with hepatocellular carcinoma (HCC). In contrast, HFHSD-fed mice with a human-like bile acid composition consistently developed MASH and subsequent HCC. We investigated the role of bile acid metabolism in driving HFHSD-induced MASH with HCC in mice with a human-like bile acid composition.

Methods: Eleven-week-old male WT and Cyp2a12/Cyp2c70 double knockout (DKO) mice were divided into normal diet (ND) and HFHSD groups. Samples were collected at 15, 30, 47, and 58 weeks for histopathological, biochemical, and immunological analyses.

Results: In the HFHSD group, DKO mice showed less severe hepatic steatosis and insulin resistance than WT mice. However, they exhibited markedly accelerated liver inflammation and fibrosis, with HCC developing in 70% of the HFHSD-fed DKO mice by 59 weeks and reaching 100% by 70 weeks. HFHSD increased the proportion of α- and β-muricholic acids (MCAs) in WT mice, whereas it increased the proportion of chenodeoxycholic acid (CDCA) in DKO mice, likely reflecting marked downregulation of Cyp8b1 due to coactivation of FXR and LXRα. Consequently, HFHSD exerted opposite effects on bile acid hydrophobicity in WT and DKO mice. Liver CDCA accumulation in DKO mice was associated with increased inflammation, reactive oxygen species production, and induction of FGF15 in hepatocytes. Moreover, HFHSD tended to activate the oncogenic transcription factor STAT3, and this effect was enhanced in the CDCA-rich environment.

Discussion/Conclusion: We developed a DKO mouse model that mimics human-like MASH with HCC. Our results indicate that, in addition to lipotoxicity, increased bile acid hydrophobicity promoted by Cyp8b1 inhibition through FXR and LXRα coactivation may drive MASH progression. This mechanism is largely overlooked in conventional mouse models, which carry high levels of MCAs, and highlights bile acid composition as a critical determinant of MASH-HCC pathogenesis.

66. Genetic variability in LPAC syndrome: Is the name still correct?

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Introduction: Gallstone formation is attributed to an imbalance of bile components. Mutations in genes essential for bile formation and secretion may underlie gallstone formation. Mutations in the ABCB4 gene are linked to low phospholipid-associated cholelithiasis (LPAC) syndrome. However, not all patients fulfilling the clinical LPAC diagnosis criteria, carry an ABCB4 mutation, suggesting a genetically multifaceted disease. For those carrying ABCB4 mutations, retrospective data found an increased risk of cholangiocarcinoma and chronic liver damage, however, prospective data remains limited.

Methods: 88 patients with clinical signs of biliary disease at Magdeburg University Hospital were included in the prospective multicenter HiChol registry. Clinical (e.g. onset of symptoms, pruritus, etc.) and quality of life data was collected, and whole-exome sequencing was performed.

Results: 35 HiChol registry patients (40%) fulfilled the diagnostic criteria for LPAC. The median age at symptom onset was 27 years. 34 LPAC patients (97%) had

undergone cholecystectomy (CCE) at a median age of 33. An ABCB4 mutation was detected in 8 LPAC patients (23%). However, an abundance of mutations in cholestasis-related genes was found. To further identify genetic gallstone diseases, a cohort of patients who underwent CCE at University Hospital Magdeburg under the age of 40 is being analyzed using a large language model as part of the ongoing ELB-STONE study.

Discussion/Conclusion: LPAC patients of the HiChol registry Magdeburg display a much lower proportion of ABCB4 mutations compared to previously reported data, suggesting greater genetic heterogeneity. Although 97% of LPAC patients underwent CCE the increased risk of gallstone recurrence remains, indicating insufficient treatment. In addition, the pending results of the ELB-STONE study are expected to provide further insights into possible complications of LPAC and the current state of care.

67. Event-free survival of maralixibat-treated patients with progressive familial intrahepatic cholestasis compared with aligned real-world data of untreated patients from NAPPED

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Introduction: Progressive familial intrahepatic cholestasis (PFIC) is a group of hepatocellular bile acid transport disorders that lead to pruritus, growth failure, lipid-soluble vitamin deficiency, and liver failure. Maralixibat, an ileal bile acid transporter inhibitor, is approved for PFIC-related cholestatic pruritus in patients ≥ 12 months of age in the US and treatment of PFIC ≥ 3 months of age in Europe. We compared event-free survival (EFS), time to surgical biliary diversion (SBD), liver transplant (LT), or death in maralixibat-treated patients from the MARCH, MARCH-ON, and RISE trials with aligned real-world data of untreated patients from the NAPPED study.

Methods: We randomly selected NAPPED eligible untreated patients with genetically confirmed biallelic non_truncating bile salt export pump (nt-BSEP) or FIC1 deficiency. To balance baseline characteristics, inverse probability of treatment weights (IPTW) were applied derived from propensity scores for receiving maralixibat (vs. being in NAPPED) estimated from age, sex, PFIC type, total bilirubin, alanine aminotransferase, and aspartate aminotransferase. EFS and SBD-free were compared using IPTW_adjusted Kaplan-Meier curves and Cox models.

