XXVII INTERNATIONAL BILE ACID MEETING: BILE ACIDS IN HEALTH AND DISEASE 2024

July 5-6, 2024

Symposium 237

EDINBURGH, UNITED KINGDOM



Preface 2
Scientific Program 4
International Adolf Windaus Award 10
List of Speakers, Moderators and Scientific Organizers 13
Information 17
Poster Abstracts 20
Full content of Poster Abstracts 25
Author Index to Poster Abstracts 71

11,5 CME CREDITS

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 Amsterdam in 2022, 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 receptors in the gut liver axis, bile acid microbiome interactions, and HCC development. FXR has evolved as a target not only for cholestatic liver disease but more recently also for NASH. Furthermore, inhibitors of bile acid transporters ASBT and NTCP have recently been approved for treatment of progressive familial intrahepatic cholestasis (PFIC), cholestatic pruritus in Alagille's syndrome as well as chronic HBV/HDV coinfection. The XXVII 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 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 another focus of this conference. The latest findings will be presented by leading scientists and clinicians in these fields. During the symposium, a poster session will also take place. In line with the tradition of the International Bile Acid Meetings some of the best poster abstracts will be selected by the scientific committee and the authors will be invited for oral presentations. The organizers of the XXVII International Bile Acid Meeting look forward to welcoming you to Edinburgh.

Ulrich Beuers Verena Keitel-Anselmino Michael Trauner

XXVII INTERNATIONAL BILE ACID MEETING: BILE ACIDS IN HEALTH AND DISEASE 2024

July 5-6, 2024

Scientific Organization:
Prof. Dr. Ulrich Beuers,
Amsterdam, The Netherlands
Prof. Dr. Verena Keitel-Anselmino,
Magdeburg, Germany
Prof. Dr. Michael Trauner,
Vienna, Austria

Congress Venue:
Edinburgh International
Conference Centre (EICC)
The Exchange, Morrison Street
Edinburgh EH3 8EE
Scotland, United Kingdom

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 4, 2024
16:00-20:00 h
at the congress office

Friday, July 5, 2024

9:00 Welcome and opening remarks
Verena Keitel-Anselmino, Magdeburg

SESSION I

Bile acid transport in health and disease

Chairs: *Stuart Forbes, Edinburgh; Verena Keitel-Anselmino, Magdeburg*

9:10 Inhibition of bile acid uptake by NTCP: From mice to men
Stan van de Graaf, Amsterdam

9:40 IBAT inhibitors in pediatric cholestatic liver disease – clinical benefits
Henkjan Verkade, Groningen

10:00 IBAT inhibitors: Does PFIC subtype matter
Richard Thompson, London

10:20 Therapy of cholemic nephropathy by inhibition of the apical sodium dependent bile acid transporter (ASBT)
Jan Hengstler, Dortmund

10:40 Identification of new correctors for traffic-defective ABCB4 variants by a high-content screening approach
Thomas Falguières, Orsay

11:00 **Coffee break with poster session**

SESSION II

Bile acids in the intestine

Chairs: *Michael Trauner, Vienna; Catherine Williamson, London*

11:30 Gut microbiota depletion impacts the development of liver pathology in mice with a human-like bile acid profile via modulation of Cyp8b1 expression
Shen Wenqiang, Groningen

Friday, July 5, 2024

11:50	State of the Art: Profiling the human intestinal environment under physiological conditions *Kerwyn Casey Huang, Stanford*
12:20	The role of the intestinal microbiota and bile acids during steatotic liver diseases *Bernd Schnabl, La Jolla*
12:40	Role of the gut-liver axis and microbial bile acid metabolism in primary sclerosing cholangitis *K. Markus Schneider, Aachen*
13:00	**Lunch with poster session**

SESSION III

Bile acids and immunity

Chairs: *Ulrich Beuers, Amsterdam; Ana Lleo, Milan*

14:00	Human gut bacteria produce TH17 modulating BA metabolites *Sloan Devlin, Boston*
14:20	MAIT cells in bile duct inflammation and hepatobiliary cancers *Espen Melum, Oslo*
14:40	The bile acid receptor Tgr5 and alcohol consumption *Jessica Ferrell, Rootstown*
15:00	Altered metabolic function of TGR5-deficient macrophages *Verena Keitel-Anselmino, Magdeburg*
15:20	Role of FXR and TGR5 signaling on immune cell function in fibrosing cholangiopathies *Alexander Miethke, Cincinnati*
15:40	**Coffee break with poster session**

Friday, July 5, 2024

INTERNATIONAL ADOLF WINDAUS AWARD

16:10 Presentation of the International Adolf Windaus Award
Ulrich Beuers, Amsterdam

16:20 International Adolf Windaus Award lecture

SESSION IV

Bile acids in health and disease

Chairs: *Peter Fickert, Graz; Nicolas Chignard, Paris*

16:50 Norucholic acid (norUDCA): Therapeutic mechanisms and clinical implications
Michael Trauner, Vienna

17:10 Bile acid signaling and liver cancer
Mathias F. Heikenwälder, Heidelberg

17:30 Novel (or old) itch mediators
Ronald J. P. Oude-Elferink, Amsterdam

17:50 State of the Art: Using single cell genomics and spatial profiling to decode cholangiopathies
Neil Henderson, Edinburgh

18:30 **End of the session**

Saturday, July 6, 2024

9:00	Welcome and Tribute to Wolfgang Gerok, Gustav Paumgartner and Jan Sjövall *Ulrich Beuers, Amsterdam*

SESSION V

Bile acids in disease

Chairs: *Dieter Häussinger, Nördlingen; Saskia van Mil, Utrecht*

9:15	Data from GWAS meta-analysis in intrahepatic cholestasis of pregnancy *Peter Dixon, London*
9:35	iPSC-derived hepatic organoids as model for intrahepatic cholestasis *Tobias Cantz, Hannover*
9:55	Identifying the variant in UNC45A underlying Aagenaes syndrome *Runar Almaas, Oslo*
10:15	Epigenetic targeted therapies based on novel UDCA-conjugates as a treatment strategy for cholangiocarcinoma *Francisco J. Caballero-Camino, San Sebastian*
10:35	Therapeutic mechanisms of tetrahydroxylated bile acids in cholestasis and beyond *Claudia Fuchs-Steiner, Vienna*
10:55	Tribute to Hanns-Ulrich Marschall *Catherine Williamson, London*
11:00	**Coffee break with poster session**

Saturday, July 6, 2024

SESSION VI

Bile acids as potential toxins

Chairs: *Claudia Fuchs-Steiner, Vienna; Emmanuel Gonzales, Paris*

11:30	Pkd1l1 knockout mice model the developmental cholangiopathy of biliary atresia *Saul J. Karpen, Richmond*
11:50	The potential roles of fetal wound healing and abnormal bile acids in biliary atresia *Rebecca Wells, Philadelphia*
12:10	Impaired bile acid defense in IgG4-related cholangitis *Ulrich Beuers, Amsterdam*
12:30	Exploring the efficacy and safety of chenodeoxycholic acid (CDCA) in adult patients with cerebrotendinous xanthomatosis (CTX): A phase 3 randomized, placebo-controlled clinical study (RESTORE) *Andrea E. DeBarber, Portland*
12:50	Presentation of Poster Awards *Michael Trauner, Verena Keitel-Anselmino, Ulrich Beuers*
13:00	**Lunch with poster session**

SESSION VII

Bile acid metabolism

Chairs: *Stan van de Graaf, Amsterdam; Rebecca Wells, Philadelphia*

14:00	Bile acid transport and metabolism in Cyp2c70 knockout mouse models *Paul Dawson, Atlanta*
14:20	Cyp2C70 mice: Gut liver axis *Folkert Kuipers, Groningen*

Saturday, July 6, 2024

14:40 Gestational bile acid signaling: Beyond cholestasis
Catherine Williamson, London

15:00 A gut microbiota-independent mechanism shapes the bile acid pool in mice with metabolic dysfunction-associated steatohepatitis
Justine Gillard, Brussels

15:20 **Coffee break with poster session**

SESSION VIII

Bile acids and the biliary tree

Chairs: *David Jones, Edinburgh; Caroline Ovadia, London*

15:40 Ursodeoxycholic acid and fibrates: Future strategies in fibrosing cholangiopathies?
Christophe Corpechot, Paris

16:00 Contributions of the gallbladder to bile homeostasis
Nicolas Chignard, Paris

16:20 Senescence and biliary disease phenotypes
Stuart Forbes, Edinburgh

16:40 Cell and gene therapy for cholangiopathies and beyond
Fotios Sampaziotis, Cambridge

17:00 Closing words
Verena Keitel-Anselmino, Magdeburg

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-third time, be presented on the occasion of the XXVII International Bile Acid Meeting, on July 5, 2024. 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)
V. Keitel-Anselmino (Magdeburg)
A. Parés (Barcelona)
R. Poupon (Paris)
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)

Coordinator of the Award Committee:

Prof. Dr. Ulrich Beuers
Dept. of Gastroenterology & Hepatology, C2-327
Univ. van Amsterdam
Tytgat Institute for Liver & Intestinal Research
Meibergdreef 9 I 1105 AZ Amsterdam
The Netherlands
u.h.beuers@amc.uva.nl

LIST OF SPEAKERS, MODERATORS AND SCIENTIFIC ORGANIZERS

Dr. Runar Almaas
Pediatric Research Institute
University of Oslo
Sognsvannsveien 20
0372 Oslo
Norway
runar.almaas@medisin.uio.no

Prof. Dr. Ulrich Beuers
Dept. of Gastroenterology & Hepatology, C2-327
Univ. van Amsterdam
Tytgat Institute for Liver & Intestinal Research
Meibergdreef 9
1105 AZ Amsterdam
The Netherlands
u.h.beuers@amc.uva.nl

Dr. Francisco J. Caballero-Camino
Dept. Of Liver and Gastrointestinal Diseases
Biogipuzkoa Health Research Institute
Donostia University Hospital
Paseo del Doctor Beguiristain
20014 San Sebastian
Spain
fcojavier.caballerocamino@biodonostia.org

Prof. Dr. Tobias Cantz
REBIRTH-Zentrum für translationale regenerative Medizin
Klinik für Gastroenterologie, Hepatologie, Infektiologie und Endokrinologie
Medizinische Hochschule Hannover
Carl-Neuberg-Str. 1
30625 Hannover
Germany
cantz.tobias@mh-hannover.de

Nicolas Chignard, PhD
Associate Professor
Centre de Recherche Saint Antoine
27 rue Chaligny
75012 Paris
France
nicolas.chignard@sorbonne-universite.fr

Dr. Christophe Corpechot
Hopital Saint-Antoine
Service Hépato-gastro-entérologie
184, rue du Faubourg
Saint Antoine
75571 Paris Cedex 12
France
christophe.corpechot@aphp.fr

Prof. Paul Dawson
Division of Pediatric Gastroenterology, Hepatology & Nutrition
Emory University School of Medicine
Health Sciences Research Building
1760 Haygood Drive
GA 30322 Atlanta
USA
paul.dawson@emory.edu

Andrea E. DeBarber, PhD
School of Medicine
Oregon Health & Science University
OH 44278 Portland
USA
debarber@ohsu.edu

Dr. Sloan Devlin
Dept. Of Biological Chemistry and Molecular Pharmacology
Harvard Medical School
Harvard University
250 Longwood Ave
MA 02115 Boston
USA
sloan_devlin@g.harvard.edu

Dr. Peter H. Dixon
Department of Metabolism, Digestion and Reproduction
2nd Floor, Institute of Reproductive and Developmental Biology
Imperial College London
Du Cane Road
London W12 0NN
United Kingdom
p.dixon@imperial.ac.uk

Thomas Falguières, PhD
UMR_S 1193 Inserm
Biliary Homeostasis and Liver Repair
Université Paris-Saclay
17, avenue des Sciences
91400 Orsay
France
thomas.falguieres@inserm.fr

Dr. Jessica Ferrell
Integrative Medical Sciences
Northeast Ohio Medical University
OH 44278 Rootstown
USA
jfrancl@neomed.edu

Prof. Dr. Peter Fickert
Klinische Abteilung für Gastroenterologie und Hepatologie
Universitätsklinikum für Innere Medizin
Medizinische Universität Graz
Auenbruggerplatz 15
8036 Graz
Austria
peter.fickert@medunigraz.at

Prof. Stuart Forbes
Centre for Regenerative Medicine
Institute for Regeneration and Repair
The University of Edinburgh
5 Little France Drive
EH16 4UU Edinburgh
United Kingdom
IRR-Director@ed.ac.uk

Dr. Claudia Fuchs-Steiner
Klinische Abteilung für Gastroenterologie & Hepatologie
Medizinische Universität Wien
Währinger Gürtel 18-20
1090 Wien
Austria
claudia.fuchs@meduniwien.ac.at

Dr. Justine Gillard
Laboratory of Hepato-Gastroenterology
Institute of Experimental and Clinical Research
Université Catholique de Louvain
Avenue Mounier 53/B1.52.01
1200 Brussels
Belgium
justine.gillard@uclouvain.be

Prof. Emmanuel Gonzales, MD, PhD
Hépatologie et Transplantation Hépatique Pédiatriques
AP-HP Université Paris Saclay
78, rue du général Leclerc
94270 Le Kremlin-Bicêtre cedex
France
emmanuel.gonzales@aphp.fr

Prof. em. Dr. Dieter Häussinger
Heinrich Heine Universität Düsseldorf
40225 Düsseldorf

Deiningerstr. 12
86720 Nördlingen
Germany
haeussin@uni-duesseldorf.de

Prof. Dr. Mathias F. Heikenwälder
Deutsches Krebsforschungszentrum
Im Neuenheimer Feld 242
69120 Heidelberg
Germany
m.heikenwaelder@dkfz-heidelberg.de

Prof. Neil Henderson
Centre for Inflammation Research
Institute for Regeneration and Repair
5 Little France Drive
EH16 4UU Edinburgh
United Kingdom
neil.henderson@ed.ac.uk

Prof. Dr. Jan Hengstler
Systemtoxikologie
Leibniz-Institut für Arbeitsforschung an der TU Dortmund
Ardeystr. 67
44139 Dortmund
Germany
hengstler@ifado.de

Prof. Kerwyn C. Huang
Bioengineering Dept.
Shriram Center
Stanford University
443 Via Ortega
CA 94305-4125 Stanford
USA
kchuang@stanford.edu

Prof. Dr. David Jones
NIHR Newcastle Biomedical Research Centre
Campus for Ageing and Vitality
Newcastle upon Tyne NE4 5PL
United Kingdom
david.jones@newcastle.ac.uk

Saul J. Karpen, MD, PhD, FAASLD
CSO, Stravitz-Sanyal Liver Institute
Virginia Commonwealth University
Richmond, VA
USA
saul.karpen@vcuhealth.org

Prof. Dr. Verena Keitel-Anselmino
Klinik für Gastroenterologie/Infektiologie
Universitätsklinikum Magdeburg
Leipziger Str. 44
39120 Magdeburg
Germany
verena.keitel-anselmino@med.ovgu.de

Prof. Folkert Kuipers
European Research Institute for the Biology of Ageing (ERIBA)
University Medical Center Groningen
Antonius Deusinglaan 1
Building 3226
9713 AV Groningen
The Netherlands
f.kuipers@umcg.nl

Prof. Ana Lleo
Humanitas Research Hospital
Via Rita Levi Montalcini, 4
20090 Pieve Emanuele (Milano)
Italy
ana.lleo@humanitas.it

Prof. Espen Melum
Research Institute for Internal Medicine
University of Oslo
Sognsvannsveien 20
4950 Oslo
Norway
espen.melum@medisin.uio.no

Alexander Miethke, MD
Associated Professor of Pediatrics
Center for Autoimmune Liver Disease (CALD)
Pediatric Gastroenterology, Hepatology & Nutrition
Cincinnati Children's Hospital Medical Center
3333 Burnet Avenue
OH 45229-3026 Cincinnati
USA
alexander.miethke@cchmc.org

Prof. Dr. Ronald J. P. Oude Elferink
Tytgat Institute for Liver & Intestinal Research
Academic Medical Center S1-162
Univ. van Amsterdam
Meibergdreef 69-71
1105 BK Amsterdam
The Netherlands
r.p.oude-elferink@amc.uva.nl

Dr. Caroline Ovadia
Women and Children's Health
King's College London
2nd Floor Hodgkin Building
Guy's Campus
SE1 1UL London
United Kingdom
caroline.ovadia@kcl.ac.uk

Dr. Fotios Sampaziotis
Cambridge Stem Cell Institute, Jeffrey Cheah Biomedical Centre
University of Cambridge
Puddicombe Way
Cambridge Biomedical Campus
CB2 0AW
United Kingdom
fs347@cam.ac.uk

Prof. Dr. Bernd Schnabl
Division of Gastroenterology
UC San Diego School of Medicine
9500 Gilman Drive
La Jolla, CA 92093
USA
beschnabl@health.ucsd.edu

Prof. Dr. Dr. K. Markus Schneider
Med. Klinik III
Uniklinik der RWTH Aachen
Pauwelsstr. 30
52074 Aachen
Germany
kmschneider@ukaachen.de

Wenqiang Shen
Dept. of Pediatrics
University Medical Center Groningen
9711 XC Groningen
The Netherlands
w.shen@umcg.nl

Prof. Richard Thompson
King's College Hospital
Institute of Liver Studies
Denmark Hill
SE5 9RS London
United Kingdom
richard.j.thompson@kcl.ac.uk

Prof. Dr. Michael Trauner
Klinische Abteilung für Gastroenterologie & Hepatologie
Medizinische Universität Wien
Währinger Gürtel 18-20
1090 Wien
Austria
michael.trauner@meduniwien.ac.at

Prof. Dr. Stan van de Graaf
Gastroenterology & Metabolism
Amsterdam UMC
Tytgat Institute for Liver & Intestinal Research
Meibergdreef 9
1105 BK Amsterdam
The Netherlands
k.f.vandegraaf@amsterdamumc.nl

Prof. Dr. Saskia van Mil
Center for Molecular Medicine
UMC Utrecht
Universiteitsweg 100
3584 CG Utrecht
The Netherlands
s.w.c.vanmil@umcutrecht.nl

Prof. Dr. Henkjan Verkade
Dept. of Gastroenterology and Hepatology
Faculty of Medical Sciences
University of Groningen
Antonius Deusinglaan 1
9713 AV Groningen
The Netherlands
h.j.verkade@umcg.nl

Rebecca G. Wells, MD
Professor of Medicine and Bioengineering
University of Pennsylvania
421 Curie Blvd.
Philadelphia, PA 19104
USA
rgwells@mail.med.upenn.edu

Prof. Dr. Catherine Williamson
Institute of Reproductive and Developmental Biology
Imperial College London
Du Cane Road
London W12 0NN
United Kingdom
catherine.williamson@imperial.ac.uk

REGISTRATION

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

CONGRESS FEES

Scientific Program of Symposium 237	EUR 300
Students (copy of student ID required)	EUR 150

The congress fees include:

- Pre-Opening and Welcome on Thursday, July 4, 2024, 19.00 h
- Refreshments during coffee breaks
- Lunch on Friday and Saturday, July 5-6, 2024
- A copy of the final program

CONGRESS OFFICE AND REGISTRATION

Opening Hours:

Thursday, July 4	16:00-20:00 h
Friday, July 5	08:00-18:30 h
Saturday, July 6	08:30-17:30 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

Edinburgh International Conference Centre (EICC)
The Exchange, Morrison Street
Edinburgh EH3 8EE
Scotland, United Kingdom

By Bus

Edinburgh's main bus terminal is located at St Andrews Square.
Bus connections stretch right across the UK. For details of these routes please visit: www.nationalexpress.com or www.citylink.co.uk.

For information on local bus services throughout Edinburgh visit www.lothianbuses.com

By Train

Edinburgh has two railway stations:

Waverley Station, which is 1.3 miles from EICC, is the city's main railway station and has direct routes to many cities across the country, including over 25 daily departures from London.

Haymarket Station, which is just 0.4 miles from EICC, is a stop for many commuter and some UK train routes. Please ensure that you check with your rail network provider to find out if your train will stop at Haymarket or Waverley.

For more information on the rail network within the UK, please visit East Coast, National Rail or Trainline; a one-stop shop for train and coach travel.

By Tram

Edinburgh Trams run between the Airport and York Place every 8-10 minutes Monday to Saturday and every 12-15 minutes on a Sunday.
The closest tram stop to the EICC is at Haymarket Station.
Please visit Edinburgh Trams website for more details.

Walk to EICC from Haymarket Station

When exiting Haymarket Station head right (east), towards the pedestrian crossing. Walk straight up Morrison Street for a few minutes and you will find the EICC on the left hand side.

SEStran

For further advice on alternative modes of transport www.sestran.gov.uk

From Edinburgh Airport by Bus

The Airlink 100 operates a frequent bus service (every 10 minutes at peak times) between Edinburgh Airport and the city centre, with designated stops en route. The service starts at 04.30 and runs until 00.22 at night, with the journey taking 20 minutes. Tickets cost £4.50 single and £7.50 return. Delegates are advised to disembark at Haymarket Railway Station and to follow signs for EICC on foot (5 minute walk). See city centre map for directions.

The N22 bus also departs from outside the Airport entrance and runs every half an hour through the night until the Airlink service starts again. For more information about these services visit www.flybybus.com.

CONFLICTS OF INTEREST

The members of the scientific committe declare the following potential conflicts of interest:
Prof. Dr. Ulrich Beuers: Abacus, Behring, GSK, Zambon

Prof. Dr. Verena Keitel-Anselmino: Dr. Falk, Astra Zeneca, Mirum, Ipsen, CSL, Behring, Abbvie, Gilead

Prof. Dr. Michael Trauner: Abbvie, Albireo, BiomX, Boehringer Ingelheim, Dr. Falk, Gilead, Genfit, Hightide, Intercept, Janssen, MSD, Novartis, Phenex, Pliant, Regulus, Siemens, Shire, BMS, Madrigal, Alnylam, Cymabay, Takeda, Ultragenyx

POSTER ABSTRACTS

1. In patients with PSC an upregulation of miR-125b is associated with more advanced liver fibrosis in a gender-dependent fashion
   J. Abramczyk, A. Laba, M. Szydlowska-Jakimiuk, P. Milkiewicz, M. Milkiewicz, A. Kempinska-Podhorodecka (Szczecin, Warsaw, PL)
2. Bridging the Gap: Therapeutic potential of liraglutide in bile acid malabsorption
   E. Alabsawy, D. Craig, K. Kejeh (Middlesbrough, Hartlepool, GB)
3. Investigation of genetic variants in a patient with rapid progressive primary sclerosing cholangitis by whole exome sequencing
   S. Alinaghi Arjas, L. Knopp, K. Stein, D. Schanze, M. Zenker, V. Keitel, C. Droege (Magdeburg, DE)
4. TGR5 and FXR activating potential of microbe-derived iso, allo and keto bile acids
   U. Ay, H. Van Eijk, M. Lenicek, S. Olde Damink, F. Schaap (Aachen, DE; Maastricht, NL; Prague, CZ)
5. Genetic variants in the bile acid synthesis gene CYP7A1 in primary bile acid diarrhoea
   S. Balesaria (London, GB)
6. The UP study confirms the neuroprotective potential of ursodeoxycholic acid (UDCA) in Parkinson's disease
   O. Bandmann, T. Payne, T. Foltnie, C. Mazza (Sheffield, London, GB)
7. Roscovitine analogues as targeted pharmacotherapies for traffic-defective ABCB4 and ABCB11 variants
   M. Banet, M. Lapalus, Y. Riahi, M. Lakli, V. Crespi, J. Elie, E. Gonzales, E. Jacquemin, L. Meijer, F. Di Meo, T. Falguieres (Orsay, Limoges, Roscoff, Le Kremlin-Bicêtre, FR)
8. Bile acids and the gut microbiome: An emerging therapeutic
   A. Basson, D. Kydd-Sinclair, A. Weymouth-Wilson, G. Gibson, K. Watson (Reading, GB)
9. Cholesterol metabolism and neurodegenerative conditions: Analysis of serum and cerebrospinal fluid concentrations of hydroxysterols and PCSK9 in patients with cognitive impairment
   M. Bertolotti, B. Papotti, M. Palumbo, G. Remaggi, L. Elviri, M. Adorni, A. Chiari, R. Bedin, M. Tondelli, E. Baldelli, G. Lancellotti, C. Mussi, M. Lupo, N. Ferri, F. Bernini, F. Zimetti (Modena, Parma, Padova, IT)
10. Enterohepatic regulation of bile acid signaling maintains body weight, lean mass, and hepatic lipid handling after vertical sleeve gastrectomy
    N. Bozadjieva-Kramer, J. Shin, A. Myronovych, R. O'Rourke, P. Henry, N. Lanthier, R. Kohli, R. Seeley (Ann Arbor, Los Angeles, US; Gosselies, Brussels, BE)
11. Challenges in interpreting rare missense variant effects in ABCB4 using ACMG recommended in silico tools, bespoke prediction algorithm Vasor and the novel AlphaMissense predictor
    A. Bracanovic, C. Williamson, J. Zollner, P. Dixon; Genomics England Research Environment, The National Genomics Research Library (London, GB)
12. A core outcome set for studies of intrahepatic cholestasis of pregnancy
    N. Capatina, C. Williamson, B. Hague, J. Chambers, C. Ovadia (London, Sutton Coldfield, GB; Adelaide, AU)
13. Bile acids drive the differential absorption of fatty acids
    A. Chan, K. Jarrett, R. Lai, M. Brearley-Sholto, A. Cheng, M. Taveras, A. Iwata, M. Steel, J. Kennelly, B. Su, K. Williams, E. Tarling, T. De Aguiar Vallim (Los Angeles, US)
14. Food nanoparticles' impact on microbial bile acid metabolism
    L. Clerbaux, V. Proot, P. Starkel, I. Leclercq (Brussels, BE)