Results: Among patients ≥ 1-year-old, 41 maralixibat-treated patients (nt-BSEP, n = 28; FIC1, n = 13) were compared with 256 untreated NAPPED patients (nt-BSEP, n

= 201; FIC1, n = 55). At 3 years, EFS was 0.74 (95% CI: 0.59–0.92) in the maralixibat cohort versus 0.42 (95% CI: 0.35–0.51) in NAPPED; the corresponding SBD-free survival probabilities were 0.98 (95% CI: 0.94–1.00) and 0.67 (95% CI: 0.6–0.76), respectively. EFS was significantly better with maralixibat (hazard ratio [HR] = 0.29; 95% CI: 0.16–0.54; p < 0.0001). The HR for SBD-free probability was 0.05 (95% CI: 0.01–0.39, p = 0.0036). In a sensitivity analysis restricted to patients who remained event-free during the first 6 months, the HRs were 0.46 (95% CI: 0.23–0.91; p = 0.0252) for EFS and 0.08 (95% CI: 0.01–0.57, p = 0.0125) for SBD-free probability.

Discussion/Conclusion: Maralixibat treatment was associated with improved event free survival in nt-BSEP- and FIC1-deficiency patients.

68. Missense variants in the ABCB11 gene and disease severity in patients with bile salt export pump deficiency

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Introduction: Patients with Bile Salt Export Pump (BSEP) deficiency have variable native liver survival (NLS) and serum bile acid (sBA) response to interruption of the enterohepatic circulation (iEHC) via surgical biliary diversion (SBD) or ileal bile acid transporter inhibition (IBATi). The missense variants E297G and D482G (ED) retain some BSEP transport functionality: patients carrying these variants have higher sBA responsiveness to iEHC and longer NLS. We aim to identify whether missense variants other than E297G and D482G (OMS) are associated with sBA response to iEHC and NLS, compatible with residual BSEP transport functionality.

Methods: In the global NAPPED registry (NCT03930810), we categorized ABCB11 variants as ED, OMS or variants leading to a truncated protein (T). Based on the combination of two allelic variants, 6 genotype groups were then compared: ED/ED, ED/OMS, ED/T, OMS/OMS, OMS/T, and T/T. We analyzed sBA response rate after iEHC (sBA concentration < 102 μM or decrease ≥ 75% within two post iEHC visits) and 5-year NLS without iEHC. Five-year NLS was assessed by Cox regression, and patients were grouped based on sBA response to iEHC.

Results: We included 75 BSEP-deficient patients with iEHC (59 SBD, 16 IBATi) and available sBA levels, and 204 without iEHC. The sBA response to iEHC was ≤ 20% in ED/T and T/T patients, but ≥ 50% in ED/ED, ED/OMS, OMS/OMS and OMS/T patients. Among patients without iEHC, the group of ED/ED, ED/OMS, OMS/OMS & OMS/T patients had a significantly higher 5-year NLS rate than ED/T and T/T (HR = 0.48 [0.32–0.72]; p < 0.001).

Discussion/Conclusion: In BSEP deficiency, missense variants other than E297G and D482G are associated with sBA responsiveness to iEHC and with 5-year NLS without iEHC, compatible with relatively high residual BSEP functionality.

We expect that the further identification of these variants will allow to optimize treatment strategies in patients with BSEP deficiency.

69. Activation of the bile acid receptor FXR changes the cellular microenvironment of human adipose tissue in obesity

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Introduction: The bile acid-sensing farnesoid X receptor (FXR) adapts to metabolic changes in obesity and metabolic dysfunction-associated steatotic liver disease. We previously demonstrated altered hepatic FXR binding and transcriptional activity in metabolic disease, promoting substrate utilization after ligand activation. Adipose tissue (AT) serves as energy reservoir and endocrine organ, but in obesity, it loses storage capacity and promotes systemic inflammation. While hepatic FXR signaling is well studied, its effects on human AT remain unclear. This study aimed to characterize, for the first time in humans, the cellular and transcriptional effects of FXR activation on subcutaneous AT (SAT) in metabolic disease.

Methods: We performed single-nuclei RNA sequencing on SAT biopsies derived from non-obese and morbidly obese (BMI > 35 kg/m2) individuals treated with the FXR agonist obeticholic acid (25 mg/d OCA for 3 weeks) or placebo.

Results: Sixteen SAT samples (four per group) yielded 34,529 quality-filtered nuclei. SAT from obese patients showed increased immune cell fractions and enriched pro-inflammatory pathways, with reduced adipocyte abundance. NR1H4 (FXR) expression was low or absent in most samples. Adipocytes and adipose-derived stromal and progenitor cells (ASPCs) each accounted for approximately 20% of nuclei. Subclustering revealed that OCA-treated obese patients exhibited reduced immune-related and angiogenic adipocyte subpopulations, accompanied by a significant downregulation of pro-inflammatory genes (CXCL17, CEBPB) and pathways involved in inflammatory response in all adipocytes, whereas metabolic effects where minor. Within ASPCs, pro-inflammatory genes (CFB, PPIA, IFI6) and humoral immune response pathways were downregulated, and PPARG-positive committed preadipocytes with inflammatory signatures were less abundant.

Discussion/Conclusion: This study provides the first single-nucleus map of FXR agonism effects on human AT. Overall, OCA reduced pro-inflammatory signaling across multiple SAT subpopulations. Minimal local FXR expression suggests that OCA-mediated effects are indirect, supporting the existence of a liver-adipose or intestine-adipose signaling axis modulating immune responses in adipocytes and progenitors.

70. Porphyran from discolored nori prevents metabolic syndrome through microbiota-bile acid-ceramide pathway

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Introduction: Nori is a dietary staple in Japan, a country with notably low obesity rates. Discolored nori is usually discarded as food waste but contains higher levels of the sulfated polysaccharide porphyran than market-quality nori. We studied porphyran's preventive effects and biological mechanisms against metabolic syndrome (MetS), metabolic dysfunction-associated steatohepatitis (MASH), and hepatocellular carcinoma (HCC).

Methods: Male C57BL/6J mice were utilized in three disease models: HFD-induced MetS (13 weeks), HFHCD-induced MASH (18 weeks), and DMBA+HFD-induced HCC (31 weeks). Diets were supplemented with 2% (w/w) porphyran. Comprehensive analyses included 16S rRNA gene sequencing for microbiota, microarray-based transcriptomics, and LC-MS/MS for bile acids and ceramides.

Results: Porphyran significantly suppressed weight gain and hepatic lipid accumulation, while improving glucose tolerance and insulin sensitivity. It also reshaped the gut microbiota composition, recovering alpha diversity and increasing the abundance of beneficial genera, particularly Prevotella and Sutterella. These changes in the microbiota correlated with the suppression of intestinal farnesoid X receptor (FXR) signaling and downregulation of ceramide synthesis-related genes such as Sptlc3 and Smpd3, as evidenced by reduced expression of Fgf15 and Ibabp. Consequently, circulating ceramide levels were significantly reduced. Suppression of intestinal FXR signaling and reduced expression of ceramide synthesis-related genes were similarly observed in the MASH model. Administration of porphyran reduced fibrosis and inflammation in the MASH model. Furthermore, in the HCC model, porphyran almost completely prevented tumor development.

Discussion/Conclusion: Porphyran has been shown to have significant preventive effects against diet-induced obesity, diabetes, MASH, and HCC. These effects are associated with mechanisms involving the gut microbiota, bile acids, and the ceramide pathway. Utilizing porphyran extracted from discarded seaweed represents a strategy that simultaneously addresses unmet medical needs and improves global environmental sustainability.

71 Long-term follow-up in women with severe intrahepatic cholestasis of pregnancy

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Introduction: Intrahepatic cholestasis of pregnancy (ICP) is associated with pathogenic genetic variants in biliary transporters (ABCB4/ABCB11). Women with ICP are known to have an increased risk of chronic liver disease later in life. We describe a cohort of women with moderate to severe ICP who were reviewed in a post-pregnancy

Methods: We identified 27 women with a history of moderate to severe ICP, (50 pregnancies). All had peak bile acid concentrations ≥ 40 μmol/L. Clinical data, family history, and genetic testing results were collected. Liver assessment included transient elastography with Controlled Attenuation Parameter (CAP) and liver stiffness measurement (kPa).

Results: The cohort ranged in age from 31 to 68 years and were assessed between 6 months and 33 years following their most recent pregnancy. Six (12%) pregnancies resulted in stillbirth. Nine (33%) women developed gallstones, and two (13.5%) developed chronic liver disease. Twelve (44%) reported a family history of gallstones or chronic liver disease.

Genetic analysis identified heterozygous pathogenic variants in ABCB4 and ABCB11 in 6 and 2 cases, respectively. Susceptibility variants were identified in ABCB11 (Val444Ala) in 25 (92%) and in SERPINA1 (Z-allele) in 2 (7%) women.

The mean CAP score was 223 dB/m (normal < 248 dB/m). Women with pathogenic ABCB4 variants demonstrated higher CAP values (mean 260 dB/m) than those without pathogenic variants in ABCB4/ABCB11. Overall mean liver stiffness was 5.4 kPa (normal < 6 kPa), while those with ABCB4 variants had higher values (mean 8.3 kPa). The average age of individuals in the ABCB4 subgroup was 47.

Discussion/Conclusion: We describe a cohort of women with moderate to severe ICP, who demonstrated a high prevalence of genetic variants associated with hepatobiliary disease. ABCB4 variants may be associated with increased hepatic steatosis and fibrosis markers on transient elastography, although age may be a confounding factor.