15. A pilot study: Recall by genotype and phenotype in a British South Asian population at high risk of cholestatic liver disease
    M. Constantinides, J. Gafton, A. Garcia, P. Dixon, C. Williamson, K. Linton, U. Gill, S. Finer, J. Zollner; Research Team Genes and Health (London, GB)
16. Chenodeoxycholic acid improves muscle insulin sensitivity
    M. Corliano, R. Singaraja (Singapore, SG)
17. The secondary bile acid, lithocholic acid, exerts antisecretory effects in colonic epithelial cells in vitro
    C. Curley, M. Mroz, S. Keely (Dublin, IE)
18. Multi-omics integration reveals a critical role of LRH-1 in the protection against progression of metabolic dysfunction-associated steatotic liver disease to metabolic dysfunction-associated steatohepatitis
    J. De Boer, W. Zhang, Y. Zhang, V. Bloks, C. Out, M. Pawlak, T. Bos, R. Havinga, N. Mulder, M. Koehorst, M. Hovingh, A. De Bruin, J. Haas, B. Staels, R. Bischoff, P. Horvatovich, J. Wolters, D. Reijngoud, A. Groen, F. Kuipers (Groningen, Utrecht, Amsterdam, NL; Lille, FR)
19. Elevated plasma bile acids coincide with cardiac stress and inflammation in young Cyp2c70-/- mice
    H. De Vries, T. Eijgenraam, V. Bloks, N. Mulder, T. Van Zutphen, H. Sillje, F. Kuipers, J. De Boer (Groningen, NL)
20. Gut microbiome composition in women with intrahepatic cholestasis of pregnancy and preeclampsia
    M. Dekker Nitert, S. Leech, E. Dorey, H. Barrett (St. Lucia, South Brisbane, Randwick, AU)
21. Inhibition of adipose triglyceride lipase alleviates NASH in diabetic mice via impaired PPAR-alpha signalling favouring hydrophilic bile acid composition
    E. Dixon, T. Claudel, A. Nardo, C. Fuchs, V. Mlitz, A. Riva, G. Busslinger, H. Schnarnagl, T. Stojakovic, J. Silva, G. Grabner, H. Verkade, G. Haemmerle, R. Zimmermann, M. Trauner (Vienna, Graz, AT; Groningen, NL)
22. First description and analysis of a genetic variant in Semaphorin 7A in a case of intrahepatic cholestasis of pregnancy
    C. Droege, M. Sgodda, T. Cantz, D. Schoeler, M. Schoeler, D. Schanze, M. Zenker, A. Bielfeld, P. Balan, T. Luedde, V. Keitel (Magdeburg, Hannover, Düsseldorf, DE)
23. Histological, molecular, and neuro-functional skin assessment reveals distinct changes in patients with pruritus in cholestatic liver diseases
    M. Duell, H. Kleinlein, V. Leibl, K. Agelopoulos, P. Dietrich, M. Vetter, M. Neurath, S. Staender, A. Sueer, M. Schmelz, R. Pellicciari, F. De Franco, B. Namer, A. Kremer (Erlangen, Münster, Mannheim, Aachen, DE; Corciano, Perugia, IT; Zürich, CH)
24. Altered urinary bile acid profile is associated with renal dysfunction in patients with liver cirrhosis and ascites
    H. El Aggan, S. Mahmoud, S. Sharaf (Alexandria, EG)
25. Increased serum bile acid levels correlate with systemic inflammatory indexes and severity of liver dysfunction in patients with liver cirrhosis
    H. El Aggan, S. Mahmoud, S. Sharaf (Alexandria, EG)
26. Primary biliary cholangitis – Evidence of familial susceptibility and associations with autoimmune disorders and malignancies
    T. Elena, I. Andreea, F. Magda, T. George, P. Mirela (Iasi, RO)
27. Dietary intervention alters the bile acid and gut microbiome profiles in a hepatocellular carcinoma model
    M. Erdem, S. Bharadwaj, A. Izcue, F. Schaap, E. Sahin, T. Cramer (Aachen, DE; Maastricht, NL; Istanbul, TR)

28. Identification of new correctors for traffic-defective ABCB4 variants by a high-content screening approach
    T. Falguieres, M. Lakli, J. Dumont, V. Vauthier, J. Charton, V. Crespi, M. Banet, Y. Riahi, A. Ben Saad, E. Mareux, M. Lapalus, E. Gonzales, E. Jacquemin, F. Di Meo, B. Deprez, F. Leroux (Orsay, Lille, Paris, Limoges, Le Kremlin-Bicêtre, FR)
29. The bile acid receptor Tgr5 and alcohol consumption
    J. Ferrell, S. Boehme, M. Dilts, S. Pokhrel, S. Ferdous, T. Olotu (Rootstown, US)
30. Elevated serum bile acids in MASH patients with fibrosis in the context of a cholestatic genetic predisposition
    A. Geier, M. Rau, T. Schmitt, M. Krawczyk, D. Luetjohann, M. Monte, J. Marin (Würzburg, Homburg, Bonn, DE; Salamanca, ES)
31. A gut microbiota-independent mechanism shapes the bile acid pool in mice with metabolic dysfunction-associated steatohepatitis
    J. Gillard, M. Roumain, C. Picalausa, M. Thibaut, A. Tailleux, B. Staels, G. Muccioli, L. Bindels, I. Leclercq (Brussels, BE; Lille, FR)
32. Bile salts exert immunosuppressive effects on macrophages in vitro independent of TGR5 and FXR receptor activation
    C. Groenen, R. Roscam Abbing, K. Schoonjans, M. Wildenberg, C. Paulusma, S. Van de Graaf (Amsterdam, NL; Lausanne, CH)
33. Prevalence of metabolic syndrome and treatment response with ursodeoxycholic acid in primary biliary cholangitis: A single-center study in Latin America
    R. Guirao, S. Mejia, R. Herrero (Mexico City, MX)
34. Serological characterization and associated autoimmune diseases in a Latin American population with primary biliary cholangitis.
    R. Guirao, S. Mejia, R. Herrero, E. Juarez, A. Avila (Mexico City, MX)
35. Measuring bile acids: The “touches of sweet harmony”
    B. Hague, S. Coat, M. Corey (North Adelaide, Bedford Park, AU)
36. The role of tissue-restricted FXR deletion in MASH development in mice
    Z. Henry, S. Maliha, B. Kong, G. Guo (Piscataway, US)
37. Characterization of the cellular expression pattern of bile acid receptors in intrahepatic cholangiocellular carcinoma
    N. Heucke, M. Reich, A. Guillot, F. Tacke, V. Keitel-Anselmino (Magdeburg, Berlin, DE)
38. Novel bile acids as a treatment for Parkinson’s disease
    L. Heywood (Sheffield, GB)
39. Expanded bile acid profiling and microbial species analysis of faecal samples reveals metabolic pathway differences in primary bile acid diarrhoea
    E. Hillman, J. Walters (Potters Bar, London, GB)
40. TAC005, a novel GalNAc-conjugated siRNA, prevents and reverses liver fibrosis in preclinical models of cholestatic liver disease via a unique mechanism
    W. Hunziker, J. Xu, C. Goh, Z. Tan (Singapore, SG)
41. Attenuating bile salt accumulation prevents dysregulation of energy metabolism in Fxr-/- mice
    W. In het Panhuis, J. Donkers, A. Nguyen, B. Porteiro, D. De Waart, R. Kunst, S. Duijst, S. Kooijman, P. Rensen, S. Van de Graaf (Amsterdam, Leiden, NL; Santiago de Compostela, ES)
42. Difficulties of NAFLD treatment. How to improve it?
    D. Janelidze, I. Lopukh (Kyiv, UA)
43. Generation of an improved murine model for progressive familial intrahepatic cholestasis type 3
    L. Ke, M. Hovingh, N. Mulder, R. Havinga, H. De Vries, V. Bloks, H. Verkade, F. Kuipers, J. De Boer (Groningen, NL)

44. Exploring the efficacy and safety of chenodeoxycholic acid (CDCA) in adult patients with cerebrotendinous xanthomatosis (CTX): A phase 3 randomized, placebo-controlled clinical study (RESTORE)
    Y. Kisanuki, P. Nobrega, R. Himes, S. Jayadev, J. Bernat, V. Prakash, J. Gibson, A. Larson, P. Sgobbi, A. DeBarber, E. Murphy, B. Fedor, C. Wong Po Foo, R. Dutta, M. Imperiale, W. Garner, P. Vig, P. Duell, S. Perez, R. Ramdhani, J. Saute (Columbus, New Orleans, Seattle, Iowa City, Orlando, Austin, Aurora, Portland, San Diego, Foster City, Hempstead, US; Ceara, Sao Paulo, Porto Alegre, BR)
45. Mice with kidney-specific deletion of the ileal bile acid transporter are protected against cholestatic liver damage
    C. Klindt, K. Pachura, A. Bennett, M. Ebert, S. Karpen, P. Dawson (Atlanta, US; Mannheim, DE)
46. Increased incidence of rectal prolapse in FGF15 transgenic mice is caused by chronic diarrhea and electrolyte imbalance
    B. Kong, K. Otersen, R. Taylor, Z. Henry, V. Basaly, G. Guo (Piscataway, US)
47. UDCA modulates TREM-2 and miR-34 expression: In vitro and in vivo study
    A. Laba, J. Abramczyk, E. Cielica, P. Milkiewicz, A. Kempinska-Podhorodecka, M. Milkiewicz (Szczecin, Warsaw, PL)
48. Trafficking rescue of a missense ABCB11 mutation responsible for progressive familial intrahepatic cholestasis type 2
    M. Lapalus, E. Mareux, R. Amzal, S. Petit, Y. Riahi, M. Lakli, M. Banet, E. Drege, T. Falguieres, I. Callebaut, D. Joseph, E. Jacquemin, E. Gonzales (Orsay, Le Kremlin-Bicêtre, FR)
49. Farnesoid x receptor deficiency impacts liver pathology in Cyp2c70-deficient mice with a human-like bile acid composition
    J. Li, H. De Vries, M. Hovingh, R. Havinga, A. Palmiotti, V. Bloks, H. Verkade, J. De Boer, F. Kuipers (Groningen, NL)
50. Constitutive androstane receptor stimulation attenuates liver accumulation of bile acids during estrogen-induced cholestasis in mice
    S. Micuda, M. Krutakova, H. Lastuvkova, J. Schreiberova, D. Manna, M. Hroch, R. Kamaraj, L. Smutna, M. Ambroz, P. Pavek (Hradec Kralove, CZ)
51. Gestational hypercholanaemia interferes with pregnancy-associated adipose mass increase
    A. Mitchell, V. Nikolova, E. Bellafante, G. Papacleovoulou, E. Jansen, A. Wahlstrom, H. Marshall, C. Williamson (London, GB; Bilthoven, NL; Gothenburg, SE)
52. The influence of hydrophobic bile acids on the onset of sclerosing cholangitis in a Cyp2c70/Cyp2a12 DKO mouse
    T. Miyazaki, H. Ueda, T. Ikegami, A. Honda (Ibaraki, JP)
53. Pharmacological inhibition of hepatic bile acid uptake to attenuate inflammation and target inflammatory bowel disease
    T. Nguyen, S. Van de Graaf, R. Roscam Abbing, P. Koelink, M. Wildenberg, C. Paulusma (Amsterdam, NL)
54. Pretreatment serum bile acid composition predicts treatment response to ileal bile acid transporter inhibition by odevixibat in patients with bile salt export pump deficiency
    M. Nomden, K. Folkert, W. Lexmond, E. Lindstrom, Q. Yu, V. Valcheva, H. Verkade (Groningen, NL; Boston, US)
55. Cholestasis in prematurely born girl, what can we find when we look deeper
    B. Perse (Zagreb, HR)
56. Listeria monocytogenes infection and membrane-bound bile acid receptor (Tgr5) deficiency leads to metabolic changes in macrophages
    M. Reich, T. Franz, P. Philippski, M. Rastegari, S. Kahlfuss, V. Keitel (Magdeburg, DE)

57. Two ABCB11 variants affecting the same amino acid have different impacts on the protein in progressive familial intrahepatic cholestasis type 2 (PFIC2)
Y. Riahi, E. Mareux, M. Banet, M. Lakli, T. Falguieres, I. Callebaut, M. Lapalus, E. Jacquemin, E. Gonzales (Orsay, Paris, Le Kremlin-Bicêtre, FR)
58. Differential systemic entry of D and L enantiomers of the microbial bile salt amidate tyrosocholic acid
F. Schaap, U. Ay, H. Van Eijk, M. Lenicek, J. Hengstler, S. Olde Damink, A. Ghallab (Maastricht, NL; Aachen, Dortmund, DE; Prague, CZ)
59. Primary and conjugated serum bile acids are increased in European women with GDM
J. Schoonejans, C. Williamson, H. Fan, M. Martineau, D. McCance (London, Belfast, GB)
60. Comparative efficacy of cholic and chenodeoxycholic acids in the treatment of cerebrotendinous xanthomatosis (CTX)
K. Setchell, W. Zhang, K. Bove, J. Wang (Cincinnati, US; Shanghai, CN)
61. Gut microbiota depletion impacts the development of liver pathology in mice with a human-like bile acid profile via modulation of Cyp8b1 expression
W. Shen, E. Verkade, M. Hovingh, N. Mulder, K. Bruyn, M. Koehorst, H. De Vries, V. Bloks, F. Kuipers, J. De Boer (Groningen, NL)
62. Characterization of cholestasis in a knock-in BsepE297G PFIC2 mouse model
J. Truong, E. Bell, Y. Jo, A. Kolpak, B. Reddy, P. Stoiber, R. Franca, E. Hoch, Y. Ren, J. Moore, A. Garfield (Cambridge, US)
63. The impact of ABCB4 rare variants on the longitudinal trajectories of bile acids concentrations in intrahepatic cholestasis of pregnancy
X. Yang, C. Ovadia, J. Chambers, J. Zollner, P. Dixon, C. Williamson (London, GB)
64. Bile acid-mediated upregulation of lncRNAH19 contributes to cholestatic liver injury through modulation of circular RNA expression in Mdr2-/- mice
H. Zhou, J. Zeng, D. Zhao, Y. Tai, E. Gurley, P. Hylemon (Richmond, US)

FULL CONTENT OF POSTER ABSTRACTS

Poster Numbers 1 – 64

1. In patients with PSC an upregulation of miR-125b is associated with more advanced liver fibrosis in a gender-dependent fashion

Joanna Abramczyk (Szczecin, PL), Alicja Laba (Szczecin, PL), Monika Szydlowska-Jakimiuk (Warsaw, PL), Piotr Milkiewicz (Szczecin, PL), Malgorzata Milkiewicz (Szczecin, PL), Agnieszka Kempinska-Podhorodecka (Szczecin, PL)

Introduction: Primary sclerosing cholangitis (PSC) is a cholestatic liver diseases with male predominance. miR-125b directly targets the co-repressor of androgen receptor (AR), and surgical castration in mice model induces a circulating level of miR-125b. Upregulation of miR-125b is associated with induction of tissue inhibitors of metalloproteinase (TIMPs) and inhibition of p53 promoting liver fibrosis. Key regulators of immune homeostasis are TGF-β, IL-10 and IL-2. We explored a possible link between AR, miR-125b and fibrosis-related genes in patients with PSC.

Methods: Expression of miR-125b and fibrosis-related genes were evaluated in liver (n = 14), peripheral blood mononuclear cells-PBMC, (female n = 14, male n = 15), and serum (female n = 16, male n = 24) of patients with PSC and healthy controls (n = 15). Liver fibrosis was assessed with FibroScan®. Serum concentrations of androgen receptor (AR) was assessed by ELISA. To induce overexpression of miR-125b the transfection with miR-125bMimic was performed in HepG2 cells.

Results: Upregulation of miR-125b was seen in both livers and serum of PSC patients ($p = 0.02$ and $p = 0.03$ vs. controls). In PBMCs expression of miR-125b was 2-fold higher in males than in females ($p < 0.0001$). A negative correlation between miR-125b and TGF-β ($r = -0.5$; $p = 0.03$), and positive correlations between TGF-β and IL-2 ($r = 0.4$; $p = 0.04$,) or IL-2 and IL-10 ($r = 0.5$; $p = 0.073$,) were observed.

In serum of PSC patients expression of miR-125b correlated positively with AST and ALT ($r = 0.5$; $p = 0.002$, and $r = 0.6$; $p = 0.0001$, respectively). However, only in male PSC level of miR-125b correlated with fibrosis ($r = 0.6$; $p = 0.009$). In PSC males serum levels of AR were lower than in age and sex-matched healthy controls ($p = 0.02$). In HepG2 the overexpression of miR-125b was associated with upregulation of TIMP-1 and the reduction of p53.

Discussion/Conclusion: Enhanced expression of miR-125b and lower level of AR in serum of male patients with PSC may favor the development of liver fibrosis.

2. Bridging the Gap: Therapeutic potential of liraglutide in bile acid malabsorption

Eman Alabsawy (Middlesbrough, GB), Darren Craig (Middlesbrough, GB), Kwerinuchi Kejeh (Hartlepool, GB)

Introduction: Bile acid malabsorption (BAM) is a disorder associated with spillover of excessive bile acids from the small intestine to the colon with subsequent osmotic-induced fluid secretion, increased stool frequency, watery diarrhea, steatorrhea, malabsorption of fat-soluble vitamins, and abdominal bloating. Unfortunately, BAM is underdiagnosed despite the fact it affects 1–2% of the general population and can account for up to 50% of patients with chronic

diarrhea. The 75selenium-homotaurocholic acid test (SeHCAT) is the gold standard to diagnose BAM by evaluating the 7-day bile acid retention using a gamma camera. The ability to retain 15% or more is considered normal, while 10–15%, 10–5%, and less than 5% retention are consistent with mild, moderate, and severe bile acid diarrhea. Current treatment options for BAM are limited and often inadequate. Bile acid sequestrants, like colesevelam, are the only approved treatment for BAM which act by binding bile acids in the intestine. However, these drugs have variable efficacy and can be poorly tolerated by many patients, which makes it of great significance to look for better modalities to treat bile acid diarrhea. Liraglutide, a glucagon-like peptide-1 receptor agonist, is primarily used in the management of type 2 diabetes and obesity. The aim of our Case series study is to explore the potential therapeutic role of liraglutide in BAM and its role in improving the quality of life among patients.

Methods: Six patients diagnosed with bile acid malabsorption (BAM) were included in this case series study. Patients were recruited from James Cook University Hospital, gastroenterology clinic between February 2023 and February 2024. BAM was diagnosed based on clinical symptoms and confirmed through SeHCAT scan. Patients received liraglutide, with an initial dosage of 0.6 mg daily, titrated up based on individual patient's response and tolerability. Baseline demographics, clinical data including BAM symptoms, previous treatments, and concurrent medications were gathered. Patients were monitored for changes in symptoms related to BAM (Bowel motions and stool type), adverse events potentially attributable to liraglutide, and quality of life (QOL).

The primary outcome was to explore the change in symptoms and QOL related to BAM following liraglutide therapy, assessed through clinical evaluation and patient-reported outcomes.

Results: In our case series (n = 6), 100% were females with mean age of 52 (9.1), and mean SeHCAT of 5.2%. Following liraglutide initiation, patients reported improvements in symptoms associated with BAM, including diarrhea and frequent bowels opening, with Mean motions of 12.8 (2.8) and 4 (1.2) before and after therapy. Clinical evaluation also revealed a reduction in the severity of gastrointestinal symptoms and significant improvement in quality of life in 83% of patients (n = 5). Liraglutide was generally well tolerated with only one patient reported intolerance to liraglutide in form of bad sickness and worsening diarrhea.

Discussion/Conclusion: Identifying an effective medication to control symptoms and improve quality of life among patients with bile acid malabsorption is crucial, especially in the current situation where there are few treatment options for BAM. The exact mechanism of action for liraglutide in bile acid malabsorption is not fully understood. However, a potential explanation might be its ability to act as a GLP-1 receptor agonist which is known to regulate gastrointestinal function. Liraglutide has also been shown to slow gastric emptying and transit of food, which can lead to altered bile acid kinetics, increased exposure of the ileum to bile acids, promoting their reabsorption and reducing fecal bile acid excretion. It also has effects on hormone secretion, gut microbiota, and intestinal barrier function which likely contribute to its therapeutic effects in BAM.

Our data suggests that liraglutide can be a promising therapeutic option for BAM, potentially alleviating its troublesome symptoms and improving patients' quality of life. However, there are limitations for our study, including its small sample size, lack of a control gro0up, and potential for confounding factors. Therefore, further research is necessary to establish the optimal dosing, exact mechanism, potential interactions, safety, and long-term effects.

3. Investigation of genetic variants in a patient with rapid progressive primary sclerosing cholangitis by whole exome sequencing

Somayeh Alinaghi Arjas (Magdeburg, DE), Lisa Knopp (Magdeburg, DE), Kerstin Stein (Magdeburg, DE), Denny Schanze (Magdeburg, DE), Martin Zenker (Magdeburg, DE), Verena Keitel (Magdeburg, DE), Carola Droege (Magdeburg, DE)

Introduction: Primary sclerosing cholangitis (PSC) is a rare, chronic cholestatic liver disease characterized by biliary strictures and peribiliary fibrosis often progressing to liver cirrhosis requiring liver transplantation. Multiple susceptibility loci were identified in Genome-wide association studies (GWAS) but the correlation between genetic background and disease pathogenesis needs further investigation. Here we provide possible candidate variants contributing to a rapidly progressive disease course of a PSC patient.

Methods: To identify potentially contributing candidate variants, variant evaluation and annotation analysis was carried out on whole exome sequencing (WES) focussing on loss of function and missense variants with an allele frequency of ≤ 1% in gnomAD. Subsequently, rare variants in cholestasis- and PSC-related genes; genes involved in bile acid synthesis, inflammation or immunity pathways in liver were analysed in silico. Prediction tools CADD, LRT, FATHMM, MetaSVM, MetaLR, M-CAP, EIGEN, REVEL, BayesDel were used to prioritize remaining variants. ClinVar database was evaluated for previous reports and classification. PhyloP100way and GREP scores were used to assess conservation of variant position among other species.

Results: Potential candidate variants in ABCC6, SPTB, SPTA1, ALDOA, HSD17B4, AHSG, ALDH2 genes were further investigated to explore correlations between genes and the patient's phenotype. The two most reasonable candidate variants are ABCC6 c.2279G>A, p.(Arg760Gln) and HSD17B4 c.1103G>C, p.(Ser368Thr). Dysfunctional ABCC6/MRP6 was described to cause BSEP and ABCG5/G8 overexpression leading to imbalanced bile composition resulting in gallstone formation and cholestasis. ABCC6 variants were described in cases of low phospholipid-associated cholelithiasis and intrahepatic cholestasis of pregnancy. HSD17B4 plays a role in bile acid synthesis.

Discussion/Conclusion: In depth analysis of WES results revealed potentially relevant variants in ABCC6 and HSD17B4 that may contribute to the progressive PSC phenotype of a young male patient.

4. TGR5 and FXR activating potential of microbe-derived iso, allo and keto bile acids

Uemran Ay (Aachen, DE), Hans Van Eijk (Maastricht, NL), Martin Lenicek (Prague, CZ), Steven Olde Damink (Aachen, DE), Frank Schaap (Aachen, DE)

Introduction: Microbial transformation of primary bile acids is increasingly recognized as contributor to regulation of host biological processes, including intestinal immunity. Recent efforts led to a vast expansion of the repertoire of secondary bile acid species. Here, we studied the ability of oxidized (keto) bile acids and C3 (iso) and C5 (allo) epimers, to activate host bile acid receptors TGR5 and FXR.

Methods: TGR5 activation was assessed in HEK293T cells transfected with human TGR5 and a cAMP-driven luciferase reporter, following exposure to solvent (0.1% DMSO) or test compounds (10 QM, 24 distinct species were tested). Similarly, an FXR reporter was used to study activation of FXRα2 in cells expressing a bile acid importer. Cell viability at end of incubations was assessed via a mitochondrial activity assay.

Results: Activation of TGR5 by lithocholic acid (LCA) was maintained upon oxidation (3-ketoLCA) or epimerization of C3 (isoLCA), but was lost in the C5 epimers alloLCA and isoalloLCA. For deoxycholic acid (DCA), epimerization of C3 (isoDCA) and oxidation of C3 or C12 were tolerated with regard to TGR5 activation. In contrast, epimerization of C3 in chenodeoxycholic acid (isoCDCA) abolished TGR5 activation, while oxidation of C3 or C7 maintained agonistic activity. DCA activated FXR, independent of the presence of a bile acid transporter (ASBT or NTCP), whereas oxidation of C3 (3-ketoDCA) required an importer. Activation of FXR by CDCA and agonistic variants thereof, strictly depended on a transporter. Oxidation of C7 (7-ketoCDCA) abolished FXR activation potential.