72. Chemical conjugation of Myrcludex B and triiodothyronine for liver-specific targeting

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Introduction: Thyroid hormones (TH) enhance lipid metabolism but therapeutic application by systemic administration is limited due to cardiovascular and skeletal toxicity. Liver-specific delivery of TH, using TH mimetics or TH receptor (TR)-beta agonists (Resmetirom), is therefore a promising frontier to improve hepatic lipid metabolism while minimizing off-target effects. Myrcludex B is a synthetic peptide

targeting liver-specific bile salt transporter sodium taurocholate co-transporting polypeptide (NTCP) and has shown anti-steatotic potential in preclinical studies. We investigated whether liver-targeted delivery of triiodothyronine (T3), the active hormone, could be achieved while mitigating systemic toxicity through chemical conjugation of T3 to Myrcludex B (Myr-T3).

Methods: FVB oatp1a/1b$^{-/-}$/hOATP1B1+/+ mice were used to mimic human hepatic bile salt dynamics. Obesity was induced by 12 weeks of high-fat diet (HFD). Mice then received daily subcutaneous injections of Myr-T3 (100 nmol/kg), Myrcludex B (100 nmol/kg), T3 (50 nmol/kg), or vehicle for 6 weeks while continuing HFD. To further assess liver specificity, a follow-up study was performed in C57BL/6J mice fed HFD, followed by 1-week treatment with lower dosing of Myr-T3 (25 nmol/kg), T3 (25 nmol/kg), or vehicle.

Results: HFD significantly increased liver-to-body weight ratios, plasma cholesterol levels, and hepatic steatosis. Myr-T3 treatment increased plasma bile acid levels (4.5-fold). Compared to T3 alone, Myr-T3 achieved greater cholesterol lowering (-15% vs. -35%) but also increased cardiac toxicity to a greater extent. Hepatic steatosis, assessed by Oil Red O staining, was similar between all groups. Dosed at 25 nmol/kg, Myr-T3 and T3 reduced plasma cholesterol to comparable levels (61% vs. 54%). Hepatic expression of T3-responsive genes was higher in the Myr-T3 group compared with T3 alone, whereas pituitary expression of T3-responsive genes was similar between these two groups.

Discussion/Conclusion: Chemical conjugation of Myrcludex B and T3 preserves the biological activity of both components. However, the current design did not prevent systemic toxicity under high doses, and no clear improvement in liver specificity over T3 alone was observed at lower doses. While the concept demonstrates hepatic efficacy, structural optimization of the fusion is required to improve the organ specificity.

73. Genetic architecture of intrahepatic cholestasis of pregnancy in a British South Asian population

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Introduction: The genetic architecture of intrahepatic cholestasis of pregnancy (ICP) remains largely uncharacterised in South Asians. We aimed to characterise common and rare genetic contributors to ICP in British Pakistani and Bangladeshi individuals, evaluate portability of a European-derived polygenic risk score (PRS), and test associations between ICP and related hepatobiliary/metabolic phenotypes.

Methods: We performed a genome-wide association study (GWAS) and exome-wide association study (ExWAS) in the Genes & Health cohort of British South Asian population (297 ICP cases/7707 controls and 249 ICP cases/6603 controls, respectively). We conducted fine mapping (Susie) and functional

mapping (FUMA) to prioritise likely causal variants and pathways. PRS transferability was assessed using an established European-ancestry ICP PRS. Gene-based rare variant burden testing (SKAT-O) focused on established ICP candidate genes. Phenotypic associations with cholelithiasis, viral hepatitis and gestational diabetes were examined using logistic regression.

Results: We identified a genome-wide significant association at the ABCG5/8 loci (lead variant rs3806471; odds ratio (OR) = 0.62, 95% CI: 0.53–0.73, p = 6.2 × 10-09), supported by ExWAS (p = 4.7 × 10-07). Functional analyses implicated the ABC transporter pathway in ICP pathogenesis, with enrichment of ICP-associated genes in cholangiocytes. The European-derived PRS showed good generalisability in this population, with a 2.23-fold increased risk of ICP per standard deviation increase in PRS (95% CI: 1.89–2.63, p < 0.0001). Rare variant analyses demonstrated significant enrichment of deleterious ABCB4 variants among cases (p = 0.0006). Clinically, ICP was associated with cholelithiasis and hepatitis B/C, but not with gestational diabetes.

Discussion/Conclusion: In British South Asians, ICP is associated with both common variation at ABCG5/8 and rare deleterious variants in ABCB4, supporting a key role for ABC transporter biology and cholangiocyte pathways. The transferability of the European-derived PRS to this underrepresented population highlights the broader applicability of European ICP genetic architecture and underscores the value of expanding genomic research in South Asian cohorts.

74. Gallstones associated with nonalcoholic steatohepatitis (NASH) and metabolic syndrome

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Introduction: We aimed to evaluate the prevalence of non-alcoholic steatohepatitis and metabolic syndrome in patients with symptomatic gallstones undergoing laparoscopic or open cholecystectomy.

Methods: A study of 95 patients was performed. Simultaneous liver biopsies were taken during cholecystectomy between 2022 and 2025. There were no postoperative complications. Patients with significant alcohol intake, hepatitis B or C (virus-positive), autoimmune diseases, and Wilson's disease were excluded. Demographics, liver function tests, lipid profile, and ultrasound findings of patients with and without non-alcoholic steatohepatitis were compared.

Results: A total of 95 patients completed the study. The mean age was 52.15 years, and 29 patients were male and 66 female. Fifty-two patients (55%) had biopsies compatible with non-alcoholic steatohepatitis.

Discussion/Conclusion: Fifty-five percent of patients with gallbladder stones had associated non-alcoholic steatohepatitis. Awareness of this association may result in an earlier diagnosis. The high prevalence of non-alcoholic steatohepatitis in patients with gallbladder stone may justify routine liver biopsy during cholecystectomy to establish the diagnosis and stage and possibly direct therapy.

75. Auxiliary role of bile acids in emergency hematopoiesis in children

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Introduction: Previous studies have shown transient elevations in total bile acids during hematopoietic recovery following chemotherapy-induced bone marrow suppression in pediatric patients with hematologic malignancies. This study aimed to identify specific bile acid fractions associated with hematopoiesis during recovery.

Methods: We conducted a prospective study involving pediatric patients with aplastic anemia and both adult and pediatric patients with hematologic malignancies treated at Toho University Omori Medical Center from January 2002 to March 2024 (Approved by the Ethics Committee, Toho University Omori Medical Center, Approval Number: M24233 22200 20304 18078). Peripheral blood and bone marrow samples were collected at diagnosis and before/after immunosuppressive therapy. Total bile acid levels and individual bile acid components were analyzed over time using liquid chromatography–mass spectrometry. Statistical analysis was performed using ANOVA.

Results: In pediatric patients with hematologic malignancies, levels of 7α-hydroxy-4-cholesten-3-one (C4-12α-ol) (0.35 ± 0.42 μmol/L) and ursodeoxycholic acid (UDCA) increased during recovery from chemotherapy-induced myelosuppression, suggesting enhanced bile acid synthesis. In pediatric aplastic anemia patients, cholic acid (CA) levels rose one month after anti-thymocyte globulin therapy. No significant bile acid changes were observed in adult patients during hematopoietic recovery.

Discussion/Conclusion: These findings indicate that in children, emergency hematopoiesis is accompanied by activation of the classical bile acid synthesis pathway and increased production of hydrophilic bile acids such as CA and UDCA. This may reflect a compensatory hepatic response to support hematopoiesis, reminiscent of fetal liver hematopoiesis.

In conclusion, hydrophilic bile acids may play an auxiliary role in supporting hematopoiesis during emergency recovery in pediatric patients.

76. Dietary protein quantity alter energy metabolism via liver-gut interaction signals

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Introduction: To date, the optimal nutrient balance remains unidentified; however, an increasing number of individuals are adopting dietary patterns that diverge

significantly from traditional averages. This study aims to investigate how alterations in nutrient balance affect gut microbiota and bile acid signalling, and how gut-liver regulatory mechanisms contribute to phenotypic differences.

Methods: C57BL/6J mice were fed diets with varying protein content (8% and 15% of energy) whilst fat intake was controlled at 30% for 22 weeks. Glucose metabolism was evaluated via oral glucose tolerance tests (OGTT) and insulin tolerance tests (IPITT). We further analysed bile acid composition, gut microbiota (via 16S rRNA gene sequence analysis), and metabolites in the caecum and plasma using CE-TOF-MS.

Results: Compared with the standard diet (15%), the low-protein diet (8%) suppressed fat accumulation and improved blood glucose control. In the low-protein group, polyamines in the caecum increased, whilst secondary bile acids decreased. Conversely, taurine and taurine-conjugated bile acids in the liver decreased. About gut-liver hormones, FGF15 decreased in the low-protein group, whilst FGF21 increased. Taurine supplementation suppressed the rise in FGF21 in the low-protein group, whereas taurine depletion in the standard diet group increased FGF21 in the liver. Since FGF21 did not increase even when cholic acid was co-administered to increase free bile acid levels in the liver, this suggests that taurine depletion was more strongly involved than the decrease in taurine-conjugated bile acids. As taurine is abundant in breast milk, a comparison was conducted between the offspring of mice whose mothers had their protein intake altered during pregnancy and lactation. This confirmed that phenotypic differences resulting from changes in protein intake were passed on to the next generation.

Discussion/Conclusion: Our findings provide new insights into the mechanisms underlying the health benefits of dietary protein quantity and its potential role in anti-aging.