Discussion/Conclusion: Gut microbial modifications of bile acids at C3, C5, C7 and C12 can affect their potential to activate TGR5 and FXR. In general, allo bile acids lose the ability to activate TGR5. The interplay between gut microbial and bile acid diversity, and crosstalk via this axis in disease states, warrants further research.

5. Genetic variants in the bile acid synthesis gene CYP7A1 in primary bile acid diarrhoea

Sara Balesaria (London, GB)

Introduction: Primary bile acid diarrhoea (PBAD) is characterised by an overproduction of bile acids (BAs), resulting in increased stool frequency, urgency and incontinence. BAs are synthesized from cholesterol in the liver, where the rate limiting step is determined by the enzyme 7α-hydroxylase (CYP7A1). CYP7A1 is negatively regulated at the transcriptional level by enterohepatic BA levels and FGF19. Several genetic variants in CYP7A1 have been shown to be associated with increased BA production and we have investigated further how these may be associated with the development of PBAD.

Methods: DNA was studied from patients with PBAD, diagnosed by a SeHCAT test retention < 15%, or idiopathic chronic diarrhoea controls (SeHCAT > 15%) described previously (Johnston 2016). Patients with secondary causes of BAD including ileal resection or cholecystectomy were not included. Genotyping for CYP7A1 polymorphisms, rs3808607 T>G in the promoter, rs8192877 A>G and putative enhancer element rs9297994 A>G, was performed using Taqman allelic discrimination assays (ThermoFisher Scientific).

Results: Results showed that the frequency of the rs3808607 T-allele was 75% in the severe PBAD group (SeHCAT ≤ 5%, n = 18) compared with 52% in those with higher SeHCAT values (> 5%, n = 119), giving an odds ratio of 2.72 (95% confidence interval: 1.23–6.02, p = 0.014). Frequency of the rs8192877 G-allele was 24% in the severe group (≤ 5%) compared with 10% in the other group (> 5%); odds ratio 2.79 (95% confidence interval: 1.19–6.56, p = 0.019). Results for the rs9297994 A-allele was 78% in the severe PBAD group (SeHCAT ≤ 5%) compared with 59% in those with higher SeHCAT values (> 5%), giving an odds ratio of 2.45 (95% confidence interval: 1.07–5.60, p = 0.034). It is not certain which are the high activity alleles of these variants, but in vitro studies (Wang et al., 2018) suggest that the rs3808607 T allele is low activity and the rs9297994 A allele is high activity. These SNPs show high linkage disequilibrium (LD). In the Caucasian population in our study, the minor allele frequencies are roughly equal (0.44 and 0.41, respectively, n = 97), with numbers of putative high activity alleles (G in rs3808607 and A in rs9297994) not significantly different. In our African Caribbean population and Asian (Indian) population (n = 24), the numbers of high activity alleles are significantly higher. However, this does not translate to SeHCAT levels, which were on average only slightly lower than that of Caucasians.

Discussion/Conclusion: Previously we showed that the genetic variants, rs3808607 and rs8192877 in CYP7A1 were significantly associated with severe PBAD (Balesaria et al, 2022). Another variant, rs9297994 indicates this is in high linkage disequilibrium with rs3808607 in our Caucasian patients. We have found a marked difference in the number of proposed high activity alleles amongst different ethnic groups which may affect how differences in CYP7A1 activity contribute to the development of bile acid diarrhoea.

6. The UP study confirms the neuroprotective potential of ursodeoxycholic acid (UDCA) in Parkinson's disease

Oliver Bandmann (Sheffield, GB), Tom Payne (Sheffield, GB), Tom Foltnie (London, GB), Claudia Mazza (Sheffield, GB)

Introduction: Mitochondrial dysfunction is a key pathogenic mechanism for Parkinson's disease. We previously identified UDCA as a powerful mitochondrial rescue compound in the first screen of an entire compound library in Parkinson's disease patient tissue. UDCA has been in clinical use for liver disorders for decades and is therefore an ideal candidate for the drug-repurposing strategy.

Methods: The UP (UDCA in Parkinson's disease) study is a phase IIa, randomised, double-blind, two centre, placebo-controlled trial of high dose ursodeoxycholic acid (30 mg/kg) in 30 participants with early Parkinson's disease for 48 weeks followed by an 8-week washout period. Randomisation was 2:1 drug to placebo.

Results: UDCA was safe and extremely well tolerated with excellent compliance. There were no serious adverse events in the treatment group. Mild, transient gastrointestinal adverse events were observed more frequently in those taking UDCA compared to placebo. Bile acid analysis confirmed a marked, stable increase of UDCA and its key conjugates throughout the treatment period. Objective quantification of motor impairment demonstrated improvement of several gait parameters such as cadence ($p = 0.019$), stride time ($p = 0.031$), stride time variability ($p = 0.031$), stance time ($p = 0.031$) and stance time variability ($p = 0.024$) in the UDCA treatment group compared to placebo. In contrast, subjective clinical assessment using standard clinical rating scales failed to detect any difference between treatment groups. Midbrain 31P-MRS revealed an improvement in Gibb's free energy of ATP hydrolysis in the UDCA group compared to placebo ($p = 0.024$).

Discussion/Conclusion: The UP Study demonstrates the utility of both sensor-based quantification of motor impairment and 31P-MRS in early phase clinical trials and provides justification for subsequent larger trials to further evaluate the disease-modifying effect of UDCA in PD.

7. Roscovitine analogues as targeted pharmacotherapies for trafficdefective ABCB4 and ABCB11 variants

Manon Banet (Orsay, FR), Martine Lapalus (Orsay, FR), Yosra Riahi (Orsay, FR), Mounia Lakli (Orsay, FR), Veronica Crespi (Limoges, FR), Jonathan Elie (Roscoff, FR), Emmanuel Gonzales (Le Kremlin-Bicêtre, FR), Emmanuel Jacquemin (Le Kremlin-Bicêtre, FR), Laurent Meijer (Roscoff, FR), Florent Di Meo (Limoges, FR), Thomas Falguieres (Orsay, FR)

Introduction: ABCB4 and ABCB11 are ATP-binding cassette (ABC) transporters expressed at the canalicular membrane of hepatocytes and involved in bile secretion of phosphatidylcholine and bile salts, respectively. Genetic variations of these transporters lead to several rare cholestatic diseases, including progressive familial intrahepatic cholestasis types 2 and

3 (PFIC2/3), which are associated with mutations in ABCB11 or ABCB4 loci, respectively. These diseases appear during the first years of life and often progress to cirrhosis and liver failure before adulthood. Medical therapy with ursodeoxycholic acid (UDCA) is available but it is inefficient (or poorly efficient) for the majority of patients who then require liver transplantation. Thus, the development of alternative pharmacotherapies is eagerly needed. As a drug candidate, we have previously shown that structural analogues of roscovitine, a tri-substituted purine able to rescue the traffic and the activity of the F508del variant of ABCC7/CFTR (Norez et al., Br J Pharmacol. 2014;171:4831–49), rescue the maturation, localization and function of several endoplasmic reticulum (ER)-retained ABCB4 variants, also categorized as class II variants (Vauthier et al., Sci Rep. 2019;9:6653). Nevertheless, in order to develop molecules with better benefit/toxicity ratios, new analogues of roscovitine were synthesized and further investigated.

Methods: So far, 56 new analogues of roscovitine were tested in cell models (10 QM) as potential correctors of three class II ABCB4 variants (I490T, I541F and L556R) and three class II ABCB11 variants (D482G, G982R and T1210P), identified in severe forms of ABCB4 and ABCB11-related liver diseases. These molecules were tested for their capacity to restore: i) the maturation of the transporters, studied by immunoblot in HEK293 cells; ii) The localization of the transporters at the canalicular membrane, analyzed by immunofluorescence in HepG2 cells; iii) The ABCB4-mediated phosphatidylcholine secretion activity, studied by a fluoro-enzymatic assay in HEK293.

Results: We show that 21 and 17 roscovitine analogues are able to significantly rescue the maturation of at least one ABCB4 variant or one ABCB11 variant, respectively. These molecules also allow a significant relocalization of ABCB4 and ABCB11 variants at bile canaliculi. Preliminary functional assays already indicate that some roscovitine analogues may partially but significantly correct the functional defects of ABCB4 variants while they would be less inhibitory of the function of wild type ABCB4 than roscovitine itself. Experimental set-up is still under construction/validation for functional testing of ABCB11.

Discussion/Conclusion: Our results allowed us to identify new structural analogues of roscovitine as drug candidates able to rescue the maturation and canalicular localization of ABCB4 and ABCB11 variants. Further functional investigations are now required in order to validate these molecules. Dose-response experiments and combination with a potentiator (e.g. VX-770/Ivacaftor) will be considered to optimize the correction efficiency of these molecules. The chemical optimization of the best molecule(s) will then allow the testing of a limited number of compounds in preclinical mouse models mimicking the human diseases.

8. Bile acids and the gut microbiome: An emerging therapeutic

Abigail Basson (Reading, GB), Dannielle Kydd-Sinclair (Reading, GB), Alex Weymouth-Wilson (Reading, GB), Glenn Gibson (Reading, GB), Kimberly Watson (Reading, GB)

Introduction: Bile acids are vital in the metabolism of glucose and lipids. Primary bile acids are converted to secondary bile acids by gut bacteria, which act as second series signalling molecules. Recent discoveries have found that bile acids increase the incidence of certain beneficial microorganisms in the gut. The aim of this research is to uncover the potential health-promoting shifts in the microbiome population due to select bile acids.

Methods: 3-stage continuous culture system, set up in sequence, simulated nutritional, temporal and physiochemical features of microbiota within the proximal, transverse and distal regions of the colon. The vessels were inoculated with faecal; samples were donated from 3 healthy adult donors. When equilibrium was reached, baseline samples were taken and bile acid treatment (400 mg/day) began.

Results: Increase the incidence of the beneficial microbes: Lactobacillus, Eubacterium rectale and Bifidobacterium spp. were observed. Changes in bacterial metabolite profiles and increases in SCFAs were also observed using GC-MS analysis and NMR spectroscopy. These results, coupled with in vitro cell culture studies, demonstrate that there may be additional mechanisms by which UDCA and UDCA-like molecules exert therapeutic effects.

Discussion/Conclusion: In addition to the role bile acids play in host metabolism, current data suggests that 7KLCA has favourable properties when used as a prebiotic substance. 7KLCA is a candidate for a human intervention trial to investigate health-promoting properties and the promotion of healthy ageing.

9. Cholesterol metabolism and neurodegenerative conditions: Analysis of serum and cerebrospinal fluid concentrations of hydroxysterols and PCSK9 in patients with cognitive impairment

Marco Bertolotti (Modena, IT), Bianca Papotti (Parma, IT), Marcella Palumbo (Parma, IT), Giulia Remaggi (Parma, IT), Lisa Elviri (Parma, IT), Maria Pia Adorni (Parma, IT), Annalisa Chiari (Modena, IT), Roberta Bedin (Modena, IT), Manuela Tondelli (Modena, IT), Enrica Baldelli (Modena, IT), Giulia Lancellotti (Modena, IT), Chiara Mussi (Modena, IT), Maria Giovanna Lupo (Padova, IT), Nicola Ferri (Padova, IT), Franco Bernini (Parma, IT), Francesca Zimetti (Parma, IT)

Introduction: Alterations of cerebral cholesterol homeostasis have been reported in Alzheimer's Disease (AD). In particular, changes in plasma and cerebrospinal fluid (CSF) levels of 24(OH)cholesterol and 27(OH)cholesterol, precursors in bile acid synthesis, have been described. Furthermore, previous evidence demonstrated higher CSF levels of PCSK9, a key regulator of cholesterol homeostasis and of plasma cholesterol levels, in AD patients compared to controls. This study aimed to evaluate the possible role of hydroxylated sterols and PCSK9 as disease biomarkers in patients with different degrees of cognitive impairment.

Methods: We recruited 83 subjects divided as follows: 28 had a diagnosis of mild cognitive impairment (MCI) stable at follow-up; 28 MCI subjects had a subsequent diagnosis of AD (MCI-AD); 27 were diagnosed as AD upon initial evaluation. Serum and CSF were analyzed; hydroxylated sterols were quantified by LC-MS/MS and PCSK9 determination was performed by ELISA. The apoE Ɛ4 carrier status was also investigated.

Results: PCSK9 CSF concentrations were similar among the three groups although higher if compared to those of control subjects previously analyzed. Within AD patients, apoE Ɛ4 carriers presented higher PCSK9 concentration as compared to non-carriers (+58%; $p = 0.04$). Moreover, CSF PCSK9 levels positively correlated with that in serum ($r = 0.521$, $p = 0.004$) and with CSF total-tau ($r = 0.339$, $p = 0.03$). Finally, again specifically in AD patients, CSF 24-OHC levels were significantly higher in apoE Ɛ4 carriers as compared to apoE Ɛ4 non-carriers (+34%; $p < 0.05$).

Discussion/Conclusion: PCSK9 levels may be increased since the early phases of AD-related cognitive decline. The positive correlation between CSF/serum PCSK9 suggests a possible increase in blood-brain barrier permeability, specifically occurring in AD, and possibly linked to the apoE Ɛ4 genotype. CSF 24-OHC levels might be associated with AD-related alterations, and thus might be considered as a potential specific disease biomarker. These findings support an interesting relationship between cholesterol and bile acid metabolism and AD.

10. Enterohepatic regulation of bile acid signaling maintains body weight, lean mass, and hepatic lipid handling after vertical sleeve gastrectomy

Nadejda Bozadjieva-Kramer (Ann Arbor, US), Jae Hoon Shin (Ann Arbor, US), Andriy Myronovych (Ann Arbor, US), Robert O'Rourke (Ann Arbor, US), Paulina Henry (Gosselies, BE), Nicolas Lanthier (Brussels, BE), Rohit Kohli (Los Angeles, US), Randy Seeley (Ann Arbor, US)

Introduction: Bariatric surgery is currently the most effective treatment for sustained weight loss. However, as the use of bariatric surgery rises, there is also greater awareness of its complications, including the development of sarcopenia, osteopenia, and liver disease in a subset of patients. Our work showed a role for the gut hormone Fibroblast-Growth Factor 15/19 (FGF15/19) which is increased in humans and mice after VSG. FGF15 INT-KO mice lost more weight after VSG due to increased lean muscle loss. Despite a substantial body weight loss, FGF15 INT-KO VSG mice had increased hepatic cholesterol content, pointing to a role of FGF15 in hepatic lipid handling after VSG. VSG resulted in increased plasma bile acid levels, but the total circulating bile acids were considerably higher in VSG-FGF15 INT-KO mice. These data pointed to an important role after VSG for intestinal FGF15 to protect from the deleterious effects of VSG by limiting the increase in circulating bile acids.

Methods: To test this hypothesis, we decreased total circulating bile acid levels by inhibiting intestinal bile acid reabsorption in WT VSG and FGF15 INT-KO VSG mice with the potent ASBT inhibitor Maralixibat (MRLX) starting at 2 weeks post-surgery for a total of 10 weeks of MRLX treatment.

Results: Our data showed that inhibiting bile acid reabsorption and thus decreasing circulating total bile acids with MRLX in WT VSG mice led to a reduction in body weight and lean mass, comparable to the changes observed in FGF15 INT-KO VSG mice. However, decreasing circulating total bile acids did not prevent the loss of body weight and lean muscle mass in FGF15 INT-KO VSG mice.

Discussion/Conclusion: These new data highlight an important role for a tightly regulated enterohepatic regulation of bile acid levels and bile acid composition after VSG to prevent complications such as great reduction in lean mass and hepatic lipid deposition.

11. Challenges in interpreting rare missense variant effects in ABCB4 using ACMG recommended in silico tools, bespoke prediction algorithm Vasor and the novel AlphaMissense predictor

Alexander Bracanovic (London, GB), Catherine Williamson (London, GB), Julia Zollner (London, GB), Peter H. Dixon (London, GB); Genomics England Research Environment, The National Genomics Research Library (London, GB)

Introduction: Intrahepatic cholestasis of pregnancy (ICP) is the most common pregnancy associated liver disorder. Despite ABCB4 (phosphatidyl floppase) heterozygous variants being associated in the aetiology of ICP, rare variant interpretation is challenging and has resulted in large numbers of variants of uncertain significance. Here we compare variant prediction in a range of in-silico tools on 9 heterozygous missense rare variants identified in women with ICP.

Methods: Using the Genomics England Research Library, a cohort of women with a previous diagnosis of ICP (n = 253) and available whole genome sequencing data were identified. Variants were filtered by minor allele frequency (< 0.05). Rare missense variants (n = 9) were selected for in-silico analysis. Five in silico tools: Sift, Polyphen, CADD, Vasor (bespoke for ABCB4) and AlphaMissense were used.

Results: Only 44% (4/9) of variants had full agreement over classification across all prediction tools. Additionally, 33% (3/9) of variants had highly contradictory predictions with significant disparity between pathogenic and benign classifications. Published studies demonstrated conflicting findings when compared to the in-silico tools. For example, a functional study using HepG2 and HEK293 cells, classed variant T175A to have no detectable effect on protein structure whilst prediction by Vasor resulted in likely pathogenic and benign by AlphaMissense classifications. In the same study, N510S was classified as having an impact on protein stability correlating with a likely pathogenic interpretation by Vasor but disagreeing with a benign interpretation by AlphaMissense.

Discussion/Conclusion: Overall, the results demonstrate conflicting evidence across in-silico tools to predict pathogenicity of rare missense variants in ABCB4. This highlights the difficulty of conclusively classifying rare variants, particularly since functional, segregation and allelic data are often not present, meaning that in-silico tools carry a large burden in overall classification.

12. A core outcome set for studies of intrahepatic cholestasis of pregnancy

Nadejda Capatina (London, GB), Catherine Williamson (London, GB), Bill Hague (Adelaide, AU), Jenny Chambers (Sutton Coldfield, GB), Caroline Ovadia (London, GB)

Introduction: Meta-analyses in intrahepatic cholestasis of pregnancy (ICP) have been limited by inconsistent source data outcome reporting. To improve the impact of future ICP studies, we aimed to define standardised outcomes, the "Core Outcome Set" (COS).

Methods: Maternal and perinatal outcomes reported in studies identified by systematic reviews, clinical trials, reviews and patient resources were collated. The importance of each outcome was ranked with three e-Delphi rounds, utilising a 9-point Likert scale. Short-listed outcomes were discussed, anonymously ranked (Google Forms) and definitions agreed (Padlet) in face-to-face consensus meetings (SOMANZ 2023), and following poster presentation at MOMS (2023). The study was registered in COMET and ethical approval granted (KCL MRA-23/24-39574).

Results: From 54 manuscripts, 97 individual clinical outcomes were attributed to ICP. A total of 64 participants from five continents answered the e-Delphi surveys and more than 80 stakeholders participated at the in-person meetings. The three rounds of e-Delphi surveys short-listed 23 outcomes, the ranking of which at the in-person meeting enabled the steering committee to select the final 12 core outcomes. Outcomes in the maternal section include: total maternal bile acid concentration (max), gestation of peak bile acid concentration, itch impact on maternal wellbeing, itch impact on quality of life, use of medication for ICP, maternal ALT. Outcomes in the birth section include: stillbirth, gestation of delivery, spontaneous premature birth vs. induced premature birth. Outcomes in the neonatal section include: perinatal death within 7 days of birth, perinatal asphyxia, neonatal unit admission.

Discussion/Conclusion: Given the variety of reported outcomes, we have confirmed the need for a COS in ICP, which aims to standardise the framework for reporting ICP outcomes.

13. Bile acids drive the differential absorption of fatty acids

Alvin Chan (Los Angeles, US), Kelsey Jarrett (Los Angeles, US), Rochelle Lai (Los Angeles, US), Madelaine Brearley-Sholto (Los Angeles, US), Angela Cheng (Los Angeles, US), Maria Taveras (Los Angeles, US), Anne Iwata (Los Angeles, US), Michelle Steel (Los Angeles, US), John Kennelly (Los Angeles, US), Baolong Su (Los Angeles, US), Kevin Williams (Los Angeles, US), Elizabeth Tarling (Los Angeles, US), Thomas De Aguiar Vallim (Los Angeles, US)

Introduction: Dietary fat absorption is a highly efficient, yet complex process that requires lipases and bile acids for intestinal uptake. Here, we report that bile acid modulation and lipase inhibition have different effects on dietary fat absorption with distinct physiological consequences.

Methods: To alter the bile acid pool, we disrupted cholesterol 7 alpha-hydroxylase (Cyp7a1), the rate-limiting enzyme in bile acid synthesis, using liver-directed AAV-CRISPR. To reduce lipase activity, we incorporated tetrahydrolipstatin (Orlistat), a gastrointestinal lipase inhibitor, into the diet. We performed parallel experiments challenging Cyp7a1 CRISPR mice as well as orlistat-treated mice with Western diet for 8 weeks to induce obesity.

Results: Loss of CYP7A1 protected from diet-induced obesity, while lipase inhibition did not, due to increased food intake. Reduction in bile acids stimulated secretion of anorectic incretin hormones (e.g. GLP-1, PYY), but lipase inhibition did not trigger hormone-dependent suppression of appetite. Both loss of CYP7A1 and lipase inhibition prevented hepatic steatosis. However, loss of CYP7A1 increased hepatic polyunsaturated fatty acid-containing lipids, blunting lipogenesis. This was not observed in lipase inhibition, which decreased all fatty acid species while also increasing lipogenesis. The alterations in hepatic lipid composition suggested potential differences in fatty acid absorption. Remarkably, the reduction of bile acids preserved the absorption of polyunsaturated fatty acids but reduced the absorption of saturated fatty acids. Mechanistically, we then show that bile acids, in particular conjugated cholic acid, preferentially solubilized polyunsaturated fatty acids into mixed micelles. In contrast, lipase inhibition decreased the absorption of all fatty acids without discrimination.

Discussion/Conclusion: Taken together, our results demonstrate the pivotal role of bile acids in mediating selective fatty acid absorption, highlighting an under-recognized aspect of metabolic physiology. These findings advance our basic understanding of dietary fat absorption and propose the role of bile acid manipulation as a potential therapeutic strategy for metabolic disease.

14.Food nanoparticles' impact on microbial bile acid metabolism

Laure-Alix Clerbaux (Brussels, BE), Valentine Proot (Brussels, BE), Peter Starkel (Brussels, BE), Isabelle Leclercq (Brussels, BE)

Introduction: Nanoparticles are present in our plates as the food industry takes advantage of their physicochemical properties to coat, color and protect the packaging or the food itself. But the use of food nanoparticles as potent bactericidal agents rises concerns about their potential impact on our gut microbiota. Mice studies administering nanoparticles showed evidence of gut dysbiosis although the long-term health significance of it remains unexplored. There is a lack of comprehensive understanding of the functional changes in gut microbiota and especially which microbial products drive pathological changes. Bile acids are such microbial metabolites.

Methods: Based on a proposed mechanistic pathway and selection of associated assays, we aimed to assess the effects of food nanoparticles on microbial bile acid metabolism and impact on steatotic livers. We primarily focused on silicon dioxide nanoparticles.

The toxicological pathway was developed based on previous results obtained in the lab and on biological plausibility. Assays associated to the main perturbed biological events were identified and performed upon nanoparticles exposure.

Results: We formulated a pathway linking gut dysbiosis to the modification of intestinal secondary bile acid biotransformation resulting in reduced bile acid-mediated anti-inflammatory response via the bile acid receptor TGR5 and subsequent escalation of inflammation in the

liver. Evidence from experimental data and literature supported these pathophysiological pathways upon dietary nanoparticles exposure and underscored knowledge gaps.

Discussion/Conclusion: Humans are exposed to nanoparticles via their food yet their effect on microbial bile acid metabolism is not well understood. Producing evidence from assays anchored in a mechanistic understanding will facilitate the risk assessment of food nanoparticles.

15. A pilot study: Recall by genotype and phenotype in a British South Asian population at high risk of cholestatic liver disease

Maria Constantinides (London, GB), Joseph Gafton (London, GB), Ana Cristina Angel Garcia (London, GB), Peter H. Dixon (London, GB), Catherine Williamson (London, GB), Kenneth Linton (London, GB), Upkar S. Gill (London, GB), Sarah Finer (London, GB), Julia Zollner (London, GB); Research Team Genes and Health (London, GB)

Introduction: Cholestatic liver disease disproportionately affects South Asians yet they remain underrepresented in genomic studies. Genes involved in bile formation such as ABCB4 and ABCB11 have been associated with a spectrum of cholestatic liver disease. This recall study aimed to prospectively recall volunteers from a British South Asian genetic Cohort (Genes and Health) that were previously identified to be at high risk of cholestatic liver disease based on their genotype. We hypothesised that high risk genetic variants or a revealed phenotype like intrahepatic cholestasis of pregnancy (ICP) would predispose to cholestatic liver disease and may manifest "silently" prior to symptomatic disease onset.