77. A human ileal organoid platform defines bile acid transport and signaling defects in Crohn's disease

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Introduction: Bile acids (BAs) are critical regulators of metabolic and immune homeostasis, and their dysregulation contributes to numerous liver, gallbladder, and intestinal diseases. Crohn's disease (CD), a chronic inflammatory disorder with increasing global prevalence, predominantly affects the ileum, impairing BA reabsorption and causing bile acid malabsorption (BAM) and diarrhea, which may increase the risk of gastrointestinal cancer. Clinically relevant human models to study BA transport and signaling are lacking, limiting mechanistic understanding and the development of targeted therapies.

Methods: Ileal organoids were derived from biopsies of healthy donors and CD patients with active ileitis. Organoids were cultured in growth and differentiation media, as well as in an air-liquid interface (ALI) system to model physiologically

relevant intestinal function. BA transporter and receptor expression were assessed by qPCR and immunostaining, while BA transport and signaling were evaluated using LC-MS, cholic acid stimulation, and functional assays. This platform was used to investigate mechanisms of BA-induced epithelial injury and to explore strategies for restoring intestinal function.

Results: Organoids recapitulated key BA transporters, receptors, and signaling pathways observed in native tissue. Organoids grown in differentiation media exhibited 1045-fold and 18-fold higher ASBT and FXR expression, respectively, compared with Caco-2 cells, while the ALI model demonstrated optimal polarity and functional BA transport from the apical to basolateral side, mimicking in vivo intestinal BA absorption. CD patient-derived ALI organoids showed downregulation of ASBT and disrupted BA signaling, confirming impaired BA absorption and pathway dysregulation.

Discussion/Conclusion: Human ileal ALI organoids provide a physiologically and clinically relevant platform to study BA transport and signaling. Observed defects in CD-derived organoids reflect disease pathophysiology and highlight mechanistic targets for therapeutic intervention. This platform enables mechanistic studies, drug testing, and development of novel therapies in patient-relevant models, offering a versatile tool for translational research in digestive and metabolic disorders.

78. Intestinal TGR5-targeted carrier enables bile acid-mediated glycemic control with reduced toxicity in mice and pigs

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Introduction: Takeda G protein-coupled receptor 5 (TGR5) is a gastrointestinal GPCR expressed on enteroendocrine L cells, where it mediates bile acid-induced regulation of glucose metabolism. TGR5 activation offers a promising strategy for type 2 diabetes mellitus (T2DM), yet systemic exposure to conventional TGR5 agonists often causes hepatobiliary toxicity, limiting clinical translation. Clinically relevant approaches to enhance intestinal TGR5 signaling while minimizing off-target effects are lacking.

Methods: We designed a TGR5-targeted carrier-drug conjugate (TGR5-CaDC) by coupling the TGR5 agonist deoxycholic acid with nonabsorbable silicon carriers. Particle shape, size, and composition were optimized to maximize intestinal retention and L cell specificity. Localization, receptor activation, and downstream signaling were assessed in vitro and in vivo in mice and Bama minipigs. Long-term effects on glycemic control, glucagon-like peptide 1 (GLP-1) secretion, and toxicity were evaluated relative to conventional TGR5 agonists and the GLP-1 agonist liraglutide.

Results: TGR5-CaDC remained confined to the intestinal lumen, providing high local agonist concentration and selective activation of TGR5 on L cells. Treatment promoted TGR5 clustering, signal amplification, and increased GLP-1

secretion. Long-term administration improved glycemic control in diabetic mice and minipigs, achieving efficacy comparable to or exceeding liraglutide, while reducing hepatobiliary and systemic toxicity. Optimization of carrier properties enhanced receptor specificity and minimized systemic absorption, providing a safe and effective intestinal-targeted approach.

Discussion/Conclusion: TGR5-CaDC establishes a physiologically and clinically relevant platform for intestinal-specific TGR5 activation. This approach demonstrates sustained glycemic benefits with reduced off-target effects and highlights the potential of localized GPCR-targeted therapies. The strategy offers a versatile framework for investigating bile acid signaling and developing translational therapies for gastrointestinal and metabolic diseases.

79. Chemoproteomic profiling reveals a novel covalent target of deoxycholic acid in tumor suppression

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Introduction: Bile acids are not only essential for intestinal lipid absorption but also serve as key signaling molecules regulating glucose and lipid metabolism, as well as overall energy homeostasis. Currently, the identification of bile acid targets is largely confined to non-covalent interactions with classical receptors, such as the farnesoid X receptor (FXR) and Takeda G-protein-coupled receptor 5 (TGR5). However, investigations into bile acid-mediated covalent protein modifications remain critically scarce. This study aims to utilize a chemoproteomic approach to identify novel covalent targets of deoxycholic acid (DCA) and explore their underlying biological functions.