Methods: Case selection included volunteers with heterozygous loss of function (LOF) variants in ABCB4 and ABCB11 or volunteers with a previous ICP diagnosis (ICD10 O26.6). Cases were matched 1:1 to controls without genetic risk, age, sex, gender, and ethnicity. A detailed medical and family history was taken from participants along with fasting anthropometric and transient elastography (TE) measurements and blood samples for biochemical analysis.

Results: Out of 22 eligible volunteers, 9 (41%) attended the re-call; 5/9 (56%) had ABCB4 LOF; 3/9 (33%) had ABCB11 LOF and 1/9 (11%) had an ICP phenotype. 6/9 (66.7%) were found to have new evidence of a cholestatic phenotype. Of these, 3/6 (50%) experienced abnormal TE (elevated liver stiffness) with 1/3 also demonstrating abnormal ALT, GGT and AST, 2/6 (33.3%) experienced at least 2 cholestatic symptoms and 1/6 (16.7%) demonstrated abnormal ALT, GGT and AST. All 6 cases with new evidence of cholestatic liver disease were consented to and referred to an NHS hepatology clinic.

Discussion/Conclusion: This pilot recall by genotype study demonstrates new evidence of cholestatic liver disease in 66.7% of volunteers. These findings suggest that targeted screening in those at highest genetic risk could provide personalised treatment and prevention of cholestatic liver disease.

16. Chenodeoxycholic acid improves muscle insulin sensitivity

Maria Corliano (Singapore, SG), Roshni Singaraja (Singapore, SG)

Introduction: We previously identified carriers of mutations in CYP8B1, a bile acid (BA) synthesis gene, who showed elevated circulatory chenodeoxycholic acid (CDCA):total BA ratio and increased peripheral insulin sensitivity. In vitro, CDCA increased glucose uptake in human primary myotubes. However, it remains unclear whether CDCA alone increases insulin sensitization and if skeletal muscle-mediated mechanisms contribute in vivo.

Methods: We generated Cyp8b1-/- mice with increased circulatory CDCA.

Results: Mimicking the human carriers of CYP8B1 mutations, these mice showed increased insulin sensitivity. As in the human CYP8B1 mutation carriers, insulin secretion was decreased and hepatic glucose metabolism unchanged, suggesting increased peripheral insulin sensitivity. Muscle insulin signaling was increased in these mice suggesting a mechanism underlying the improved whole body insulin sensitivity. Muricholic acids (MCA), primary BAs in mice but not humans, were also increased in Cyp8b1-/- mice. In mice, Cyp2c70 generates MCAs from CDCA. To exclude contributions by MCAs to insulin sensitivity, we generated Cyp2c70-/- mice with a more human-like bile acid profile of almost absent MCAs and increased CDCA. These mice also showed increased insulin sensitivity, and glucose uptake was specifically increased in muscle by FDG-PET imaging, further suggesting that CDCA increases muscle insulin sensitization. Wild-type mice administered CDCA reproduced these phenotypes, confirming that CDCA increases muscle insulin sensitization. In Cyp8b1-/-, Cyp2c70-/-, and CDCA-administered wild-type mice, muscle Insulin receptor expression increased, as did activity of its transcription factor Forkhead Box O1 (FOXO1).

Discussion/Conclusion: Our data suggest that CDCA signals in skeletal muscle through FOXO1 to increase whole organism insulin sensitization.

17. The secondary bile acid, lithocholic acid, exerts antisecretory effects in colonic epithelial cells in vitro

Caitriona Curley (Dublin, IE), Magdalena Mroz (Dublin, IE), Stephen Keely (Dublin, IE)

Introduction: Bile acids, classically known for their roles in facilitating lipid digestion, are now also appreciated as a family of enterocrine hormones that modulate many aspects of intestinal and metabolic function. We have previously shown lithocholic acid (LCA), a secondary bile acid formed in the colon, to be protective against colonic inflammation. Here, we sought to investigate if LCA might also regulate colonic epithelial fluid and electrolyte transport.

Methods: T84 cell monolayers were mounted in Ussing chambers for measurements of Cl- secretion, the primary driving force for colonic fluid secretion. qRT-PCR and Western blotting were used to analyze mRNA and protein expression. To assess the effects of LCA on CFTR promoter activity, we used a luciferase promoter/reporter system in HEK293. Results were expressed as mean 3 SEM and data were analyzed by one way ANOVA and Tukey's post-hoc test or by mixed-effects analysis and Dunnett's post-hoc test.

Results: Pretreatment of T84 cell monolayers with LCA inhibited subsequent Cl- secretory responses to the cAMP-dependent agonist, forskolin (FSK; 10 M), in a concentration (1 – 10 M) and time-dependent (3–24 hrs) manner. Maximal effects of LCA were observed at a concentration of 10 M after treatment for 24 hrs, when responses to FSK were reduced to 50.9 3 8.5% of those in controls (n = 6; p < 0.01). LCA (10 M; 24 hrs) also inhibited responses to the Ca2+-dependent secretagogues, thapsigargin (2 M) and histamine (100 M), by 59.4 3 2.4% (n = 4; p < 0.001) and 52.2 3 1.9% (n = 5; p < 0.001), respectively. In further experiments, using nystatin-permeabilized T84 monolayers to isolate apical Cl- conductances, LCA (10 M; 24 hrs) reduced FSK-stimulated responses to 72.7 3 6.6% (n = 17; p < 0.001) of those in control cells. Analysis of CFTR expression, the primary exit pathway for Cl- in colonic epithelial cells, revealed that LCA treatment reduced mRNA and protein expression of the channel to 0.65 3 0.05 (n = 7; p < 0.01) and 0.43 3 0.06 (n = 6; p < 0.001) fold of controls, respectively. In CFTR promoter assays, LCA (10 M) reduced CFTR promoter activity to 0.7 3 0.02 fold of that in control cells (n = 5; p < 0.01), with expression of FXR being required for this effect to be observed. Finally, while LCA activated both FXR and the vitamin D receptor (VDR) in T84 cells, its effects in downregulating CFTR expression and Cl- conductances were mimicked by the FXR agonist, GW4064, but not by the VDR agonist, calcitriol.

Discussion/Conclusion: LCA, at physiologically-relevant concentrations, inhibits Cl- secretion across colonic epithelial cells, likely through a mechanism involving FXR activation and inhibition of CFTR expression. These data add to the growing pool of knowledge regarding regulatory actions of LCA in the colon and its potential role as a target for the treatment of intestinal disorders.

18. Multi-omics integration reveals a critical role of LRH-1 in the protection against progression of metabolic dysfunction-associated steatotic liver disease to metabolic dysfunction-associated steatohepatitis

Jan Freark De Boer (Groningen, NL), Wenxuan Zhang (Groningen, NL), Yang Zhang (Groningen, NL), Vincent W. Bloks (Groningen, NL), Carolien Out (Groningen, NL), Michal Pawlak (Lille, FR), Trijnie Bos (Groningen, NL), Rick Havinga (Groningen, NL), Niels L. Mulder (Groningen, NL), Martijn Koehorst (Groningen, NL), Milaine V. Hovingh (Groningen, NL), Alain De Bruin (Utrecht, NL), Joel T. Haas (Lille, FR), Bart Staels (Lille, FR), Rainer P. Bischoff (Groningen, NL), Peter L. Horvatovich (Groningen, NL), Justina C. Wolters (Groningen, NL), Dirk-Jan Reijngoud (Groningen, NL), Albert K. Groen (Amsterdam, NL), Folkert Kuipers (Groningen, NL)

Introduction: Liver receptor homolog-1 (LRH-1/NR5A2) has been identified as a core transcription factor in the liver, orchestrating hepatocyte-specific functions such as bile acid and lipid metabolism. Low hepatic expression of LRH-1 has been reported in metabolic dysfunction-associated steatotic liver disease (MASLD) patients, but whether this is a cause or consequence of disease development is unclear.

Methods: In this study, relationships between low Lrh-1 expression and liver pathology were assessed in conditional whole-body Lrh-1 knockdown (LRH1-KD) mice, fed either regular chow or a high-fat/high-cholesterol/sucrose (HFHCS) diet for 12 weeks, using a multi-omics approach.

Results: Lrh-1 knockdown in chow-fed female mice led to a mild fatty liver phenotype, yet, with a distinct signature of gene and protein expression and lipid composition. Feeding a HFHCS substantially increase hepatic triglyceride contents in wild-type (WT) and LRH-1-KD mice. Intriguingly, LRH-1-KD, but not WT, mice developed features of metabolic dysfunction-associated steatohepatitis (MASH), with marked bridging fibrosis and inflammation. In line, plasma transaminases as well as bile acid levels were increased in HFHSC-fed Lrh1-KD mice compared to controls. Integration of hepatic mRNA, protein and lipid profiles unveiled distinct differences in control of hepatic lipid metabolism between HFHCS-fed LRH-1-KD and WT mice. In addition to the anticipated effects on bile acid synthesis, impaired cholesterol transport and esterification, altered very long-chain fatty acid and phosphatidylcholine synthesis, and increased ceramide biosynthesis were identified, with corresponding changes in hepatic lipid profiles regarding the latter pathways.

Discussion/Conclusion: Our results indicate a pivotal role of LRH-1 in protection of the liver from developing MASH under Western-type diet feeding conditions, supposedly by mediating adequate adaptations of hepatic lipid metabolism to cope with excess dietary nutrients.

19. Elevated plasma bile acids coincide with cardiac stress and inflammation in young Cyp2c70-/- mice

Hilde De Vries (Groningen, NL), Tim Eijgenraam (Groningen, NL), Vincent Bloks (Groningen, NL), Niels Mulder (Groningen, NL), Tim Van Zutphen (Groningen, NL), Herman Sillje (Groningen, NL), Folkert Kuipers (Groningen, NL), Jan Freark De Boer (Groningen, NL)

Introduction: High plasma bile acids (BAs), for instance due to intrahepatic cholestasis of pregnancy or neonatal cholestasis, are associated with cardiac abnormalities. Here, we exploited the variability in plasma BA levels in Cyp2c70-/- mice with a human-like BA composition to investigate the acute effects of elevated circulating BAs on the heart.

Methods: RNA sequencing was performed on hearts of 3-week-old Cyp2c70-/- mice lacking mouse-specific BA species that show features of neonatal cholestasis. Cardiac transcriptomes were compared between wild-type pups, Cyp2c70-/- pups with low or high plasma BAs, and Cyp2c70-/- pups from dams that were perinatally treated with ursodeoxycholic acid (UDCA).

Results: Cyp2c70-/- pups with high plasma BA concentrations showed significantly increased expression of cardiac ventricular gene expression of natriuretic peptide A (Nppa), indicating that the elevated concentrations of human-like BAs caused stress to the heart of these pups. In total, we identified 1355 genes that were differentially expressed in hearts of Cyp2c70-/- mice with high versus low plasma BAs with enrichment of inflammatory processes. Strikingly, expression of 1053 (78%) of those genes was normalized in hearts of pups of UDCA-treated dams. Moreover, 645 cardiac genes strongly correlated to plasma BAs, of which 172 genes were associated with cardiovascular disease.

Discussion/Conclusion: Elevated plasma BAs alter gene expression profiles of hearts of mice with a human-like BA profile, revealing cardiac stress and inflammation. Our findings support the notion that high plasma BAs induce cardiac complications in early life.

20. Gut microbiome composition in women with intrahepatic cholestasis of pregnancy and preeclampsia

Marloes Dekker Nitert (St. Lucia, AU), Sophie Leech (St. Lucia, AU), Emily Dorey (South Brisbane, AU), Helen Barrett (Randwick, AU)

Introduction: Intrahepatic cholestasis of pregnancy (ICP) is an uncommon, serious complication of pregnancy characterised by high bile acids. Bile acid metabolism is complex but is partially regulated by the deconjugation, dihydroxylation, dehydrogenation and epimerisation of primary bile acids by the gut microbiome. Pregnancy is associated with significant changes to the composition of the gut microbiome. In this small study, we compared the gut microbiome of two women with ICP and preeclampsia with the gut microbiota in matched women with and without preeclampsia from a study of the gut microbiome in women with hypertensive disorders of pregnancy.

Methods: A stool sample was collected in the late third trimester from women with ICP treated with ursodeoxycholic acid and preeclampsia (n = 2) and from women matched for age, BMI and gestational age at delivery with (n = 4) and without preeclampsia (n = 5). Microbial DNA was isolated and gut microbiota composition was determined with metagenomic sequencing. Alpha and beta diversity were compared as was the abundance of Bacteroides fragilis, which previously has been reported to be increased in women with ICP.

Results: The groups were similar for pre-pregnancy BMI and Gestational age at delivery but the women with PE had higher blood pressures than the control women ($p < 0.05$). There were no significant differences in the evenness and the richness of the gut microbiome samples between the groups. There was also no distinct grouping of the women with ICP nor of the women with PE but the women in the control group clustered together. In contrast to a previous study, only 3 participants carried B. fragilis in their gut microbiome of whom one had ICP and PE and the two others had PE only whereas none of the women in the control group carried B. fragilis.

Discussion/Conclusion: In summary, in this very small study, there were no differences in the gut microbiomes of women with ICP and PE as compared to women with PE or women with healthy pregnancies. While B. fragilis was only found in the gut microbiomes of women with pregnancy complications, it was not unique for women with ICP indicating the importance of careful matching of pregnant women to take into account other potential modulators of the gut microbiome.

21. Inhibition of adipose triglyceride lipase alleviates NASH in diabetic mice via impaired PPAR-alpha signalling favouring hydrophilic bile acid composition

Emmanuel Dauda Dixon (Vienna, AT), Thierry Claudel (Vienna, AT), Alexander Nardo (Vienna, AT), Claudia Fuchs (Vienna, AT), Veronika Mlitz (Vienna, AT), Alexandra Riva (Vienna, AT), Georg Busslinger (Vienna, AT), Hubert Schnarnagl (Graz, AT), Tatjana Stojakovic (Graz, AT), Joana Silva (Vienna, AT), Gernot Grabner (Graz, AT), Henkjan Verkade (Groningen, NL), Guenter Haemmerle (Graz, AT), Robert Zimmermann (Graz, AT), Michael Trauner (Vienna, AT)

Introduction: Metabolic comorbidities such as diabetes, obesity, and metabolic syndrome play a crucial role in the pathogenesis and progression of metabolic dysfunction associated steatotic liver disease (MASLD). Inhibition of adipose triglyceride lipase (ATGL/PNPLA2) activity in insulin resistance and MASLD is an attractive therapeutic target. This study evaluated the impact of Atglistatin-mediated ATGL inhibition on non-alcoholic steatohepatitis (NASH) development in diabetic and hyperlipidaemic mice.

Methods: Streptozotocin-injected male mice were fed an HFD to induce NASH. Atglistatin (ATGLi) was administered on week 4 for four weeks. We analysed liver histology, hepatic lipid content, immunohistochemistry, RNA sequencing, and serum biochemistry. Mechanistically, we employed a gel shift and luciferase assay system on HepG2 and Hepa 1c1c7 cells. Additionally, we treated Caco2 cells, human primary extrahepatic cholangiocyte organoids (ECO), and HepG2 ATGL KD cells with the human ATGL inhibitor, NG-497, and Ppar agonist WY14643.

Results: Atglistatin reduced body weight (-2.54 g) despite a comparable food intake between the STAM and Atglistatin groups. The bodyweight reduction was due to fat (-40%) and liver mass (-23%) reduction. Atglistatin treatment significantly improved fasting blood sugar (-12%). In line with a trend for reduced ALT (-45%), AP (-25%), total acylglycerol, and cholesterol contents, there was a significant improvement in histological liver injury assessed by H&E staining and NAFLD score (-45%). The NAFLD score revealed improvement of steatosis (-60%) and inflammation (-35%) but not ballooning in the Atglistatin group. Immunochemistry showed that Atglistatin treatment significantly reduces Mac2-positive cells in the liver (-50%) and gonadal white adipose tissues (gWAT) (-60%) compared to the untreated STAM group, indicating a reduced infiltration of immune cells into the liver and gWAT. Mechanistically, the NGS data revealed that Atglistatin reduced Cpt1a, Acox1, and Ppara gene expression, suggesting impaired Ppar signalling that favours hydrophilic bile acids synthesis. In line, Cyp7a1, Cyp27a1, and Cyp2c70 were increased while Cyp8b1 was reduced. Accordingly, Intestinal lipid transporters in the Atglistatin mice, like Abca1 and Cd36, were reduced, consistent with the reduction of liver TAG species, mostly linoleic acids. Because of the lack of efficacy of Atglistatin in humans, we used the novel human-specific ATGL inhibitor NG-497 to validate the in vivo findings. The NG-497-treated Caco2 cells showed reduced ATGL, ABCA1, FATP5, and MTTP mRNA transcripts. PPARA signalling was impaired in ECO treated with NG-497 and HepG2 ATGL KD cells since PPARA, CPT1A, and ABCA1 were reduced, and CYP7A1 increased. Furthermore, gel-shift analysis showed reduced PPARA binding activity in Hepa 1c1c7 and HepG2 cells treated with the Atglistatin

and NG-497 respectively. Luciferase analysis also revealed reduced Ppara transcriptional activity in response to the Atglistatin treatment in Hepa 1c1c7 cells, demonstrating Atglistain-mediated repression of PPARA transcriptional activity.

Discussion/Conclusion: Impaired Ppara signalling translated into hydrophilic BA synthesis that interferes with dietary lipid absorption, improving the metabolic disturbances in the STAM model of NASH associated with diabetes. The validation with the human-specific ATGL inhibitor should open a new clinical avenue for MASLD clinical trial and treatment.

22. First description and analysis of a genetic variant in Semaphorin 7A in a case of intrahepatic cholestasis of pregnancy

Carola Droege (Magdeburg, DE), Malte Sgodda (Hannover, DE), Tobias Cantz (Hannover, DE), David Schoeler (Düsseldorf, DE), Miriam Schoeler (Düsseldorf, DE), Denny Schanze (Magdeburg, DE), Martin Zenker (Magdeburg, DE), Alexandra Bielfeld (Düsseldorf, DE), Percy Balan (Düsseldorf, DE), Tom Luedde (Düsseldorf, DE), Verena Keitel (Magdeburg, DE)

Introduction: Hereditary intrahepatic cholestasis encompasses a spectrum of clinical phenotypes including intrahepatic cholestasis of pregnancy (ICP), low phospholipid-associated cholelithiasis (LPAC) or progressive familial intrahepatic cholestasis (PFIC). First, genetic variants in ATP8B1, ABCB11, and ABCB4 were described to be associated with hereditary intrahepatic cholestasis. Next generation sequencing techniques led to the identification of further cholestasis-related genes.

Methods: Whole exome sequencing (WES) and subsequent virtual panel analysis was performed to test for variants in a patient with a personal and family history of recurrent gallstones, cholecystectomy and acute symptoms of intrahepatic cholestasis of pregnancy (ICP). For further characterization of the identified variant, patient-specific iPSCs were generated from patient's blood.

Results: After recurrent miscarriages, the woman presented in her third pregnancy at week 30 suffering from severe pruritus despite normal serum bile acid levels (7.8 Qmol/l, [< 8.0 Qmol/l]). In the further course, pruritus progressed and urine got dark-colored. Serum bile acids increased to 97.0 Qmol/l at week 36. Ursodeoxycholic acid (UDCA) treatment was initiated immediately. Four days prepartum, bilirubin (2.39 mg/dl [1.0 mg/dl]) and liver enzymes (AST 51 U/l [< 31 U/l], ALT 56 U/l [< 35 U/l], AP 294 U/l [35–104 U/l]) were elevated. Short-term UDCA treatment led to normalization of bilirubin and AST, while ALT and AP decreased to 44 U/l and 216 U/l, respectively. C-section was carried out at 36 + 4 weeks. Two weeks postpartum, liver enzymes normalized, despite slightly elevated AP (123 U/l). WES revealed a heterozygous in frame duplication p.(Arg32_Leu33dup) in the Semaphorin 7A (SEMA7A) gene. A homozygous missense variant was described by Pan et al. in PFIC.

Discussion/Conclusion: So far, no ICP-related SEMA7A variant is known in literature and the impact of the detected SEMA7A variant on ICP and gallstone disease needs to be elucidated. Therefore, the patient is included in the HiChol registry and patient-derived iPSC-based organoids are currently used for functional analysis regarding hepatobiliary secretion.

23. Histological, molecular, and neuro-functional skin assessment reveals distinct changes in patients with pruritus in cholestatic liver diseases

Miriam M. Duell (Erlangen, DE), Hannah Kleinlein (Erlangen, DE), Victoria Leibl (Erlangen, DE), Konstantin Agelopoulos (Münster, DE), Peter Dietrich (Erlangen, DE), Marcel Vetter (Erlangen, DE), Markus F. Neurath (Erlangen, DE), Sonja Staender (Münster, DE), Aysenur Sueer (Münster, DE), Martin Schmelz (Mannheim, DE), Roberto Pellicciari (Corciano, Perugia, IT), Francesca De Franco (Corciano, Perugia, IT), Barbara Namer (Erlangen/Aachen, DE), Andreas E. Kremer (Zürich/Erlangen, CH)

Introduction: Chronic pruritus is common in cholestatic liver diseases and remains a major unmet clinical need. The interplay between cutaneous sensory C-nerve fibers and potential chemical mediators including bile acids is incompletely understood. This study assessed skin biopsies for histological neuroanatomy, bile acid content as well as tested sensory nerve fiber function in patients with cholestatic liver disorders with and without pruritus.

Methods: We included 59 clinically well-characterized patients with cholestatic liver diseases (PBC, PSC, other causes of cholestasis). Pruritus was evaluated with questionnaires including a numeric rating scale (NRS). Patients underwent a transcutaneous electrical stimulation protocol using sine-wave stimuli, which selectively activate C-fibers in the skin. Skin punch biopsies at the forearm were analyzed for neuro-histology (intra-epidermal nerve fiber density; IENFD) and quantification of bile acid sub-species via UPLC-ESI-MS.

Results: Patients were divided into those with significant itching (defined as mean itch rating of NRS ≥ 3; pruritus high) and those without clinically significant itching (NRS < 3; pruritus low). Both patient groups were comparable regarding age, liver and kidney function tests, disease entity and stage of liver disease including proportion of patients with liver cirrhosis. Electrical sine-wave stimulation applied to the skin caused a dose-dependent itch sensation in the high pruritus group, while low-pruritus patients characterized the sensation as painful. In skin biopsies, the mean IENFD was strongly reduced ($p < 0.001$) in patients with cholestatic liver diseases compared to age- and gender-matched healthy controls, with an even lower tendency in the pruritus high group. Quantification of bile-acid subspecies was successfully established and performed in a sub-set of skin samples, now rendering further analyses and correlation to functional and histological findings.

Discussion/Conclusion: Patients with chronic cholestatic pruritus exhibit neuro-anatomical and -functional changes of the skin, warranting further investigation into the interplay with potential chemical mediators of pruritus.

24. Altered urinary bile acid profile is associated with renal dysfunction in patients with liver cirrhosis and ascites

Hayam El Aggan (Alexandria, EG), Sabah Mahmoud (Alexandria, EG), Samia Sharaf (Alexandria, EG)

Introduction: Various renal diseases are accompanied by alterations in bile acid (BA) concentrations. Renal disease may impact BA metabolism, while changes in the secretion of BAs can exacerbate renal disease. The present study was designed to evaluate urinary levels of BAs in patients with liver cirrhosis and ascites in relation to renal dysfunction.

Methods: Thirty patients with liver cirrhosis (14 patients without ascites and 16 patients with ascites) were prospectively studied and compared with 15 healthy subjects. The severity of liver disease was assessed by the Child-Pugh classification, Model for End Stage Liver

Disease Sodium (MELDNa) score and albumin-bilirubin (ALBI) score. Renal function was evaluated by serum creatinine and estimated glomerular filtration rate (eGFR). Urinary 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 and were expressed as BAs to creatinine ratio (mol per gram creatinine).

Results: The urinary levels of total BAs, free CA, GCA, TCA, and TGCA were significantly higher in patients with liver cirrhosis than in healthy subjects and in patients with ascites than in patients without ascites ($p < 0.001$). About 50% of urinary total BAs was composed of the sulphated fraction TGCA in patients with liver cirrhosis regardless of the presence of ascites. The total and individual BAs were positively correlated with serum albumin concentration, serum creatinine levels, and Child-Pugh, MELDNa, and ALBI scores and were negatively correlated with eGFR ($p < 0.05$).

Discussion/Conclusion: Liver cirrhosis is associated with altered urinary bile acid profile particularly in the presence of ascites, which may contribute to renal dysfunction. Renal clearance of sulfated fraction of BAs is greater than the clearance of non-sulfated BAs.

25. Increased serum bile acid levels correlate with systemic inflammatory indexes and severity of liver dysfunction in patients with liver cirrhosis

Hoda El Aggan (Alexandria, EG), Sabah Mahmoud (Alexandria, EG), Samia Sharaf (Alexandria, EG)

Introduction: Disturbances in bile acid metabolism play a major role in liver disease and may be involved in the inflammatory response. The present study was designed to evaluate serum levels of bile acids (BAs) in patients with liver cirrhosis and their correlation with systemic inflammatory indexes and severity of hepatic dysfunction.