Methods: We designed and synthesized a novel chemical probe by introducing an alkyne handle at the 3-OH position of DCA. Target proteins were enriched utilizing click chemistry and streptavidin-biotin affinity pull-down. To achieve deep proteomic coverage, the enriched samples were sequentially digested with trypsin and formic acid prior to LC-MS/MS analysis. We conducted deoxycholic acid competition assays, dose-dependent labeling assays and site-directed mutagenesis coupled with Western blot (WB) analysis to validate the covalent nature and specific binding site of this modification. Furthermore, knock-down and rescue experiments were performed to investigate its biological consequences at the cellular level.

Results: Initial mass spectrometry analysis identified approximately 50 candidate targets. Following the validation of these candidates through cellular overexpression, pull-down assays, and Western blot (WB) analysis, we successfully pinpointed “Protein A” as a covalent target. The existence and covalent nature of this modification were firmly established through DCA competition and dose-dependent labeling assays. Further mechanistic studies revealed that this covalent conjugation is actively mediated by SLC27A5; the knockdown of SLC27A5 significantly attenuated the modification level. Interestingly, contrary to traditional carboxyl-directed modifications that typically target lysine residues, we identified

the specific modification site on a histidine residue located near the active pocket of Protein A. This was corroborated by site-directed mutagenesis, where the mutant exhibited an almost complete loss of the probe-labeling signal in WB analysis. Functionally, treatment with 100 μM DCA led to an approximate 20% reduction in the enzymatic activity of Protein A, which consequently decelerated the proliferation of HepG2 tumor cells by roughly 50% through the modulation of oxidative stress-related pathways.

Discussion/Conclusion: This study provides the first evidence that DCA can target a specific protein through an atypical, SLC27A5-mediated covalent histidine modification. The DCA-induced enzymatic inhibition of Protein A uncovers a novel pathway for suppressing tumor cell proliferation via oxidative stress regulation, thereby significantly broadening our understanding of bile acid biology. The profound impacts of this specific covalent modification on downstream cellular functions are currently under further investigation.

80. Bile acid-S1PR2 signaling drives circAFF3 biogenesis and hepatic stellate cell activation in cholestatic liver fibrosis

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Introduction: Cholestatic liver fibrosis is characterized by the accumulation of toxic bile acids, which directly drive the activation of hepatic stellate cells (HSCs). While bile acid receptors such as S1PR2 are known to be involved, the specific downstream epigenetic mechanisms, particularly the role of circular RNAs, remain poorly defined. This study investigates the biogenesis, function, and therapeutic potential of circAFF3 in bile acid-mediated HSC activation.

Methods: Bile acid–induced circRNA regulation was investigated in mouse (JS-1) and human (LX-2) hepatic stellate cells treated with taurocholic acid (TCA). S1PR2-dependent signaling pathways controlling circRNA biogenesis were examined using pharmacologic inhibition and RNA interference approaches. RNA pull-down assays combined with mass spectrometry and immunoblot validation were performed to identify circAFF3-interacting RNA-binding proteins. In vivo relevance was evaluated in cholestatic liver injury models, including multidrug resistance protein 2 knockout (Mdr2$^{-/-}$) mice and bile duct ligation (BDL) mouse model. Single-nucleus RNA sequencing (snRNA-seq) defined HSC-specific transcriptional changes during disease progression. Functional roles of circAFF3 in HSC activation were assessed using wound-healing migration, transwell migration, and collagen gel contraction assays.

Results: Our data reveal that bile acid (TCA) accumulation significantly upregulates circAFF3 expression in HSCs in both a dose- and time-dependent manner. Importantly, snRNA-seq revealed a significant upregulation of the S1PR2 receptor (Fold Change = 1.58) specifically in activated HSCs from Mdr2-/- mice. Furthermore,

circAFF3 expression positively correlates with disease progression over time in both BDL (days 3–14) and Mdr2-/- (days 90–180) models, tightly mirroring the expression of key fibrotic markers ACTA2 (α-SMA) and COL1A1. Mechanistically, TCA induces circAFF3 biogenesis via an S1PR2–MEK/ERK–dependent signaling axis. In vitro pharmacological validation definitively showed that pretreatment with the ERK phosphorylation inhibitor SCH772984 completely abolishes TCA-induced circAFF3 upregulation. Downstream of ERK, the activation of the MAPK/ERK cascade directly enhances the binding of the RNA-binding protein QKI to specific flanking introns (intron-2 and intron-5) of the AFF3 transcript, thereby driving its circularization into circAFF3. Functionally, wound healing and transwell migration assays confirmed that circAFF3 promotes HSC activation, migration, and contractile functions. Transcriptomic profiling (RNA-seq) of HSCs combined with RNA pull-down assays definitively identified the RNA-binding protein PTBP1 as a direct downstream binding partner of circAFF3. From a translational perspective, targeted inhibition of this pathway using the S1PR2 antagonist JTE-013 (1.5 mg/kg i.p.) and small interfering RNA against circAFF3 (si-circAFF3) successfully suppressed HSC activation in vitro and in vivo. This intervention drastically reduced serum markers of liver injury, including total bilirubin (TBIL) and alkaline phosphatase (ALP), and markedly attenuated cholestatic liver fibrosis as evidenced by Sirius Red staining.

Discussion/Conclusion: We have identified a novel TCA-S1PR2-ERK-QKI-circAFF3 signaling axis that plays a fundamental role in driving HSC activation during cholestatic liver injury. Dual inhibition of this pathway via an S1PR2 antagonist and circAFF3 siRNA represents a highly promising, novel therapeutic strategy for the treatment of cholestatic liver fibrosis.

81. Alterations in bile acid biosynthesis in liver cirrhosis

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Introduction: Physiologically, the synthesis of bile acids is mediated by classical and alternative pathways in a ratio of approximately 3:1. However, under pathological conditions, this ratio may shift in favor of the alternative pathway. Both the classical and alternative pathways generate specific intermediate metabolites that can serve as markers of bile acid biosynthesis. Our study aimed to determine whether bile acid synthesis is altered in alcoholic cirrhosis and whether these changes differ from those observed in fatty liver disease. In addition, we assessed the biological effects of these intermediates.

Methods: The study included 36 patients with alcoholic liver cirrhosis, 27 patients with MASLD (metabolic dysfunction–associated steatotic liver disease), and 22 patients with MASH (metabolic dysfunction–associated steatohepatitis) and 11 healthy controls. In all participants, serum concentrations of 7α-hydroxy-4-cholesten-3-one (C4), as a marker of the classical pathway, and 3β,7α-dihydroxy-5-cholestenoic acid (CHA), a product of the alternative pathway, were analyzed

using LC–MS. The biological effects of these intermediates were evaluated by measuring the expression of nuclear receptors (FXR, LXR, PXR, PPARα and THRβ) by qPCR in the HepaRG cell line.

Results: Measurements revealed that patients with alcoholic liver cirrhosis exhibited a significant increase in CHA concentrations (median 0.55 vs. 1.23 µmol/L), while C4 concentrations decreased (2.5 vs. 0.68 ng/mL) compared to controls. In patients with MASH, a significant increase in CHA concentrations was observed (median 0.55 vs. 1.02 µmol/L), while changes in C4 concentrations remained insignificant (2.5 vs. 3 ng/mL) compared to healthy controls. In contrast, patients with MASLD did not show statistically significant differences in either marker. CHA was found to decrease THRβ expression, while C4 led to increase in its expression.

Discussion/Conclusion: Our results suggest that alcoholic liver cirrhosis is associated with increased activity of the alternative bile acid biosynthesis pathway at the expense of the classical pathway. On the other hand, in MASH, bile acid synthesis via the alternative pathway is increased, while the activity of the classical pathway remains unchanged. Hence, it appears that increased activity of the alternative pathway may be a general feature of liver steatosis, whereas reduced activity of the classical pathway is specific to liver cirrhosis. Reduced expression of THRβ caused by CHA may suggest a potential mechanism that contributes to the progression of MASLD.

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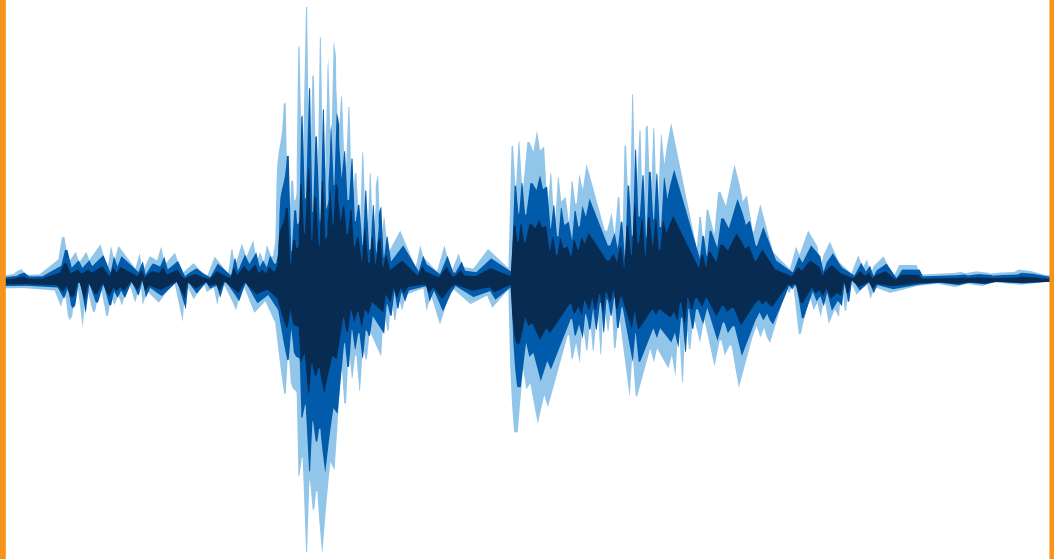

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