Methods: Bile acids (BAs) in fasting serum samples were quantified in 30 patients with liver cirrhosis and 15 healthy subjects. 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. The severity of hepatic dysfunction was assessed by the Child-Pugh classification, Model for End Stage Liver Disease 3.0 (MELD 3.0), and albumin-based liver reserve models [albumin-bilirubin (ALBI), easy (EZ)-ALBI, platelet-albumin-bilirubin (PALBI), and platelet-albumin (PAL) scores]. Systemic inflammatory indexes [neutrophil-to-lymphocyte ratio (NLR), platelet-to-lymphocyte ratio (PLR), systemic immune-inflammatory index (SII), and systemic inflammation response index (SIRI)] were calculated.

Results: Patients with liver cirrhosis showed significant increases in serum total BAs, free CA, GCA, TCA, and TGCA and a significant decrease in GCA/TCA ratio compared with healthy subjects ($p < 0.01$). As percentages of total BAs, the proportions of free CA and TGCA were significantly higher than those of GCA and TCA in patients with liver cirrhosis while the proportion of GCA was significantly higher than those of TCA and TGCA in healthy subjects ($p < 0.01$). Serum levels of total and individual BAs were positively correlated with Child-Pugh score, MELD 3.0 score, ALBI score, PAL, NLR, and SIRI ($p < 0.05$).

Discussion/Conclusion: Increased serum BA levels in liver cirrhosis are linked to hepatic dysfunction and are associated with systemic inflammation. The usefulness of serum BAs as potential biomarkers for monitoring liver disease progression need to be validated in large-population trials.

26. Primary biliary cholangitis – Evidence of familial susceptibility and associations with autoimmune disorders and malignancies

Toader Elena (Iasi, RO), Iacob Andreea (Iasi, RO), Filioreanu Magda (Iasi, RO), Tila George (Iasi, RO), Piscuc Mirela (Iasi, RO)

Introduction: Primary biliary cholangitis (PBC), a chronic progressive cholestatic liver disease characterized by the destruction of small intrahepatic bile ducts, is considered a complex phenomenon combining polygenic traits, immune and environmental factors. The genetic background of PBC is supported by high rates of familial PBC occurrence, the presence of antimitochondrial antibodies (AMA), and the coexistence of autoimmune diseases and/or other autoantibodies in first-degree relatives.

Methods: We present the case of a family with three sisters and one brother, in which four individuals were diagnosed with symptomatic and asymptomatic PBC, positive AMA, elevated transaminase levels, IgM, IgG, autoimmune diseases, and malignancies. Following the index patient's diagnosis at 63 years – the mother with PBC in cirrhosis stage – three sisters were diagnosed with PBC (middle-aged).

Results: One sister diagnosed with PBC at 42 years initially presented with acute hepatitis associated with insulin-dependent diabetes since the age of 22 years, another sister with asymptomatic PBC at 40 years but with cholestasis and hepatocytolysis syndrome, positive AMA, F0; a sister at 48 years with positive AMA, a history of rheumatoid arthritis, Sjogren's syndrome, renal carcinoma associated with isolated elevation of cholestatic liver enzymes suggestive of the Stauffer syndrome. On the paternal lineage, the father diagnosed with intestinal GIST, hepatic metastases, without clinical or laboratory evidence of PBC, deceased at 67 years, and the brother with normal clinical and laboratory status. Except for the mother with advanced PBC, deceased at 68 years, the clinical, imaging, and laboratory evolutions of PBC in the daughters' cases were favorable under treatment with ursodeoxycholic acid.

Discussion/Conclusion: Our case indicating familial clustering of the disease suggests the possibility of maternal inheritance of PBC susceptibility. For the early detection and management of PBC in families of index patients with PBC, screening with AMA and liver tests, especially for first-degree female relatives, is recommended.

27. Dietary intervention alters the bile acid and gut microbiome profiles in a hepatocellular carcinoma model

Merve Erdem (Aachen, DE), Sraddha Bharadwaj (Aachen, DE), Ana Maria Izcue (Aachen, DE), Frank Schaap (Maastricht, NL), Eray Sahin (Istanbul, TR), Thorsten Cramer (Aachen, DE)

Introduction: Hepatocellular carcinoma (HCC) is characterized by strong therapy resistance and poor prognosis. Dietary approaches and modifications are actively investigated regarding their impact on both tumor metabolism and the host. In previous studies, we have observed the inhibitory effect of different diets on HCC progression and diet-induced changes in the tumor immune microenvironment. In this part of the study, we have focused on the systemic effects of various diet treatments in HCC, including changes in bile acid (BA) profile and gut microbiome.

Methods: In our study, we used a transgenic mouse model of HCC, known as ASV-B. In this model, mice develop HCC through the T oncogene of SV40 in the liver. ASV-B mice were subjected to two different diet regimens: a "low carb high fat" (LCHF, ketogenic diet) and a "low carb high protein" (LCHP) chow. The effects of these diet approaches on the composition of BAs in blood (via LC-MS) and gut microbiome (via 16S rRNA sequencing) were analyzed.

Results: Total bile salt levels in ASV-B mice sera samples were comparable in control and diet-treated groups. Diet application however altered the BA composition, resulting in an increased primary to secondary BA ratio. This increase may be attributed to changes in the intestinal microbiome profile, the diversity of which was drastically reduced in both LCHF and LCHP diets. In the microbiome, Firmicutes-to-Bacteriodetes ratio decreased significantly in both diets, while Compilobacterota and Proteobacteria increased in ketogenic diet. In the serum, taurine-conjugated muricholic acid forms (a-, b- and wMCA) were the dominant BAs in the profile of all groups, with the majorly affected BAs by the diet were: TwMCA, cholic acid, glycocholic acid and wMCA with significantly decreased levels in both diets. Additionally, HCC-associated bile acids (deoxycholic acid, chenodeoxycholic acid, lithocholic acid, and taurachenodeoxycholic acid) were lower after the LCHF diet, whereas liver cytoprotective Bas, ursodeoxycholic acid and its taurine-conjugated form, were increased in only LCHP-applied mice.

Discussion/Conclusion: Changes in the gut microbiome composition can affect bile acid homeostasis and signaling. While the initial impact of diet-induced changes on the gut microbiome or bile acids remains ambiguous, their bidirectional relationship likely constitutes a crucial aspect of the tumor inhibitory effects, especially of the LCHF diet. Both dietary interventions show tumor inhibition in the liver, potentially providing a promising adjuvant therapy approach, and taken together with these results, the underlying mechanisms of this inhibition are likely diverse in across different diet types.

28. Identification of new correctors for traffic-defective ABCB4 variants by a high-content screening approach

Thomas Falguieres (Orsay, FR), Mounia Lakli (Orsay, FR), Julie Dumont (Lille, FR), Virginie Vauthier (Paris, FR), Julie Charton (Lille, FR), Veronica Crespi (Limoges, FR), Manon Banet (Orsay, FR), Yosra Riahi (Orsay, FR), Amel Ben Saad (Orsay, FR), Elodie Mareux (Orsay, FR), Martine Lapalus (Orsay, FR), Emmanuel Gonzales (Le Kremlin-Bicêtre, FR), Emmanuel Jacquemin (Le Kremlin-Bicêtre, FR), Florent Di Meo (Limoges, FR), Benoit Deprez (Lille, FR), Florence Leroux (Lille, FR)

Introduction: ABCB4 is an ATP-binding cassette (ABC) transporter located at the canalicular membrane of hepatocytes and responsible for the secretion of phosphatidylcholine into bile. Genetic variations of this transporter are correlated with rare cholestatic liver diseases, the most severe being progressive familial intrahepatic cholestasis type 3 (PFIC3). PFIC3 patients most often require liver transplantation. In this context of unmet medical need, we developed a high-content screening approach to identify small molecules of interest able to correct ABCB4 molecular defects.

Methods: High-content screening was developed to identify small molecule correctors of a tagged defective ABCB4. Intracellularly-retained variants of ABCB4 were expressed in cell models and their maturation, cellular localization and function were analyzed after treatment with the molecules identified by high-content screening. In addition, we performed in silico molecular docking calculations to describe the possible interaction of the new drug candidates with ABCB4.

Results: In total, six hits were identified by high-content screening. Three of them were able to correct the maturation and canalicular localization of two distinct intracellularly-retained ABCB4 variants; one molecule was able to significantly restore the function of two ABCB4 variants. In silico molecular docking calculations suggest that the hits may interact with ABCB4 residues involved in ATP binding/hydrolysis.

Discussion/Conclusion: We identified new compounds as correctors for intracellularly-retained ABCB4 variants. Our results pave the way for their optimization in order to provide new drug candidates as potential alternative to liver transplantation for patients with severe forms of ABCB4-related diseases, including PFIC3.

29. The bile acid receptor Tgr5 and alcohol consumption

Jessica Ferrell (Rootstown, US), Shannon Boehme (Rootstown, US), Matthew Dilts (Rootstown, US), Sabita Pokhrel (Rootstown, US), Shifat-E Ferdous (Rootstown, US), Titilayo Olotu (Rootstown, US)

Introduction: Both alcohol and high fat diet consumption can lead to steatosis, which can progress to serious liver disease. Bile acids are made in the liver and aid in nutrient absorption. They also regulate metabolism by binding to bile acid receptors, including the G protein-coupled receptor Tgr5 expressed in the liver, gut, adipose tissue, and brain. Bile acid-binding to Tgr5 potentiates insulin secretion and regulates energy metabolism. Our previous research indicates Tgr5-/- mice were protected from alcohol-induced liver damage, possibly due to altered fibroblast growth factor 21 signaling. This master metabolic regulator mediates the consumption of ethanol; we therefore aimed to determine if the bile acid receptor Tgr5 plays a role in the regulation of ethanol consumption.

Methods: Female C57BL/6J wild type mice (WT) and Tgr5-/- mice were fed a high fat (40% kcal), high fructose diet (HFS) plus 10% ethanol in drinking water for 20 weeks. Separate cohorts of mice were subjected to a 2-bottle choice paradigm to determine ethanol consumption (10% v/v in water) and preference with oral gavage of the specific Tgr5 agonist BAR-501 (30 mg/kg/day) for 2 weeks. Metabolic phenotypes were characterized through examination of bile acid metabolism in the liver and metabolic feedback pathways in liver, adipose tissue, and brain.

Results: Female Tgr5-/- mice fed HFS diet + ethanol gained weight similarly to WT mice and food intake was not significantly different. However, Tgr5-/- mice surprisingly consumed less ethanol than their WT counterparts, which likely contributed to the observed phenotype: Tgr5-/- mice had significantly less body fat and greater lean mass after 20 weeks HFS diet + ethanol. Tgr5-/- mice consuming ethanol also had reduced serum ALT and AST, reduced serum free fatty acids and serum bile acids, and reduced bile acids and Sirius red positive staining in liver. Cyp7a1 expression was significantly reduced by ethanol in both groups of mice, indicating potential dysregulation of the biliary system in response to ethanol. Expression of lipolysis genes in brown adipose (Cpt1a, Pnpla2) were upregulated in Tgr5-/- mice and downregulated with ethanol. To determine a true reduced preference for ethanol, mice were subjected to a 2-bottle choice experiment, which revealed Tgr5-/- mice voluntarily drink less ethanol without reduction in water consumption. These mice also exhibited non-significantly elevated Fgf21 expression in liver, indicating regulation of ethanol consumption may be independent of Fgf21 feedback. Hypothalamic mRNA expression of glucose transport genes (Glut1, Glut2, Glut3), TCA cycle genes (Sdha, Mdh1/2) and metabolic mediators of leptin homeostasis (Agrp, Npy) were significantly reduced in Tgr5-/- mice drinking ethanol. Finally, gavage of the Tgr5 agonist BAR-501 in female WT mice increased voluntary ethanol consumption as determined by 2-bottle choice, overall indicating a potential role for Tgr5 in the gut-brain crosstalk that regulates ethanol consumption and its use as a fuel.

Discussion/Conclusion: The bile acid receptor Tgr5 may regulate ethanol consumption and its use as a metabolic fuel.

30. Elevated serum bile acids in MASH patients with fibrosis in the context of a cholestatic genetic predisposition

Andreas Geier (Würzburg, DE), Monika Rau (Würzburg, DE), Theresa Schmitt (Würzburg, DE), Marcin Krawczyk (Homburg, DE), Dieter Luetjohann (Bonn, DE), Maria J. Monte (Salamanca, ES), Jose Marin (Salamanca, ES)

Introduction: Drug development in MASLD patients show promising results for bile acid (BA) based therapeutic approaches. Activation of BA receptors appears to be protective against MASLD progression, but their retention could also promote liver injury. The genetic variant V444A (1331T>C) of the bile-salt export pump (BSEP/ABCB11) as the most frequent pro-cholestatic polymorphism represents a genetic predisposition for BA retention under pathologic conditions and may render carriers more susceptible to cholestatic insults.

Aim: To analyse the relationship between serum BAs and BSEP-dependent genetic susceptibility in MASLD patients.

Methods: In total, 70 MASL, 124 MASH patients and 165 clinically diagnosed (Fibroscan, CAP) MASLD patients were included in this study. Histological steatosis, inflammation, ballooning and fibrosis scoring was available for 70 MASL and 124 MASH patients. The ABCB11 1331T>C variant was genotyped in all patients using TaqMan assays. Serum BAs were analysed by mass-spectrometry in 33 NAFL, 58 NASH, 146 clinically diagnosed NAFLD patients.

Results: Serum BA levels were significantly higher in MASH patients compared to MASL (2.64 3 2.24 vs. 1.77 3 1.63 QM, p = 0.02) although within the normal range. No significant association was observed between serum BAs and steatosis, inflammation or ballooning in liver histology. Analysis of BA profile showed higher levels of primary (1.60 3 1.35 vs. 0.93 3 1.1 QM, p = 0.013) and secondary BAs (1.09 3 1.07 vs. 0.75 3 0.83 QM, p = 0.035), particularly cholic acid (CA) (0.57 3 0.48 vs. 0.40 3 0.36 QM, p = 0.044) und chenodeoxycholic acid (CDCA) (0.98 3 0.91 vs. 0.61 3 0.78 QM, p = 0.017), in MASH patients compared to MASL. Histology proven MASLD patients with advanced disease (F3/F4) had significantly higher BAs compared to those with F0–F2 (3.05 3 2.38 vs. 2.27 3 1.76, p = 0.023). In agreement, MASLD patients with elevated BAs > 10 QM had significantly higher liver stiffness values compared to those with BAs < 10 QM (41.1 3 28.6 vs. 8.4 3 9.1 kPa, p < 0.001). Clinically, elevated BAs > 10 QM were significantly associated with coronary heart disease (p = 0.002). A trend towards lower CAP values as a measure of steatosis was observed in MASLD patients with BAs > 10 QM (307.0 3 83 vs. 350.5 3 81 dB/m, p = 0.064). No significant elevation in serum BAs was observed in ABCB11 1331 wildtype TT compared to heterozygous CT or homozygous CC variant carriers (1.90 3 1.02 vs. 2.36 3 2.01, p = 0.294). However, a significant correlation between serum BAs and liver stiffness was observed for CT_CC patients (R = 0.261, p < 0.05), but not for TT patients (R = 0.104, p > 0.05). In line, significantly higher liver stiffness was observed for CC patients compared to TT_CT (10.00 3 11.30 vs. 7.80 3 6.80, p = 0.049). No difference in steatosis degree could be detected for any ABCB11 genotype.

Discussion/Conclusion: The presence of MASH and advanced fibrosis are associated with higher serum BAs indicating the relevance of inflammation and/or tissue damage for systemic BA levels. Genetic susceptibility at ABCB11 1331T>C may become functionally relevant as a potential pathogenetic co-factor of fibrosis progression as the degree of liver stiffness elevation correlates with this variant in MASLD patients. Elevated BA levels and respective genetic predisposition play no detectable role on steatosis per se. Whether the ABCB11 1331T>C variant affects systemic and intrahepatic BA levels under BA based therapy – particularly in those with advanced fibrosis - remains to be elucidated.

31. A gut microbiota-independent mechanism shapes the bile acid pool in mice with metabolic dysfunction-associated steatohepatitis

Justine Gillard (Brussels, BE), Martin Roumain (Brussels, BE), Corinne Picalausa (Brussels, BE), Morgane Thibaut (Brussels, BE), Anne Tailleux (Lille, FR), Bart Staels (Lille, FR), Giulio Muccioli (Brussels, BE), Laure Bindels (Brussels, BE), Isabelle Leclercq (Brussels, BE)

Introduction: An imbalance between 7alpha-hydroxylated and 7alpha-dehydroxylated bile acids contributes to the development of metabolic dysfunction-associated steatohepatitis (MASH) (1,2). However, the precise mechanisms underpinning changes in the bile acid pool in MASH have yet to be elucidated. Given that gut bacteria convert 7alpha-hydroxylated bile acids to 7alpha-dehydroxylated bile acids, we investigated the contribution of the gut microbiota and its metabolizing activities to bile acid alterations in MASH.

Results: To distangle the influence of MASH from environmental and dietary factors, high fat diet-fed foz/foz mice were compared to their wildtype littermates fed the same diet. While mice with MASH exhibited lower levels of 7alpha-dehydroxylated bile acids (22 3 9% vs. 34 3 7% of the portal bile acids in controls, p = 0.019), the caecal microbial composition was similar in mice with and without MASH, as assessed by 16S rRNA gene sequencing (3). We developed functional assays to extend the analyses beyond a mere study of gut microbiota composition. Unexpectedly, similar bile salt hydrolase and 7alpha-dehydroxylating activities could not explain the low levels of 7alpha-dehydroxylated bile acids (3). By exploring alternative mechanisms, we identified an increased bile acid 7alpha-rehydroxylation mediated by liver CYP enzymes, that reduces 7alpha-dehydroxylated bile acid levels in MASH (3). Key findings were confirmed in an independent mouse model of MASH (i.e. C57BL/6J fed a western and high fructose diet).

Discussion/Conclusion: In summary, our study reveals a gut microbiota-independent mechanism that regulates 7alpha-dehydroxylated bile acid levels in mice, challenging the conventional notion that the gut microbiota is the exclusive regulator of the balance between 7alpha-hydroxylated and 7alpha-dehydroxylated bile acids. Moreover, this study provides an explanation for the low levels of 7alpha-dehydroxylated bile acids in MASH, opening avenues for innovative therapeutic opportunities.

References:

(1) Gillard et al. JHEP Reports. 2021
(2) Gillard and Leclercq. Clin Sci. 2023
(3) Gillard et al. JHEP Reports. 2024

32. Bile salts exert immunosuppressive effects on macrophages in vitro independent of TGR5 and FXR receptor activation

Claire Groenen (Amsterdam, NL), Reinout Roscam Abbing (Amsterdam, NL), Kristina Schoonjans (Lausanne, CH), Manon Wildenberg (Amsterdam, NL), Coen Paulusma (Amsterdam, NL), Stan Van de Graaf (Amsterdam, NL)

Introduction: Bile salts are recognized as potent signaling molecules exerting downstream effects on metabolism and inflammation. Activation of bile salt sensing proteins including Takeda G-protein-coupled receptor 5 (TGR5) and Farnesoid X receptor (FXR) has previously been associated with inhibition of inflammation. Here, we investigated the direct effects of the bile salt taurochenodeoxycholic acid (TCDCA) on inflammatory cytokine production in bone marrow derived macrophages (BMDM) and the dependency of potential effects on TGR5 and/or FXR activation.

Methods: BMDMs from wild type (WT), TGR5-/-, and FXR-/- mice were stimulated with LPS and ATP and simultaneously incubated with TCDCA. mRNA expression and protein levels of TNFα, IL-1β, and IL-10 were determined by qPCR and ELISA. Finally, a bile salt uptake assay was performed to determine whether TCDCA enters macrophages and a caspase activity assay was performed to assess potential bile salt toxicity.

Results: The administration of TCDCA in LPS-stimulated WT BMDMs reduced the expression of TNFα and IL-1β, while IL-10 expression was unaffected. TNFα and IL-1β concentrations were reduced, while an increase was observed for IL-10 in BMDM WT supernatant by TCDCA. TCDCA significantly reduced the concentration of TNFα and IL-1β in the medium of TGR5-/- and FXR-/- BMDMs. TCDCA administration led to a significant increase in IL-10 in the medium in FXR-/- BMDMs and a similar trend was observed in TGR5-/- BMDMs. BMDMs do not actively take up bile salts and can handle high concentrations of bile salts without going into apoptosis.

Discussion/Conclusion: Bile salts exert immunomodulatory actions on BMDMs in vitro independent of TGR5 and FXR activation. Our findings suggest the potential for targeting of bile salt signaling as a treatment for inflammatory disorders. Further research is warranted to identify other bile salt receptors that can explain the observed TGR5- and FXR-independent immunomodulatory effects of bile salts.

33. Prevalence of metabolic syndrome and treatment response with ursodeoxycholic acid in primary biliary cholangitis: A single-center study in Latin America

Rodrigo Guirao (Mexico City, MX), Scherezada Mejia (Mexico City, MX), Rosario Herrero (Mexico City, MX)

Introduction: Metabolic risk factors and the prevalence of metabolic syndrome in primary biliary cholangitis (PBC) have not been adequately described in the Latin American population and their implications on treatment response are unknown. In this study, we aim to determine the prevalence of metabolic syndrome and determine the frequency of treatment response.

Methods: We retrospectively studied a Mexican population of 80 patients with PBC between January 2019 and December 2023, determining the presence of metabolic syndrome according to the criteria established by the Adult Treatment Panel III (ATP III), and evaluated the response one year after the start of treatment according to the standardized dose with ursodeoxycholic using the Barcelona Scoring System.

Results: 80 patients, 72 women (90%) and 11 men (10%) with a mean age at diagnosis of 53 years. 57 (71.25%) responded to treatment and 23 (28.75%) did not respond. Metabolic syndrome was diagnosed in 13, representing 16.25%, of which 92.3% did not respond to treatment (12 patients). Obesity was determined in 19 patients (23.75%) of which 57.9% were non-responders, 25 were overweight (16 responders and 9 non-responders) and 36 patients with normal BMI (33 responders and 3 non-responders).

Discussion/Conclusion: Except for one, patients with metabolic syndrome did not respond to treatment with ursodeoxycholic acid. When divided into groups according to BMI, the highest response rate was in the group with normal BMI and the lowest in the obesity group. An intentional search for metabolic syndrome in this population and multidisciplinary management are required to achieve adequate control of metabolic risk factors.

34. Serological characterization and associated autoimmune diseases in a Latin American population with primary biliary cholangitis

Rodrigo Guirao (Mexico City, MX), Scherezada Mejia (Mexico City, MX), Rosario Herrero (Mexico City, MX), Eumir Juarez (Mexico City, MX), Anel Avila (Mexico City, MX)

Introduction: Primary biliary cholangitis is a prototypical autoimmune disease that leads to cholestasis and cirrhosis if untreated. In our population, there is a lack of epidemiological studies that determine the main autoimmune and serological associations. This study aims to determine this lack of information in a Mexican population.

Methods: A population of 104 patients was retrospectively studied between February 2018 and January 2024, determining the presence of other autoimmune diseases and plasma analysis of autoantibodies: antimitochondrial (AMAs), anti-smooth muscle (ASMAs) and antinuclear (ANAs), as well as immunoglobulins determined by immunofluorescence.

Results: A total of 104 patients were included, 93 women (89.4%) and 11 men (10.5%) with an average age at diagnosis of 54.1. 50 (48%) patients presented associated autoimmune diseases, in 35 cases only one autoimmune disease was determined, 10 presented 2, 3 presented 3 associations and 2 patients presented 4. In order of frequency: hypothyroidism in 27 (25.9%), Sjögren's syndrome in 18 (17.3%), systemic sclerosis in 10 (9.6%), rheumatoid arthritis in 7 (6.73%), systemic lupus erythematosus in 3. 94 patients (90.3%) presented positive AMAs and 10 were negative for AMAs, anti-gp210 and anti-sp100 who underwent liver biopsy. AMAs in 32 patients (30.7%) and 96 were positive for ANAs (92.3%). 60% had high titers of immunoglobulin (Ig) G, 46.2% of IgM and 20% of IgA.

Discussion/Conclusion: Our study provides the best evidence of association of autoimmune diseases reported in Mexico and the high frequency of ANAs in this population. Despite not being one of the main objectives of the study, it was determined that patients who had adequate control of the associated autoimmune diseases (by activity scales and/or euthyroidism) had an adequate response to treatment with ursodeoxycholic acid, for this reason it is necessary to continue studying these findings.

35. Measuring bile acids: The "touches of sweet harmony"

Bill Hague (North Adelaide, AU), Suzette Coat (Adelaide, AU), Markus Corey (Bedford Park, AU)

Introduction: Assay standardisation is underappreciated in relation to clinical trials, particularly when patient results come from different laboratories. The Bile Acid Comparison and Harmonisation (BACH) study was established as a sub study of the international Trial of URsodeoxycholic acid vs. RIFampicin in severe early onset Intrahepatic Cholestasis of pregnancy (TURRIFIC).

Methods: Fifteen laboratories providing bile acid (BA) measurements for TURRIFIC were invited to participate after completing a methodological questionnaire. Pooled serum comparison samples were manufactured, some spiked with individual BA species. Harmonisation was investigated with regression-based approaches using the All-Procedure Median (APM) as the consensus value.

Results: All 15 participating laboratories responded to the questionnaire: each assayed 60 comparison samples. At least two quality-control (QC) samples were tested; despite some inappropriately low QC target concentrations, precision was satisfactory. Results from one reagent displayed minor assay non-linearity. Harmonisation decreased between-laboratory variability by 62%, most results falling within Royal College of Pathologists of Australia (RCPA) analytical performance specifications. At clinically relevant harmonised values (40

Qmol/l – increased risk of spontaneous preterm birth and neonatal asphyxia, and 100 Qmol/l – increased risk of stillbirth), estimated values were 35–46 Qmol/l and 88–110 Qmol/l respectively. Samples spiked with glycodeoxycholic, chenodeoxycholic and cholic acids showed between-result variability similar to that of native serum samples, unlike serum spiked with lithocholic acid.

Discussion/Conclusion: Harmonisation of BA assays is achievable, with feasibility demonstrated in silico and with native patient samples. Standardisation with a universal calibrator, e.g., glycodeoxycholic acid, combined with mass-spectrometry, is the preferred approach; however, harmonisation with a serum sample reference panel is also viable. Reagent manufacturers need encouragement to improve post-market surveillance, addressing non-linearity, inappropriate calibrator target setting and commutability.

Meta-analysis of previous studies using harmonised units may allow re-evaluation of the effect sizes.

36. The role of tissue-restricted FXR deletion in MASH development in mice

Zakiyah Henry (Piscataway, US), Syeda Maliha (Piscataway, US), Bo Kong (Piscataway, US), Grace Guo (Piscataway, US)

Introduction: Metabolic dysfunction associated steatohepatitis (MASH) is a chronic liver condition characterized by steatosis, inflammation, hepatocyte ballooning, and varying degrees of fibrosis. If not resolved, cirrhosis or hepatocellular carcinoma can develop. Currently, MASH-induced cirrhosis is the leading indicator for liver transplantation in the US and its prevalence is rapidly rising. Treatment is limited as there is only one FDA approved therapeutic; however, an emerging target is the Farnesoid X Receptor (FXR). FXR is a type II nuclear receptor and ligand-activated transcription factor that is found within various cell types and organs, but primarily in the liver and intestine. FXR is the major homeostatic regulator of bile acids (BAs) which have been implicated in the pathogenesis of MASH. Because FXR negatively regulates BA production along with mitigating MASH effects, synthetic ligands that are global FXR agonists have been developed to treat MASH. Although beneficial, these whole-body modulators contribute to unfavorable side effects such as cholesterol homeostasis imbalance and pruritus, thereby explaining the importance of FXR tissue- or cell-restricted activation in the development of novel therapeutics for MASH to negate any potential harmful consequences and off-target effects. The objective of this study was to determine the tissue-specific role of FXR in MASH development in mice.

Methods: For this study, six to eight-week old male and female mice in 4 genotypes: Wild-Type (WT, C57BL/6J, Fxr+/+), Liver FXR KO ((Fxrfloxed/floxed, Albumin Cre (+), Fxr LKO), Intestinal FXR KO (Fxrfloxed/floxed, Villin Cre (+), Fxr IKO), and Whole Body FXR KO (WB FXR KO, Fxr -/-), were fed either a low-fat control diet or a MASH “Fast Food” (FF) diet (Western diet with 21% milk fat, 1.25% cholesterol, and 34% sucrose) for 16 weeks.

Results: We discovered that Fxr-/- and Fxr LKO were more resistant to weight gain for both sexes, in comparison to the WT and Fxr IKO mice. Regarding the female mice, serum levels of alanine aminotransferase (ALT), alkaline phosphatase (ALP), total cholesterol, triglycerides, and total bile acids were all elevated in Fxr LKO mice. Fxr IKO displayed lower values for each serum parameter, compared to all other groups, including the WT. The male Fxr LKO mice displayed significantly increased serum lipids compared to Fxr IKO and surprisingly no significant changes were observed in the injury markers among all FXR deficient mice. In females, expression of genes involved in inflammation and fibrosis were significantly increased in LKO mice fed the FF diet compared to IKO. A concrete trend was not seen in males.

Discussion/Conclusion: In conclusion, hepatic, but not intestinal, deficiency of FXR is associated with more severe liver injury suggesting that hepatic FXR is more critical in suppressing liver injury during MASH development in females. This same trend is not clear in the male cohorts, highlighting sex-differences and potential roles that sexual dimorphism may play in MASH development overall as it relates to liver injury.

37. Characterization of the cellular expression pattern of bile acid receptors in intrahepatic cholangiocellular carcinoma

Niklas Heucke (Magdeburg, DE), Maria Reich (Magdeburg, DE), Adrien Guillot (Berlin, DE), Frank Tacke (Berlin, DE), Verena Keitel-Anselmino (Magdeburg, DE)

Introduction: Cholangiocellular carcinoma (CCA) arises from the epithelial cells of the bile ducts and can be classified according to its location along the biliary tree as intrahepatic (iCCA), perihilar or distal. Each subtype has a distinct epidemiology, biology, prognosis, and strategy for clinical management.

Pathogenesis remains elusive, and only recently, bile acids as potent signalling molecules have been linked to CCA development. Nevertheless, the spatial expression pattern of bile acid receptors in human CCA is unknown. However, this knowledge could be the cornerstone of potential new bile acid receptor-associated therapeutic strategies.

Methods: In a cohort of 35 iCCA patients, the expression pattern of bile acid receptors was studied using quantitative reverse transcription PCR (RT-qPCR) as well as multiplex immunostaining. This technique is a sequential immunostaining protocol consisting of repetitive cycles of staining and chemically induced antibody stripping followed by image processing. By staining cell type specific markers (e.g. T-cells, macrophages) together with the bile acid receptors (TGR5, FXR, S1PR2), the cellular landscape of iCCA can be deciphered. Ultimately, we aim to correlate our experimental findings with clinical parameters such as therapy response or survival of the patients.

Results: First results indicate a downregulation of FXR (NR1H4), a nuclear receptor responsive to BA, in CCA tissue while TGR5 expression seems to be maintained within the tumor tissue.

38. Novel bile acids as a treatment for Parkinson's disease

Louise Heywood (Sheffield, GB)

Introduction: Parkinson's disease is a progressive neurodegenerative disease caused by the loss of dopaminergic neurons in the substantia nigra and characterised by a syndrome of motor and non-motor symptoms. Constipation is a key non-motor symptom, often presenting up to a decade before the onset of motor symptoms, and as such is considered a potential prodromal indicator of PD. PD patients show alterations in gut microbiota and bile acid compositions, further indicating the involvement of the gut in the etiopathology of PD. Here we show that novel bile acids and bile acid metabolites can be used to treat the mitochondrial deficits observed in patient-derived PD cells.

Methods: 83 compounds comprised of 58 novel bile acids and 25 secondary bile acid metabolites were screened in two assays for effects on ATP production and mitochondrial membrane potential (MMP). 20 compounds were shortlisted based on activity in the ATP assay and further assayed at 11 concentrations ranging from 100pM to 10 uM. A final selection of 6 compounds was made to be tested in patient-derived dopaminergic neurons and astrocytes.

Results: Of the 83 compounds screened, 36 showed an increase in total ATP production, whilst 13 showed an increase in MMP. Of these active compounds, 20 were selected for further assay, showing increased ATP at 11 concentrations; the final 6 chosen from this cohort show increased activity in a total of 6 patient derived fibroblast lines and are now being assessed for activity in patient-derived neurons and astrocytes.

Discussion/Conclusion: These results indicate that bile acids possess a potential therapeutic benefit for Parkinson's disease. Further work is needed to understand the mechanisms through which bile acids and their metabolites achieve this effect.

39. Expanded bile acid profiling and microbial species analysis of faecal samples reveals metabolic pathway differences in primary bile acid diarrhoea

Evette Hillman (Potters Bar, GB), Julian Walters (London, GB)

Introduction: Primary bile acid diarrhoea (PBAD) represents a challenging gastrointestinal ailment marked by excessive bile acid secretion culminating in persistent diarrhoea. This investigation endeavours to examine alterations in faecal bile acids in PBAD diagnosed through SeHCAT testing.

Methods: The comparative analysis involved faecal samples from 26 PBAD subjects and 21 healthy controls. Leveraging ultra-high-performance liquid chromatography-mass spectrometry and shotgun metagenomic sequencing, a comprehensive analysis of 38 bile acids and serval species was performed. The range of measured bile acids was expanded to encompass various epimers including iso-forms, oxo-intermediates, allo-species, and 3-sulfated forms.

Results: The total measured bile acid median value was 2.87-fold higher in the PBAD group. Consistent with previous findings, most bile acids were unconjugated with low levels of glycol- and tauro-conjugated bile acids. Primary bile acids constituted a median of 6% in controls and 11% in PBAD when encompassing their iso variants. Deoxycholic emerged as the prevailing entity in both groups (50% in controls, 60% in PBAD). Following suit was lithocholic, accounting for 25% in controls but only 10% in PBAD, where 12-oxo-lithocholic was the second most prevalent, occupying 11% in both cohorts. Various iso-forms were slightly more abundant in PBAD, with iso-ursodeoxycholic exhibiting an 8-fold elevation, alongside the intermediate 7-oxo-lithocholic. The most substantial relative increase in sulfated forms was observed for ursodeoxycholic-3-sulfate, with a median ratio of 8.9. Notably, bile acids within the allo-pathway (3-oxo-cholanic, 5α-cholanic acid-3-one, allolithocholic, and isoallolithocholic) demonstrated diminished abundance in the PBAD cohort. Particularly striking was the PBAD: control ratio of medians for isoallolithocholic, standing at only 0.1 ($p < 0.0001$). We observed that several microbial species exhibited notable differences in their frequency of being detectable in primary BAD patients and healthy controls. Specifically, Ruminococcus gnavus (R. gnavus) exhibited a particularly high odds ratio of 72.8 of detection in primary BAD patients. There was a negative correlation between the abundance of R. gnavus and SeHCAT values (Spearman Rho -0.53, $p = 0.007$).

Discussion/Conclusion: These findings have identified differences in various bile acids in SeHCAT-diagnosed PBAD subjects, indicating variances in bile acid metabolism mediated by the faecal microbiome. Identification of bacterial species, such as R. gnavus harbouring enzymes capable of modifying these bile acids underscores a promising avenue for further exploration. Subsequent targeted investigations into specific bile acids hold potential for shedding light on the pathogenesis, diagnosis, and therapeutic strategies for PBAD.

40. TAC005, a novel GalNAc-conjugated siRNA, prevents and reverses liver fibrosis in preclinical models of cholestatic liver disease via a unique mechanism

Walter Hunziker (Singapore, SG), Jianliang Xu (Singapore, SG), Christine Meng Fan Goh (Singapore, SG), Zhi Wei Tan (Singapore, SG)

Introduction: Pediatric cholestatic liver diseases (CLD) such as progressive familial intrahepatic cholestasis (PFIC), Alagille syndrome (ALGS) or biliary atresia (BA) pose significant challenges in clinical management due to limited therapeutic options that often require liver transplantation. Here, we use preclinical mouse models to present the therapeutic potential of TAC005, a novel GalNAc-conjugated small interfering RNA (siRNA), in mitigating CLD, focusing on its efficacy in preventing and reversing cholestasis-induced liver fibrosis.

Methods: Rodent models of cholestatic liver diseases that recapitulate human PFIC3 (Abcb4 KO mice), ALGS (Yap cKO mice) and BA (bile duct ligation in mice and rats) were used to evaluate the therapeutic effect of TAC005. In mouse models with advanced CLD (e.g. Abcb4 KO or Yap cKO mice), TAC005 was administered s.c. in bi-weekly to monthly doses of 10 mg/kg. In the acute bile duct ligation model, rodents were injected the compound s.c. one week before ligation. After treatment, animals were sacrificed, and plasma and liver biochemistry, liver histopathology and liver enzyme expression analyzed.

Results: In Abcb4 KO or Yap cKO mice with advanced CLD, TAC005 reversed liver fibrosis and improved liver function. In bile duct ligated mice or rats, TAC005 pre-treatment suppressed liver necrosis. Mice treated with TAC005, or carrying a liver-specific deletion of the TAC005 target gene, showed no overt detrimental phenotype. Mechanistically, we postulate that TAC005 enhances bile acid hydroxylation and sulfation and thereby reduces bile cytotoxicity. Thus, TAC005 may attenuate a key driver of liver injury that is common to all types of CLDs, irrespective of their aetiology.

Discussion/Conclusion: These findings underscore the possible therapeutic potential of TAC005 for pediatric and other CLDs and warrants further pre-clinical assessment of clinical suitability. Its efficacy in preventing and reversing liver fibrosis in relevant mouse models offers potential prospects for improving clinical outcomes and addressing the unmet medical needs in this vulnerable patient population.

41. Attenuating bile salt accumulation prevents dysregulation of energy metabolism in Fxr-/- mice

Wietse In het Panhuis (Amsterdam, NL), Joanne Donkers (Amsterdam, NL), Anh Nguyen (Amsterdam, NL), Begona Porteiro (Santiago de Compostela, ES), Dirk De Waart (Amsterdam, NL), Roni Kunst (Amsterdam, NL), Suzanne Duijst (Amsterdam, NL), Sander Kooijman (Leiden, NL), Patrick Rensen (Leiden, NL), Stan Van de Graaf (Amsterdam, NL)

Introduction: Bile salts regulate energy metabolism through systemic signaling and activation of bile salt receptor Farnesoid X receptor (FXR) is considered beneficial for cardiometabolic health, but underlying mechanisms are unclear and often contradicting. Effects of FXR signaling are often studied in Fxr-/- mice, which display glucose intolerance, body weight loss, hepatosteatosis and are prone to hepatocellular carcinoma. Since FXR activation represses hepatic bile salt synthesis and uptake, we hypothesized that bile salt accumulation-induced hepatotoxicity underlies the dysregulated metabolism in Fxr-/- mice. Here, we aimed to assess if preventing hepatic bile salt accumulation by genetic deletion

of hepatic bile salt transporter Na+ taurocholate cotransporting polypeptide (NTCP) prevents the dysregulated metabolism in Fxr-/- mice.

Methods: WT, Fxr-/-, Ntcp-/-, and Fxr-/- Ntcp-/- mice received a high-fat diet (60%) for 15 weeks or a single injection of DEN at age of 2 weeks and chow for 36 weeks.

Results: FXR deletion elevated toxicity markers ALT, AST, and ALP in plasma, lowered body weight and delayed glucose clearance during an oral glucose tolerance test. NTCP deletion attenuated ALT, AST, and ALP levels and fully prevented the reduction in body weight and the delayed glucose clearance. Carcinoma marker AFP was elevated in the DEN-treated Fxr-/- group, and this also normalized upon genetic NTCP deletion.

Discussion/Conclusion: These data indicate that dysregulated metabolism in Fxr-/- mice may be a consequence of hepatotoxicity, due to hepatic bile salt accumulation. We are currently investigating underlying mechanisms.

42. Difficulties of NAFLD treatment. How to improve it?

David Janelidze (Kyiv, UA), Igor Lopukh (Kyiv, UA)

Introduction: Treatment of NAFLD requires a decision of which patients need to be treated, because not all patients progress to steatohepatitis, fibrosis or cirrhosis. Aim of our study was to evaluate the role of UDCA and vitamin E in improvement of NAFLD evolution.

Methods: We included 60 patients with NAFLD evaluated by FibroMax at the beginning and in the end of the study and excluded the patients with others conditions associated with hepatic steatosis. We divided the patients into two groups matched by gender, weight and fibrosis degree: group A (31 patients treated with UDCA 10 mg/kg/day) and group B (29 patients with UDCA 10 mg/kg/day + 400 mg vitamin E per day). All patients were also placed on a 1200-calorie diet during the period 6 months. In all patients we calculated BMI, fatty Liver Index (FLI), NAFLD Liver Fat Score (NAFLD-LFS) before medication and after 6 months. In both groups we determined aminotransferases, GGT, lipid profile, albumin, glucose, at the beginning and at the end of the study. No significant differences between the two groups in ALT, AST, GGT, and lipid profile at the beginning.

Results: After 6 months' treatment aminotransferases and GGT levels diminished in UDCA group compared with the initial values, without statistical significant from the beginning and were normal in the combination group, with significant statistical difference between the start and the end of treatment ($p < 0.001$). FLI and NAFLD-LFS were significant better compared with the beginning in the group B but we also observed a better score in UDCA group. No improvement for lipid profile in both groups. No differences during the study period in BMI, presence of diabetes mellitus or metabolic syndrome in all patients. We noticed an improvement of fibrosis degree (evaluated by FibroMax) in the group B but without statistical significance compared with the start.

Discussion/Conclusion: UDCA in dose 10 mg/kg/day improves aminotransferases levels and fatty liver scores in patients with NAFLD but the combination of UDCA with vitamin E seems to be more efficient in normalizing aminotransferases and also in fibrosis progress during 6 months.

43. Generation of an improved murine model for progressive familial intrahepatic cholestasis type 3

Lixin Ke (Groningen, NL), Milaine V. Hovingh (Groningen, NL), Niels L. Mulder (Groningen, NL), Rick Havinga (Groningen, NL), Hilde D. De Vries (Groningen, NL), Vincent W. Bloks

(Groningen, NL), Henkjan J. Verkade (Groningen, NL), Folkert Kuipers (Groningen, NL), Jan Freark De Boer (Groningen, NL)

Introduction: Progressive familial intrahepatic cholestasis type 3 (PFIC3) is a cholestatic liver disease that is caused by a deficiency in Abcb4 (MDR3), which transports phospholipids (PL) into the bile. Thereby, Abcb4 facilitates the formation of bile acid (BA)/PL micelles, which reduces BA toxicity. Abcb4-knockout (KO) mice have been generated, but the presence of hydrophilic, non-cytotoxic muricholic acids in mice complicates extrapolation of experimental observations to the human situation. Therefore, the aim of the current study was to generate a preclinical model for PFIC3 with improved translational ability.

Methods: The Abcb4 gene was inactivated in livers of Cyp2c70-KO mice with a human-like, hydrophobic BA composition and in heterozygous control (CTRL) mice using CRISPR/Cas9-technology. The consequences hereof on liver pathology and BA metabolism were investigated.

Results: Inactivation of Abcb4 (Abcb4-KD) significantly reduced biliary PL secretion and PL:BA molar ratios in Cyp2c70-KO as well as in CTRL mice. Cyp2c70-KO/Abcb4-KD mice had high abundances of hydrophobic chenodeoxycholic acid (CDCA) in their BA pools, translating into a significantly higher hydrophobicity index of biliary BAs as compared to CTRL/Abcb4-KD mice. In keeping with the higher cytotoxicity of hydrophobic BAs, liver fibrosis and ductular reactions were markedly aggravated in Cyp2c70-KO mice as compared to CTRLs upon Abcb4-inactivation. Furthermore, plasma transaminase levels were substantially higher in Cyp2c70-KO/Abcb4-KD mice than in CTRL/Abcb4-KD mice. Gene set enrichment analysis (GSEA) on the hepatic transcriptome revealed that the Hallmark epithelial mesenchymal transition pathway was the most upregulated pathway in Cyp2c70-KO/Abcb4-KD mice as compared to CTRL/Abcb4-KD mice (NES = 2.32, Padj = 0.004), while bile acid metabolism was among the most downregulated pathways (NES = -2.42, Padj = 0.017) in these animals.

Discussion/Conclusion: BA composition profoundly impacts the consequences of Abcb4-deficiency. Hence, this study emphasizes the importance of taking species differences in BA metabolism into consideration when generating preclinical models for human BA-related diseases.

44. Exploring the efficacy and safety of chenodeoxycholic acid (CDCA) in adult patients with cerebrotendinous xanthomatosis (CTX): A phase 3 randomized, placebo-controlled clinical study (RESTORE)

Yaz Kisanuki (Columbus, US), Paulo Nobrega (Ceara, BR), Ryan Himes (New Orleans, US), Suman Jayadev (Seattle, US), John Bernat (Iowa City, US), Vikram Prakash (Orlando, US), James Gibson (Austin, US), Austin Larson (Aurora, US), Paulo Sgobbi (Sao Paulo, BR), Andrea DeBarber (Portland, US), Edward Murphy (San Diego, US), Brian Fedor (San Diego, US), Cheryl Wong Po Foo (San Diego, US), Rana Dutta (Foster City, US), Michael Imperiale (Foster City, US), Will Garner (Foster City, US), Pamela Vig (Foster City, US), P. Barton Duell (Portland, US), Sarah Perez (New Orleans, US), Ritesh Ramdhani (Hempstead, US), Jonas Saute (Porto Alegre, BR)

Introduction: CTX is a bile acid synthesis disorder caused by biallelic pathogenic variants in CYP27A1, resulting in abnormal bile acid synthesis and toxic accumulation of cholestanol, bile alcohols and metabolites. CDCA treatment is considered standard-of-care for CTX. The purpose is to evaluate the efficacy and safety of CDCA on biomarkers of CTX in the first phase 3, randomized withdrawal, double-blind (DB), placebo (PBO)-controlled, two-period x two-treatment cross-over study with rescue medication.

Methods: This phase 3 trial with a 30-day safety follow-up was conducted in adult patients (≥ 16 years of age) with CTX who received 250 mg CDCA TID for two 8-week open label (OL) periods of the study and either blinded CDCA or PBO for two 4-week DB periods. The primary end point was change in urine 23S-pentol from Baseline (BL) to the end of each DB treatment period. Secondary end points included: percent changes in plasma cholestanol and 7αC4 at end of each DB period, and proportion of patients requiring rescue medication (CDCA) during the DB periods. Safety parameters were assessed.

Results: 14 participants with CTX (clinical diagnosis and biochemical confirmation) and median (min, max) age of 41.5 (16,55) years were analyzed. CDCA withdrawal after the OL treatment period resulted in a statistically significant 20-fold increase (95% confidence interval [CI]: 10.3–43.5 $p < 0.0001$) in urine 23S-pentol (ng/ml), a 2.8-fold increase (95% CI: 1.5–5.2; $p = 0.0083$) in plasma cholestanol (Qg/ml), and a 50-fold increase (95% CI: 25.0–66.7; $p < 0.0001$) in plasma 7αC4 (ng/ml; placebo relative to CDCA). A significant proportion of participants on PBO (61.5% [95% CI: 31.6–86.1]; $p = 0.0006$) required blinded rescue medication during DB withdrawal periods. Common TEAEs on CDCA were diarrhea (n = 5) and headache (n = 3) with the majority mild to moderate in severity and not considered treatment-related.

Discussion/Conclusion: CDCA treatment is well-tolerated, effectively suppresses abnormal bile acid synthesis, and prevents accumulation of cholestanol and bile alcohols.

45. Mice with kidney-specific deletion of the ileal bile acid transporter are protected against cholestatic liver damage

Caroline Klindt (Atlanta, US), Kimberly J. Pachura (Atlanta, US), Ashley L. Bennett (Atlanta, US), Matthias Ebert (Mannheim, DE), Saul J. Karpen (Atlanta, US), Paul A. Dawson (Atlanta, US)

Introduction: The ileal bile acid transporter (Ibat) is not only the most importand bile acid (BA) uptake transporter in the intestine, but also in the proximal tubule of the kidney. Several cholestatic liver diseases like primary sclerosing cholangitis are accompanied by disrupted excretion of hepatic bile into the gut and increased amounts of circulating bile acids filtrated by the kidney. The aim of our project was to determine if kidney-specific deficiency of Ibat in a model of cholestatic liver disease would be able to significantly decrease the bile acid pool size and hence the severity of liver damage and if these mice might be prone to develop cholemic nephropathy.

Methods: We fed 8-week-old male and female Ibat-Fl/Fl (control) and Ibat-Ki/Kil-Pax8+ (IbatKi/Ki) chow or a diet containing 0.1% DDC (3,5-diethoxycarbonyl-1,4-dihydrocollidine) for 7 days. Upon sacrifice, urine, serum and feces was collected for biochemical analysis. Liver, kidneys and ileum was collected for histology and RT-qPCR.

Results: As compared to control, Ibat Ki/Ki mice fed with DDC had significantly lower levels of serum liver injury markers and serum bile acids. This corresponded with reduced signs of inflammation, ductular reaction and fibrosis in liver histology of these mice in comparison to control. The amount of bile excreted in urine was significantly higher in the Ibat Ki/Ki mice compared to control. Urine markers for kidney injury were elevated in all animals fed with DDC, but there were no signs of an increase in kidney damage in Ibat Ki/Ki mice.

Discussion/Conclusion: Renal deficiency of Ibat is protective against liver injury in this mouse model for cholestatic liver damage, demonstrating the use of systemic or kidney specific inhibition of Asbt in patients with cholestatic liver disease in the future.

46. Increased incidence of rectal prolapse in FGF15 transgenic mice is caused by chronic diarrhea and electrolyte imbalance

Bo Kong (Piscataway, US), Katherine Otersen (Piscataway, US), Rulaiha Taylor (Piscataway, US), Zakiyah Henry (Piscataway, US), Veronia Basaly (Piscataway, US), Grace Guo (Piscataway, US)

Introduction: Fibroblast growth factor (FGF) 15 (human homolog, FGF19) is highly expressed in the small intestine, and acts as an endocrine hormone to regulate bile acid homeostasis. Our previous studies demonstrated that overexpression of FGF15 in both liver and intestine promotes liver regeneration in mice directly through activating Fibroblast Growth Factor Receptor 4 (FGFR4), and indirectly by reducing bile acid (BA) toxicity after partial hepatectomy. Moreover, a high incidence of rectal prolapse was observed in these FGF15 transgenic mice. However, the etiology is unclear. A variety of factors including weakness of sphincters, chronic diarrhea and constipation, intestinal inflammation and colitis, and pathogenic bacteria are suggested to be involved in the development of rectal prolapse.

Methods: Group-housed wild-type (WT), FGF15 transgene mice with (Tg-prolapse) and without rectal prolapse (Tg) from the same cage were selected for the study. Intestine and colon tissues were collected. Histology and immunohistochemistry were performed by standard procedures. Transcriptome in both intestine and colon was analyzed by RNA sequencing and further confirmed by quantitative real-time PCR.

Results: Compared to WT, morphological abnormalities of tissue architecture including intestinal and colonic dilation and enteric crypts alterations were observed in Tg mice, which tended to be worse in Tg-prolapse mice. H&E staining showed muscle thickness was reduced in both Tg and Tg-prolapse mice compared to WT, which was further evident by the decreased expression levels of myosin related genes. However, no significant difference in inflammation was found between WT and Tg mice. Increased weight of wet feces was also observed in Tg mice, and relative expression levels of transporters involved in colonic electrolyte secretion and absorption were disturbed as well, indicating imbalance between luminal secretion and absorption of fluids and ions in the Tg mice.

Discussion/Conclusion: FGF15 overexpression in mice increased the incidence of rectal prolapse. BA and FGF15 related increase of chronic diarrhea and weakness of sphincter muscles, but not the inflammation might drive the disease progression. Imbalance between the secretion and absorption of water and salts contributes to the chronic diarrhea. However, the underline mechanisms need to be further investigated.

47. UDCA modulates TREM-2 and miR-34 expression: In vitro and in vivo study

Alicja Laba (Szczecin, PL), Joanna Abramczyk (Szczecin, PL), Eliza Cielica (Szczecin, PL), Piotr Milkiewicz (Szczecin/Warsaw, PL), Agnieszka Kempinska-Podhorodecka (Szczecin, PL), Malgorzata Milkiewicz (Szczecin, PL)

Introduction: Backgrounds and aims: Triggering-receptor-expressed-on-myeloid-cells-2 (TREM-2) suppresses pro-inflammatory cytokines, therefore it was recognized as a potential therapeutic target in patients with primary biliary cholangitis (PBC). Ectodomain shedding by enzyme ADAM17 releases a secreted variant of TREM-2. MicroRNAs (miRNAs) regulate gene expressions by degradation of target mRNAs. TREM2 is a known target of miR-34. MiR-34a is a critical mediator in the cholestasis-associated ductular reaction and liver fibrosis. This study aims to evaluate the effect of UDCA on miR-34 and TREM-2 expression in cholangiocytes and serum of PBC patients.

Methods: The miR-34 and TREM-2 expressions were evaluated in three human cholangiocyte cell lines NHC (n = 4), H69 (n = 4) and H69miR-506 (PBC-like cholangiocytes with miR-506-over-expression) (n = 4) both at basal level, and after incubation with lipopolysaccharide (LPS) and/or ursodeoxycholic acid (UDCA). In PBC the expressions of miR-34 and TREM-2 were analyzed in serum before and after UDCA-treatment (n = 9).

Results: We confirmed that cholangiocytes produce TREM-2 receptor, and the highest level of TREM-2 mRNA was observed in H69miR-506 cell line (9-fold increase vs. NHC, $p = 0.04$). The basal expressions of miR-34 was 3.5-fold higher in H69miR-506 than in H69 or NHC cells ($p = 0.02$, $p = 0.04$). LPS stimulation of NHC cells led to the induction of miR-34 expression (2-fold, $p = 0.023$ vs. control) which was blocked by co-treatment with UDCA ($p = 0.044$ vs. LPS). LPS did not enhance TREM-2 expression but co-treatment with UDCA resulted in the 1.7-fold increase vs. LPS or controls (both $p < 0.001$). In serum of patients with PBC miR-34 was upregulated (3.5-fold vs. control; $p = 0.04$). UDCA-treatment suppressed serum level of miR-34 (60% reduction, $p < 0.001$) and TREM-2 ($p = 0.004$).

Discussion/Conclusion: miR-34a and TREM-2 are effectively modulated by UDCA in NHC cells. Serum level of TREM-2 is suppressed by UDCA independently of miR-34. Whether this suppression is a result of lower TREM-2 shedding due to inhibition of ADAM17 activity by UDCA remains to be determined.

48. Trafficking rescue of a missense ABCB11 mutation responsible for progressive familial intrahepatic cholestasis type 2

Martine Lapalus (Orsay, FR), Elodie Mareux (Orsay, FR), Rachida Amzal (Orsay, FR), Sylvain Petit (Orsay, FR), Yosra Riahi (Orsay, FR), Mounia Lakli (Orsay, FR), Manon Banet (Orsay, FR), Emmanuelle Drege (Orsay, FR), Thomas Falguieres (Orsay, FR), Isabelle Callebaut (Orsay, FR), Delphine Joseph (Orsay, FR), Emmanuel Jacquemin (Le Kremlin-Bicêtre, FR), Emmanuel Gonzales (Le Kremlin-Bicêtre, FR)

Introduction: Progressive familial intrahepatic cholestasis type 2 (PFIC2) is a severe hepatocellular cholestasis due to mutations in ABCB11 gene encoding the canalicular bile salt export pump (BSEP/ABCB11). Missense mutants such as ABCB11-R1128C identified in some patients with PFIC2 are unable to traffic to the canalicular membrane. 4-Phenybutyrate (4-PB) has been shown, in vitro and in vivo, to partially correct mistrafficking as well as clinical improvement. However, because of its galenic, treatment with sodium 4-PB requires the child to take daily an important number of pills. We aimed at assessing the ability of 4-PB analogous or homologous drugs and of non-4-PB related chemical correctors to restore the canalicular expression and the activity of the previously reported folding-defective Abcb11-R1128C mutant.

Methods: New compounds were identified by screening of the BioCIS chemical library (contributed to The French National Compound Library) by structural homology with 4-PB (or its metabolites) or synthetized by medical chemistry. These new compounds and other non-4-PB related chemical correctors already known to promote proper trafficking, have been evaluated for: i) correcting the canalicular localization of Abcb11-R1128C after transfection in hepatocellular Can 10 or HepG2 cell lines (immunodetection assays); ii) restoring the activity of the Abcb11-R1128C protein by measuring the 3H-taurocholate transport in Madin-Darby canine kidney (MDCK) cells stably co-expressing Abcb11 and the sodium taurocholate co-transporting polypeptide (Ntcp/Slc10A1).

Results: Three-dimensional structure analysis predicted a folding defect of the Abcb11-R1128C mutant. Glycerol phenylbutyrate (GPB, Ravicti®), phenylacetate (PA, the active compound of 4-PB), 3-hydroxy-2-methyl-4-phenylbutyrate (HMPB, a 4-PB metabolite analog chemi-

cally synthetized) and 4-oxo-1,2,3,4-tetrahydro-naphthalene-carboxylate (OTNC, from the library screening) increased significantly the proportion of canalicular Abcb11-R1128C protein in Can 10 cells. Nevertheless, only GPB and PA restored partially the function of the mutant in MDCK cells. Ursodeoxycholic acid (UDCA) alone or in combination with 4-PB, suberoylanilide hydroxamic acid (SAHA), C18, VX-445 or/and VX-661 corrected significantly both the traffic and the activity of Abcb11-R1128C.

Discussion/Conclusion: We identified several compounds, analogous or not to 4-PB, able to rescue the traffic and activity of a missense mutation of Abcb11. Such correctors could represent new pharmacological insights for improving the condition of patients with ABCB11 deficiency due to missense mutations affecting the traffic of the transporter.

49. Farnesoid x receptor deficiency impacts liver pathology in Cyp2c70-deficient mice with a human-like bile acid composition

Jinxiao Li (Groningen, NL), Hilde De Vries (Groningen, NL), Milaine Hovingh (Groningen, NL), Rick Havinga (Groningen, NL), Anna Palmiotti (Groningen, NL), Vincent Bloks (Groningen, NL), Henkjan Verkade (Groningen, NL), Jan Freark De Boer (Groningen, NL), Folkert Kuipers (Groningen, NL)

Introduction: Farnesoid x receptor (FXR) is a bile acid (BA)-activated nuclear receptor that governs BA homeostasis. Loss-of-function mutations in human FXR cause progressive familial intrahepatic cholestasis type 5 (PFIC5), characterized by severe liver pathology at early age. However, FXR-deficiency in mice does not lead to severe liver disease at early age, possibly due to the abundant presence of hydrophilic muricholic acids (MCAs) in their BA pool, produced by CYP2C70. Mice lacking Cyp2c70 are not able to synthesize MCAs and display a hydrophobic, human-like BA composition. In this study, we investigated the impact of Fxr-deficiency in mice in the context of a human-like BA composition.

Methods: Fxr/Cyp2c70-double knock-out (DKO) mice were generated. BA metabolism and liver function were studied in male and female WT, Fxr-KO, Cyp2c70-KO and DKO mice.

Results: In Fxr-KO and DKO mice, hepatic expression of genes encoding BA synthesis enzymes was increased, whereas expression of BA transporters was decreased. However, DKO mice unexpectedly excreted less BAs into the feces compared to other groups, implying decreased hepatic BA synthesis. Mild hypercholanemia was observed in male and female Fxr-KO and DKO mice and in female Cyp2c70-KO mice. Markers of liver damage were elevated in plasma of DKO mice, which coincided with elevated white blood cell counts. Liver and spleen weights were increased in both Fxr-KO and DKO mice. Surprisingly, the hydrophobic, human-like BA composition did not exacerbate liver fibrosis in DKO mice. Moreover, cholangiocyte proliferation, that was particularly evident in female Cyp2c70-KO mice, was reduced by Fxr-deficiency. Cholic acid dominated the biliary BA composition of DKO mice, translating into a decreased BA hydrophobicity as compared to Cyp2c70-KO mice (chenodeoxycholic acid predominant).

Discussion/Conclusion: In Cyp2c70-KO mice with a human-like BA composition, FXR-deficiency aggravates hepatocyte damage but reduces liver fibrosis. Consequently, liver pathology in Cyp2c70/Fxr-DKO mice is considerably less severe than that observed in PFIC5 patients.

50. Constitutive androstane receptor stimulation attenuates liver accumulation of bile acids during estrogen-induced cholestasis in mice

Stanislav Micuda (Hradec Kralove, CZ), Maria Krutakova (Hradec Kralove, CZ), Hana Lastuvkova (Hradec Kralove, CZ), Jolana Schreiberova (Hradec Kralove, CZ), Dina Manna (Hradec Kralove, CZ), Milos Hroch (Hradec Kralove, CZ), Rajamanikkam Kamaraj (Hradec Kralove, CZ), Lucie Smutna (Hradec Kralove, CZ), Martin Ambroz (Hradec Kralove, CZ), Petr Pavek (Hradec Kralove, CZ)

Introduction: Bile acids (BAs) are steroid compounds secreted primarily by the bile with numerous endocrine and exocrine functions. BAs accumulate during cholestasis and contribute to liver damage. The constitutive androstane receptor (CAR) is a nuclear receptor involved in the regulation of BA homeostasis, but the effect of its activation during different forms of cholestasis has not been comprehensively studied.

Methods: In the present study, we analyzed the changes in BA homeostasis induced by CAR activation in mice with estrogen-induced (EE) cholestasis, a model mimicking intrahepatic cholestasis in pregnancy (ICP). Mice received ethinylestradiol and CAR was stimulated by TCPOBOP administered on the first and fourth days of cholestasis.

Results: TCPOBOP restored EE-induced impairment of bile flow by increasing biliary secretion of glutathione and BAs. These activations were possible due to the induction of Slc10a1 (Ntcp), and Abcc2 (Mrp2) transporters and Cyp7a1, Cyp8b1, Cyp27a1, and Cyp3a11 genes mRNA, which are involved in BA homeostasis in the liver. The total BA content in the liver was subsequently reduced in cholestatic mice treated with TCPOBOP compared to mice treated with estrogen alone. In contrast, TCPOBOP did not alter BA in the plasma and feces of EE-treated mice.

Discussion/Conclusion: In conclusion, our results suggest that CAR receptor activation may protect against EE-induced cholestatic liver injury by attenuating BA accumulation in the organ. However, net plasma concentrations of BA are not reduced by CAR agonism, indicating that this approach may not be suitable for attenuating BA systemic toxicity during cholestasis, such as ICP.

This research was funded by GACR (22-05167S) and NETPHARM project NO. CZ.02.01.01/00/22_008/0004607 funded by EU.

51. Gestational hypercholanaemia interferes with pregnancy-associated adipose mass increase

Alice L. Mitchell (London, GB), Vanya Nikolova (London, GB), Elena Bellafante (London, GB), Georgia Papacleovoulou (London, GB), Eugene Jansen (Bilthoven, NL), Annika Wahlstrom (Gothenburg, SE), Hanns-Ulrich Marshall (Gothenburg, SE), Catherine Williamson (London, GB)

Introduction: Intrahepatic cholestasis of pregnancy (ICP) is typically diagnosed in the third trimester of pregnancy through elevated total serum bile acids (TSBA). It is also associated with an increased risk of dyslipidaemia. We investigated how cholic acid supplementation in mice impacts adipose tissue function during pregnancy.

Methods: Female mice were fed 0.5% cholic acid (CA)-supplemented or normal chow diet from identification of copulatory plug until sacrifice at gestational day 14 (n = 10–11/group). Non-pregnant mice were matched for duration and diet (n = 7/group). Mouse and organ

weight were measured at sacrifice. Serum was analysed for bile acid and lipid concentrations alongside adipokine expression. Adipocytes were extracted from subcutaneous or gonadal white adipose tissue (scWAT/ gWAT) and mRNA expression analysed.

Results: Adipose tissue weight was increased during pregnancy which was inhibited by CA supplementation; CA diet did not alter pregnancy-associated liver expansion. Pup weight was decreased by 10% in mice fed CA. Supplementation with CA increased serum LDL-cholesterol and reduced HDL-cholesterol in both pregnant and non-pregnant mice but did not affect triglyceride concentration. Both pregnancy and CA diet reduced expression of most transcripts involved in lipid uptake and lipolysis breakdown in both scWAT and gWAT, with an interaction between pregnancy and CA diet observed in scWAT. CA supplementation in pregnant mice reduced serum IGF-1 levels to 0.38-fold of those fed normal chow.

Discussion/Conclusion: Bile acid overload disturbs adipose tissue expansion in pregnancy. CA supplementation alters LDL- and HDL-cholesterol levels but not triglycerides, suggesting cholesterol-specific processing or storage changes. Enhanced suppression of adipose tissue lipid uptake and lipolysis mRNAs with CA feeding in pregnancy could indicate failure of early pregnancy expansion or increased lipid export. Reduced IGF-1 as a result of CA feeding alongside altered lipid availability could impact the observed reduction in fetal weight.

52. The influence of hydrophobic bile acids on the onset of sclerosing cholangitis in a Cyp2c70/Cyp2a12 DKO mouse

Teruo Miyazaki (Ibaraki, JP), Hajime Ueda (Ibaraki, JP), Tadashi Ikegami (Ibaraki, JP), Akira Honda (Ibaraki, JP)

Introduction: Sclerosing cholangitis (SC) is characterized by fibrosis around the intrahepatic bile duct (onion-skin lesions) and frequently complicates with inflammatory bowel diseases (IBD). A cell adhesion molecule integrin (Itg) αVβ6 that activates TGFβ, which is the master regulator of fibrosis, is highly expressed in the epithelial cells of the large intestine in IBD and the bile duct in SC. Bile acids (BAs) have been considered as the principal cause of SC, but it is still unclear because of unestablished mouse models due to the species difference of BA types to humans. The present study evaluated the influences of hydrophobic BA on the onset of PC in the Cyp2c70/Cyp2a12 double KO (DKO) mouse with human-type BA composition.

Methods: Wild-type (WT) and DKO mice (Male, 5 weeks of age) were treated with or without LCA solution (75 mg/l) for 15 weeks in drinking water. The frequency of onion-skin lesions in the liver, the expression of Itgs αV/β6 in the liver and large intestine, BAs in the liver and serum, and LPS in serum were evaluated. FITC-dextran loading test was carried out for evaluation of intestinal membrane permeability.

Results: The frequency of onion-skin lesions around the large interlobular bile duct was 88% in the DKO livers and increased to 100% by the LCA treatment. Itgs αV and β6 mRNA expression were significantly higher in the DKO livers than in the WT livers, and the highly expressed Itgβ6 protein was immunohistochemically observed in the portion of the fibrotic bile duct in the DKO livers. In the DKO mice, Itgs αV/β6 mRNA expression in the large intestine, and LPS and FITC-dextran concentrations in serum were also significantly enhanced by the LCA treatment.

Discussion/Conclusion: Severe fibrosis around the large intrahepatic bile ducts was developed in the DKO livers, and further aggravated by LCA treatment, even at a low dose. Hydrophobic bile acids such as LCA may be highly associated with the onset of SC through the intestine-liver correlation by the promotion of the endotoxin influx into the liver via increased

intestinal membrane permeability with abnormal endothelial cell adhesion, and the activation of TGFβ around the intrahepatic bile duct via increased ItgαVβ6 expression.

53. Pharmacological inhibition of hepatic bile acid uptake to attenuate inflammation and target inflammatory bowel disease

Thuc-Anh Nguyen (Amsterdam, NL), Stan Van de Graaf (Amsterdam, NL), Reinout Roscam Abbing (Amsterdam, NL), Pim Koelink (Amsterdam, NL), Manon Wildenberg (Amsterdam, NL), Coen Paulusma (Amsterdam, NL)

Introduction: Inflammatory bowel disease (IBD) is characterized with chronic intestinal inflammation, and macrophages are known to contribute to the development of IBD. Bile salts, which have an established role in fat digestion, also act as hormones, activating bile salt-responsive signaling receptors and exerting beneficial metabolic and immunosuppressive effects on various cells and organs, including macrophages. We hypothesized that raising systemic bile salt levels by blocking the sodium taurocholate cotransporting polypeptide (NTCP), a hepatic bile salt uptake transporter, could stimulate bile salt signaling, rendering macrophages less inflammatory and attenuating intestinal damage in IBD.

Methods: Mice that lack the organic anion transporting polypeptide (OATP)1a/1b were used to mimic human (NTCP-centered) hepatic bile salt dynamics. NTCP was inhibited by using a peptidic drug called bulevirtide.

Results: Pharmacological inhibition of NTCP by bulevirtide increased plasma bile salts levels and dampened immune responses in OATP1a/1b KO mice injected with lipopolysaccharide (LPS), as shown by reduced levels of inflammatory cytokine tumor necrosis factor α (TNFα) and increased levels of anti-inflammatory cytokine interleukin 10 (IL-10) in the plasma. Furthermore, bulevirtide treatment also protected OATP1a/1b KO mice from colitis induced by dextran sodium sulfate (DSS). Bulevirtide-treated mice displayed an attenuated goblet cell loss, crypt loss, a smaller affected area and less submucosal inflammation, culminating in a lower disease activity score. Flow cytometry analysis, however, did not show altered colonic macrophage polarization by bulevirtide treatment.

Discussion/Conclusion: Targeting hepatic bile acid uptake to increase bile acid signaling is a promising approach to attenuate inflammation and treat IBD.

54. Pretreatment serum bile acid composition predicts treatment response to ileal bile acid transporter inhibition by odevixibat in patients with bile salt export pump deficiency

Mark Nomden (Groningen, NL), Kuipers Folkert (Groningen, NL), Willem S. Lexmond (Groningen, NL), Erik Lindstrom (Boston, US), Qifeng Yu (Boston, US), Velichka Valcheva (Boston, US), Henkjan J. Verkade (Groningen, NL)

Introduction: Bile salt export pump (BSEP) deficiency (PFIC2) is a genetic liver disease caused by defective secretion of bile acids (BAs). Ileal bile acid transporter inhibition (IBATi) by odevixibat inhibits intestinal reabsorption of conjugated BAs and reduces the serum BA concentration (sBA) in about 50% of PFIC2 patients. Responsiveness to IBATi in PFIC2 patients has been impossible to predict. We investigated if the sBA composition before IBATi predicts responsiveness to IBATi in PFIC2 patients, because the sBA composition reflects residual biliary BA secretion.

Methods: 41 odevixibat-treated (24 weeks) PFIC2 patients were included. Individual sBA concentrations were measured by liquid chromatography-tandem mass spectrometry. Per-

centual contributions per sBA species were calculated by dividing the individual sBA concentration by total sBA concentration. We compared pretreatment sBA composition in patients who responded to treatment (sBA reduction ≥ 70% or a total sBA concentration ≤ 70 Qmol/l after 24 weeks; Rs) with non-responders (patients not meeting responder criteria; NRs).

Results: Rs had higher percentages of unconjugated cholic acid (CA) (median [range]: 0.04% [0.01–0.4%]) and chenodeoxycholic acid (CDCA) (0.03% [0.01–0.7%]) and higher CA+CDCA concentration (0.14 Qmol/l [0.07–0.34 Qmol/l]) compared with NRs (CA: 0.02% [0.01–0.05%]; p = 0.03; CDCA: 0.02% [0.01–0.05%]; p = 0.01; CA+CDCA concentration: 0.09 Qmol/l [0.06–0.18 Qmol/l]; p = 0.004). The areas under the ROC curve for percentage of CA, percentage of CDCA, and CA+CDCA concentration were 0.70 (95% confidence interval [CI]: 0.52–0.87), 0.73 (95% CI: 0.56–0.90), and 0.76 (95% CI: 0.61–0.92), respectively. When a patient that met at least 1 of the 3 optimal thresholds was classified as R, 36 of 41 PFIC2 patients (87.8%) were correctly classified as Rs or NRs (sensitivity: 89.5%; specificity; 86.4%).

Discussion/Conclusion: Pretreatment unconjugated sBA levels are strongly associated with (non)responsiveness to IBATi treatment by odevixibat in PFIC2 patients. The results suggest that IBATi treatment responsiveness can be predicted and requires a threshold of residual BSEP BA secretion.

55. Cholestasis in prematurely born girl, what can we find when we look deeper

Barbara Perse (Zagreb, HR)

Introduction: Parenteral nutrition (PN)-related cholestasis is present in 20% of neonates receiving PN, however cholestasis should always be considered pathological and other causes ruled out. Alpha-1-Antitrypsin deficiency (A1ATD) is the most common cause of inherited neonatal cholestasis. 10–15% of neonates with A1ATD present with cholestasis (combination of hepatocellular and obstructive). Some patients may develop cirrhosis, but mostly jaundice clears by 4 months of age.

Methods: Analysis of the medical records of patient presented with neonatal cholestasis due to prolonged PN, but also had contributing factor A1ATD.

Results: Our patient was born from the complicated pregnancy, conceived by in vitro fertilization from donated blastocyst, as third triplet. Born in 31st gestational week, she had 1500 g, Apgar scores 6/7. Surfactant was substituted once endotracheally, she was put on nCPAP. She received balanced parenteral nutrition (PN) with minimal enteral feeding (EF) from the first day of life. EF volumes were increased gradually till 26th day of life when she was exclusively fed enterally (initially via nasogastric tube, then bottle), with satisfactory weight gain. Due to elevated total bilirubin level on the 4th day of life, discontinuous phototherapy was administered. During that period, she developed neonatal cholestasis (elevated levels of direct bilirubin, bile acids and GGT) and ursodeoxycholic acid (UDCA) was introduced. After an evaluation, biliary atresia, infection, cystic fibrosis, metabolic diseases, hypopituitarisam were excluded. A1AT levels were low and A1ATD phenotype was done which came in PiZZ form. Therapy UDCA was gradually decrease as bile acid normalised, jaundice cleared but chronic hepatitis still persists.

Discussion/Conclusion: PN is cause of cholestasis in most neonates receiving it, however these children also need workup as other ethology can be cause as well. Unfortunately, A1ATD does not have a specific therapy, but patients need to be closely monitored as they may develop serious liver disease.

56. Listeria monocytogenes infection and membrane-bound bile acid receptor (Tgr5) deficiency leads to metabolic changes in macrophages

Maria Reich (Magdeburg, DE), Tobias Franz (Magdeburg, DE), Paulina Philippski (Magdeburg, DE), Mahroo Rastegari (Magdeburg, DE), Sascha Kahlfuss (Magdeburg, DE), Verena Keitel (Magdeburg, DE)

Introduction: Tgr5 (Gpbar-1) is a membrane-bound bile acid receptor considered a metabolic regulator with the ability to enhance energy expenditure (Broeders et al., 2015; Watanabe et al., 2006) and improve glucose tolerance (Thomas et al., 2009). Tgr5 is primarily expressed in monocytes and macrophages, and its activation plays an immunomodulatory role.

The present study aimed to determine the contribution of Tgr5 to metabolic changes in macrophages during bacterial infection.

Methods: Tgr5 knockout (Tgr5-/-) and wildtype (WT) mice were injected with Listeria monocytogenes (L.m.). The RNA-seq approach was used to investigate the differential expression of transcripts involved in metabolic pathways in L.m.-infected bone marrow-derived macrophages (BMDMs). Glucose uptake was determined using 2-NBDG and measured by FACS. Glycolytic and oxidative phosphorylation capacity was measured by a Seahorse analyzer. BMDMs were treated with 5mM 2-hydroxycitrate (2-HC) followed by 2 or 6 h with L.m. Expression of cytokines in treated BMDMs were quantified by qPCR.

Results: Tgr5-/- mice are more susceptible to L.m. infection, as demonstrated by increased mortality rates, higher Listeria titers, increased liver inflammation, and serum inflammatory cytokines. The RNA-seq analysis indicated that gene expression of multiple pathways, including glycolysis, oxidative phosphorylation, and the tricarboxylic acid (TCA) cycle, was distinct between WT and Tgr5-/- BMDMs after infection. Tgr5 deficiency caused reduced glycolytic rates and glucose transporter type 1 (Glut1) expression in macrophages after stimulation with L.m. The inhibition of acetyl-CoA generation reversed the Tgr5-dependent pro-inflammatory cytokine suppression.

Discussion/Conclusion: Changes in the expression of genes related to glucose and oxidative phosphorylation in both WT and Tgr5-/- macrophages, coupled with the modified metabolic profile of Tgr5-/- macrophages after an infection contribute to the beneficial role of Tgr5 in the immune response.

57.Two ABCB11 variants affecting the same amino acid have different impacts on the protein in progressive familial intrahepatic cholestasis type 2 (PFIC2)

Yosra Riahi (Orsay, FR), Elodie Mareux (Orsay, FR), Manon Banet (Orsay, FR), Mounia Lakli (Orsay, FR), Thomas Falguieres (Orsay, FR), Isabelle Callebaut (Paris, FR), Martine Lapalus (Orsay, FR), Emmanuel Jacquemin (Le Kremlin-Bicêtre, FR), Emmanuel Gonzales (Le Kremlin-Bicêtre, FR)

Introduction: Bile secretion plays a pivotal role in maintaining biliary homeostasis, primarily facilitated by the canalicular bile acids transporter ABCB11 (ATP-binding cassette subfamily B member 11), also known as BSEP (Bile Salt Export Pump). Variations in the ABCB11 gene are associated with a severe form of cholestatic disease known as progressive familial intrahepatic cholestasis Type 2 (PFIC2). Herein, we investigate two variants of ABCB11, T463I and T463P, affecting the same residue within a highly conserved region of the protein.

Methods: Clinical data from two patients carrying these variations were analyzed and compared to assess the severity of each phenotype. Utilizing a comparative 3D structure analysis, we predicted the impact of each variation on protein localization and function. Subsequently, we studied the variants in cellular models: i) quantifying protein expression via Western blotting in HEK cells and ii) assessing localization in HepG2 and Can10 cells. Based on the characterization results, we tested the efficacy of drugs previously effective on ABCB11 variants with similar defects, to correct the ABCB11-T463P protein.

Results: The patient with the T463I variation exhibited a less severe phenotype compared to the T463P patient, who ultimately required liver transplantation. Structural analysis suggested that, besides the loss of function observed in the T463I variant, T463P also displayed a folding defect due to the loss of an additional hydrogen bond. In cell models, T463I exhibited correct canalicular localization in polarized cells, while T463P predominantly remained cytoplasmic, indicating entrapment in the endoplasmic reticulum due to folding defects. Western blot analysis revealed a mature band for T463I, contrasting with an immature band observed for T463P, consistent with results in polarized cells. Furthermore, we demonstrated that certain correctors such as GPB and C18 could restore, partially, the trafficking of the T463P variant.

Discussion/Conclusion: Our findings highlight significant differences between the T463I and T463P variants, with the latter presenting a more severe phenotype attributed to a substantial folding defect, resulting in the absence of the protein at the canaliculus. Further investigations are underway to elucidate the residual activity of the ABCB11-T463P protein and explore potential strategies for its potentiation if defective. These insights contribute to a deeper understanding of protein structure and offer avenues to enhance targeted pharmacotherapy of patients with PFIC2.

58. Differential systemic entry of D and L enantiomers of the microbial bile salt amidate tyrosocholic acid

Frank Schaap (Maastricht, NL), Umran Ay (Aachen, DE), Hans Van Eijk (Maastricht, NL), Martin Lenicek (Prague, CZ), Jan Hengstler (Dortmund, DE), Steven Olde Damink (Maastricht, NL), Ahmed Ghallab (Dortmund, DE)

Introduction: We recently showed that N-amidates of (chenodeoxy)cholic acid ((CD)CA) with leucine, phenylalanine or tyrosine, activate bile salt receptors at the cell surface (TGR5) and intracellularly (FXR), and are transporter substrates of human ASBT and NTCP. Despite potential for intestinal absorption, we found levels of these microbial bile salt conjugates (MBSCs) to be negligible in mesenteric and portal blood of patients, while being detected readily in human bile. In this pilot study, we investigated luminal degradation as possible cause of marginal presence of MBSCs in the circulation, and explored whether occurrence in bile is common in mammals.

Methods: Male C57BL/6J mice were gavaged with a single dose of D-TyrCA or L-TyrCA (50 mg/kg BW, n = 3 per group), and blood was sampled from the jugular vein at 0, 30, 60, 120 and 240 min after gavage. Animals were sacrificed after 4 hrs, and portal and hepatic venous blood and bile were collected. TyrCA levels were determined by quantitative LC-MS.

Results: Neither of the TyrCA enantiomers was detected at baseline. Gavage of L-TyrCA did not result in quantifiable levels in the systemic circulation (i.e. < 2.5 nmol/l), contrasting with readily detection of D-TyrCA from the earliest time point after gavage, with median levels reaching 36 nmol/l at sacrifice. Portal TyrCA levels were > 200-fold higher in mice gavaged with the D enantiomer. Porto-hepatic venous differences indicate that both TyrCA enantiomers underwent marked hepatic extraction. Only the D enantiomer was recovered in bile,

approaching low millimolar concentration. Other MBSCs uncovered in mouse bile included LeuCA and PheCA. Similar and additional MBSCs were observed in bile samples from a further six mammals.

Discussion/Conclusion: Following gavage, a plasma response was only observed for D-Tyr-CA. Earlier reported resistance of this enantiomer to degradation by pancreatic carboxypeptidases and/or bacterial bile salt hydrolases, may contribute to the divergent plasma excursions. MBSCs undergo hepatobiliary secretion and may further circulate enterohepatically.

59. Primary and conjugated serum bile acids are increased in European women with GDM

Josca Schoonejans (London, GB), Catherine Williamson (London, GB), Hei Man Fan (London, GB), Marcus Martineau (London, GB), David McCance (Belfast, GB)

Introduction: We have previously shown that in the PRiDE study, late gestation serum bile acids were increased in women with gestational diabetes mellitus (GDM) of European, but not South Asian, ethnicity. This study aimed to confirm whether serum bile acid profiles were altered in a separate cohort of European women with GDM.

Methods: This study used a subset of participants from the Hyperglycemia and Adverse Pregnancy Outcomes (HAPO) study with fasting glucose in the lowest (≤ 4.3 mmol/l) or highest (≥ 5.1 mmol/l) quartile (n = 82 Control, n = 84 GDM). GDM was diagnosed if fasting plasma glucose was ≥ 5.1 mmol/l and/or if 2-hour plasma glucose was ≥ 8.5 mmol/l. Serum BAs were measured with UPLC-MS/MS in fasted serum samples collected between 25–31 weeks' gestation from women. Log-transformed bile acid data were analysed using multiple linear regression corrected for maternal age.

Results: Total serum BA were elevated 1.29-fold [95% confidence interval [CI]: 1.02–1.64] in women with GDM (2.36 [1.36–4.09] GDM vs. 1.99 [1.23–3.47] control, p = 0.035). Specifically, concentrations of individual bile acid species G-CDCA (1.61-fold [1.20–2.15], p = 0.002), G-CA (1.85-fold [1.32–2.59], p < 0.001), and T-CDCA (1.93-fold [1.16–3.21], p = 0.012) were significantly increased, as well as concentrations of total CA species (1.51-fold [1.11–2.06], p = 0.009), total CDCA species (1.55-fold [1.12–2.14], p = 0.008), total conjugated bile acids (1.49-fold [1.18–1.87], p < 0.001), total G-conjugated bile acids (1.50-fold [1.20–1.88], p < 0.001), and total primary bile acids (1.40 [1.08–1.80], p = 0.010).

Discussion/Conclusion: This study confirmed our previous findings that serum bile acids are increased in European women with GDM. In this cohort, specific elevation of primary and glyco-conjugated bile acids was observed, which is novel. Further work is required to determine the cause of this potential compositional change, including investigation of the gut microbiome.

60. Comparative efficacy of cholic and chenodeoxycholic acids in the treatment of cerebrotendinous xanthomatosis (CTX)

Kenneth Setchell (Cincinnati, US), Wujuan Zhang (Cincinnati, US), Kevin Bove (Cincinnati, US), Jian-she Wang (Shanghai, CN)

Introduction: A deficiency in the activity of sterol 27-hydroxylase (CYP27A1) manifests as the rare lipid-storage disease of cerebrotendinous xanthomatosis (CTX). This autosomal recessive disease presents with neurological abnormalities, xanthomas in tendons, premature atherosclerosis, dementia, diarrhea, and cataracts. In infancy, CTX presents with transient, severe, or even fatal cholestasis. chenodeoxycholic acid (CDCA) lowers 5-alpha-cholestanol

and bile alcohol levels and has been the standard treatment for decades, although long-term improvements in neurological status have proven variable. CDCA is intrinsically hepatotoxic and cathartic. Cholic acid, is more hydrophilic and is approved for treatment of bile acid synthesis disorders (BASD), including CTX, yet despite its superior safety profile has not been the first treatment choice. The argument against its use has been that it does not bind well to the nuclear receptor FXR to suppress CYP7A1 expression, the ultimate target of therapy. However, the in vivo ability of cholic acid to activate FXR is unquestionable based on its clinical effectiveness in suppressing atypical bile acids and improving liver function in patients with BASD.

Methods: A study of 26 patients with CTX compared the effects of treatment with either cholic acid (n = 16) or CDCA (n = 10) on key biochemical endpoints of efficacy and on liver histology.

Results: Treatment with either cholic acid (n = 16) or CDCA (n = 10) resulted in a clinically meaningful lowering from baseline of the key bile alcohol 5β-cholestane-3α,7α,12α,25-tetrol-3-O-β-glucuronide in urine. The average % reduction from baseline was 70% and 90% respectively, indicating both bile acids effectively reduce bile acid/alcohol synthesis with the difference unlikely to be clinically significant. Cholic acid reduces 5alpha-cholestanol levels, and in CTX patients with neonatal cholestasis led to normalization of liver histology and resolution of the inflammatory activity observed pre-treatment.

Discussion/Conclusion: We now confirm that cholic acid is as effective as CDCA on the key biochemical endpoints in CTX. Based on its superior safety profile, cholic acid should be the treatment of choice for pediatric CTX patients, especially those presenting with neonatal cholestasis.

61. Gut microbiota depletion impacts the development of liver pathology in mice with a human-like bile acid profile via modulation of Cyp8b1 expression

Wenqiang Shen (Groningen, NL), Esther Verkade (Groningen, NL), Milaine Hovingh (Groningen, NL), Niels Mulder (Groningen, NL), Krisztina Bruyn (Groningen, NL), Martijn Koehorst (Groningen, NL), Hilde De Vries (Groningen, NL), Vincent Bloks (Groningen, NL), Folkert Kuipers (Groningen, NL), Jan Freark De Boer (Groningen, NL)

Introduction: Cyp2c70-deficient mice have a human-like bile acid (BA) composition due to their inability to convert chenodeoxycholic acid (CDCA) into rodent-specific muricholic acids (MCAs). However, the hydrophobic BA composition in these animals is associated with liver pathology. Although Cyp2c70-ablation has been shown to alter gut microbiome composition, the impact of gut bacteria on liver pathology in Cyp2c70-/- mice remains to be established.

Methods: We treated young-adult male and female wild-type (WT) and Cyp2c70-/- mice with antibiotics (AB) with broad specificity to deplete the gut microbiota and assessed the consequences on BA metabolism and liver pathology.

Results: Female Cyp2c70-/- mice did not tolerate AB treatment, necessitating premature termination of the experiment. Male Cyp2c70-/- mice did tolerate AB but showed markedly augmented liver pathology after 6 weeks of treatment. Dramatic downregulation of hepatic Cyp8b1 expression (-99%) caused a reduction in the proportions of 12a-hydroxylated BAs in the circulating BA pools of AB-treated male Cyp2c70-/- mice. Interestingly, the resulting increased BA hydrophobicity strongly correlated with various indicators of liver pathology. Moreover, genetic inactivation of Cyp8b1 in livers of male Cyp2c70-/- mice increased liver

pathology, while addition of ursodeoxycholic acid to the diet prevented weight loss and liver pathology in AB-treated female Cyp2c70-/- mice.

Discussion/Conclusion: Depletion of gut microbiota in Cyp2c70-/- mice aggravates liver pathology at least in part by increasing the hydrophobicity of the circulating BA pool. These findings highlight that the potential implications of AB administration to cholestatic patients should be evaluated in a systematic manner.

62. Characterization of cholestasis in a knock-in BsepE297G PFIC2 mouse model

Jennifer Truong (Cambridge, US), Eric Bell (Cambridge, US), Youhwa Jo (Cambridge, US), Adrianne Kolpak (Cambridge, US), Bharat Reddy (Cambridge, US), Patrick Stoiber (Cambridge, US), Renata Franca (Cambridge, US), Eitan Hoch (Cambridge, US), Yong Ren (Cambridge, US), Jonathan Moore (Cambridge, US), Alastair Garfield (Cambridge, US)

Introduction: Progressive Familial Intrahepatic Cholestasis type 2 (PFIC2) is a rare pediatric liver disorder arising from genetic mutations in the bile salt export pump (BSEP, ABCB11). E297G is the most prevalent mutation found in PFIC2 patients, which when inherited biallelically or in trans with another defective ABCB11 allele presents with compromised bile acid transport, subsequent hepatic accumulation, and toxicity. A PFIC2 mouse model homozygous for the BsepE297G mutation was generated, as well as a polyclonal antibody that recognizes both human and mouse Bsep protein. Characterization of this mouse model demonstrates the recapitulation of key translational aspects of the human disease and offers advancements for the evaluation of novel PFIC2 therapeutic interventions.

Methods: CRISPR/Cas9-mediated gene editing was used to create BsepE297G knock-in mice (C57BL/6N). Male and female wildtype (WT), heterozygous (HET), and homozygous (HOM) mice were phenotypically characterized starting at 6 weeks. The mice were assessed for clinically relevant markers associated with progressive cholestasis, liver damage, and PFIC2 pathophysiology. Parameters included serum, biliary, and hepatic bile acid concentration, bile acid composition, liver enzymes, gene expression profiles, and liver histopathology. Antibody production was carried out against mouse Bsep recombinant protein in NZW rabbits and antisera were affinity purified for all applications.

Results: The specificity of the polyclonal BSEP antibody was validated by comparisons of wildtype and BsepE297G mouse liver using western blot, ELISA and IHC. The homozygous BsepE297G mice present with the essential clinical features of PFIC2 of cholestasis and hepatic toxicity. Progressive escalation in serum total bile acids (TBA) and indicators of liver damage were observed from 6 to 12 weeks of age in female HOM BsepE297G mice, which exhibit a more robust phenotype as compared to male animals of the same genotype. By the 12th week, serum TBA levels had increased 92%, while ALT and ALP levels rose by 48% and 88%, respectively. Biliary TBA content exhibited a remarkable 82% decrease, juxtaposed with a 203% increase in hepatic TBA levels, indicative of Bsep insufficiency and cholestasis. Liver immunohistochemistry confirms the absence of endogenous Bsep in HOM BsepE297G mice.

Discussion/Conclusion: The BsepE297G homozygous mouse serves as a translational model of PFIC2 which exhibits several of the key pathophysiological hallmarks of the human disease. This model can further an understanding of the relationship between deficits in BSEP and the resultant cholestasis that develops from that deficiency. The model thus provides an additional framework in which therapeutics aimed at PFIC2 can be evaluated.

63. The impact of ABCB4 rare variants on the longitudinal trajectories of bile acids concentrations in intrahepatic cholestasis of pregnancy

Xi Yang (London, GB), Caroline Ovadia (London, GB), Jenny Chambers (London, GB), Julia Zollner (London, GB), Peter Dixon (London, GB), Catherine Williamson (London, GB)

Introduction: To understand the difference in longitudinal trajectories of serum bile acids in intrahepatic cholestasis of pregnancy (ICP) patients with and without likely pathogenic ABCB4 variants.

Methods: Maternal demographics and serial measurements of bile acid concentrations were collected for participants of the NIHR BioResource Rare Disease cohort, which recruited patients with severe early onset ICP (< 33 weeks' gestation, and bile acid concentration > 40 Qmol/l). These were compared based upon identification of ABCB4 pathogenic rare (MAF < 0.01) variants (ACMG criteria). Mann-Whitney U test was used to assess the difference in bile acids between the two groups.

Results: ICP onset was earlier in 31 pregnancies with ABCB4 rare variants than in 186 pregnancies without ABCB4 rare variants (median [IQR], 26.0 [18.5–29.5] vs. 30.0 [26.0–32.0] weeks, p = 0.003). Initial bile acid concentration did not differ between the two groups, although the peak bile acid concentration was higher in the group with ABCB4 rare variants (median [IQR], 121.0 [55.0–259.5] vs. 79.0 [45.0–155.5] Qmol/l, p = 0.034). Amongst pregnancies with initial bile acid concentration below 40 Qmol/l, a higher degree of bile acid concentration increase occurred after one month in the group with ABCB4 rare variants than those without (p = 0.027). There was no difference in the bile acid concentration before UDCA treatment, while bile acid concentrations after UDCA treatment were higher in the group with ABCB4 rare variants compared to those without (median [IQR], 70.0 [35.5–161.3] vs. 35.4 [23.7–81.6] Qmol/l, p = 0.006).

Discussion/Conclusion: In this cohort, patients with pathogenic ABCB4 rare variants had more severe bile acid increases than those without. Similarly, bile acid concentrations were markedly higher following UDCA treatment in those women with ABCB4 rare variants.

64. Bile acid-mediated upregulation of lncRNAH19 contributes to cholestatic liver injury through modulation of circular RNA expression in Mdr2-/- mice

Huiping Zhou (23298, US), Jing Zeng (Richmond, US), Derrick Zhao (Richmond, US), Yunling Tai (Richmond, US), Emily Gurley (Richmond, US), Phillip Hylemon (Richmond, US)

Introduction: Chronic cholestatic liver diseases, such as primary sclerosing cholangitis (PSC), frequently progress to end-stage liver conditions. Currently, there is a lack of effective therapeutic agents, primarily due to an incomplete understanding of the disease's pathogenesis. Circular RNAs (circRNAs), characterized as covalently closed continuous RNA loops, have gained attention due to recent advancements in high-throughput RNA sequencing (RNAseq) and specialized bioinformatics algorithms. These developments have led to the identification of numerous circRNAs exhibiting tissue-specific expression. Emerging as a significant class of non-coding RNAs (ncRNAs), circRNAs are increasingly recognized for their roles in regulating various biological and disease processes. They function as molecular 'sponges' for miRNAs and RNA-binding proteins (RBPs), modulating their interaction with target molecules. Yet, the specific role of circRNAs in liver pathologies, particularly in cholestatic liver diseases, remains largely unexplored. Previous research, including our own, has indicated that bile acid-induced upregulation of the long non-coding RNA (lncRNA) H19

significantly contributes to cholestatic liver injury. The mechanisms underlying this process, however, are not well-defined. This study aims to investigate the potential involvement of H19 in regulating circRNA expression under cholestatic conditions.

Methods: Wild type (WT), Mdr2-/- mice, and Mdr2-/-H19-/- mice (C57BL6, male, 10-week old, n = 3) were used in this study. Total liver RNA was isolated for circRNA proofing using Arraystar Mouse circRNA Array V2 analysis). The potential miRNA targets of each circRNA were predicted using Arraysrar's prediction software together with TargetScan and Mirnet 2.0 databases. Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis were conducted to annotate the biological functions of host linear transcripts of differentially expressed (DE) circRNAs. The hepatic miRNAs were profiled using the NanoString nCounter and analyzed using Rosalind Software. The potential targets of DE-miRNAs were identified using miRNet. Protein-protein interaction (PPI) of the identified target genes was further analyzed using the STRING database. Cytoscape was used to visualize Hub genes and construct the H19-circRNA-miRNA-hub gene regulatory network. The human cholangiocarcinoma patient data were downloaded from the Cancer Genome Atlas (TCGA) database.

Results: Our analysis revealed that a significant proportion (74%) of circRNAs identified are exonic. We identified 201 upregulated and 43 downregulated circRNAs (fold change ≥ 1.3, $p < 0.05$) in Mdr2-/- mice compared to WT mice. In Mdr2-/-H19-/- mice, there were 37 significantly upregulated and 124 downregulated circRNAs compared to Mdr2-/- mice. Interestingly, we identified 33 cirRNAs which were upregulated in Mdr2-/- mice but downregulated in Mdr2-/-H19-/- mice. The Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis further suggest that these DE-cirRNAs were linked to multiple signaling pathways related to cholestatic liver injury. By using Arraystar's prediction software, along with theTargetScan and mirnet2.0 databases, we identified 25 DE-miRNAs that are regulated by H19. Furthermore, the PPI network analysis of the potential target genes of these DE-miRNAs highlighted 10 hub genes potentially contributing to cholestatic liver injury. Remarkably, 9 out of these 10 identified hub genes were also found to be significantly upregulated in the CCA patient data from the TCGA database, suggesting a potential correlation between our findings in the mouse model and human cholestatic liver disease.

Discussion/Conclusion: This study identified 33 circRNAs potentially regulated by H19. Compared to other members of ncRNAs and mRNA, circRNAs are remarkably stable. Understanding the regulation of tissue- or cell-specific expression of circRNAs offers vital information for their potential use. Specifically, circRNAs hold promise as novel diagnostic and prognostic markers, as well as therapeutic targets for cholestatic liver diseases.

AUTHOR INDEX TO POSTER ABSTRACTS

(Name - Poster Number)

Abramczyk, J. 1, 47
Adorni, M. 9
Agelopoulos, K. 23
Alabsawy, E. 2
Alinaghi Arjas, S. 3
Ambroz, M. 50
Amzal, R. 48
Andreea, I. 26
Avila, A. 34
Ay, U. 4, 58

Balan, P. 22
Baldelli, E. 9
Balesaria, S. 5
Bandmann, O. 6
Banet, M. 7, 28, 48, 57
Barrett, H. 20
Basaly, V. 46
Basson, A. 8
Bedin, R. 9
Bell, E. 62
Bellafante, E. 51
Ben Saad, A. 28
Bennett, A. 45
Bernat, J. 44
Bernini, F. 9
Bertolotti, M. 9
Bharadwaj, S. 27
Bielfeld, A. 22
Bindels, L. 31
Bischoff, R. 18
Bloks, V. 18, 19, 43, 49, 61
Boehme, S. 29
Bos, T. 18
Bove, K. 60
Bozadjieva-Kramer, N. 10
Bracanovic, A. 11
Brearley-Sholto, M. 13
Bruyn, K. 61
Busslinger, G. 21

Callebaut, I. 48, 57
Cantz, T. 22
Capatina, N. 12
Chambers, J. 12, 63
Chan, A. 13
Charton, J. 28
Cheng, A. 13
Chiari, A. 9
Cielica, E. 47
Claudel, T. 21
Clerbaux, L. 14
Coat, S. 35
Constantinides, M. 15
Corey, M. 35
Corliano, M. 16
Craig, D. 2
Cramer, T. 27
Crespi, V. 7, 28
Curley, C. 17

Dawson, P. 45
De Aguiar Vallim, T. 13
De Boer, J. 18, 19, 43, 49, 61
De Bruin, A. 18
De Franco, F. 23
De Vries, H. 19, 43, 49, 61
De Waart, D. 41
DeBarber, A. 44
Dekker Nitert, M. 20
Deprez, B. 28
Di Meo, F. 7, 28
Dietrich, P. 23
Dilts, M. 29
Dixon, E. 21
Dixon, P. 11, 15, 63
Donkers, J. 41
Dorey, E. 20
Drege, E. 48
Droege, C. 3, 22
Duell, M. 23
Duell, P. 44
Duijst, S. 41
Dumont, J. 28
Dutta, R. 44

Ebert, M. 45
Eijgenraam, T. 19
El Aggan, Ha. 24
El Aggan, Ho. 25
Elena, T. 26
Elie, J. 7
Elviri, L. 9
Erdem, M. 27

Falguieres, T. 7, 28, 48, 57
Fan, H. 59
Fedor, B. 44
Ferdous, S. 29
Ferrell, J. 29
Ferri, N. 9
Finer, S. 15
Folkert, K. 54
Foltnie, T. 6
Franca, R. 62
Franz, T. 56
Fuchs, C. 21

Gafton, J. 15
Garcia, A. 15
Garfield, A. 62
Garner, W. 44
Geier, A. 30
George, T. 26
Ghallab, A. 58
Gibson, G. 8
Gibson, J. 44
Gill, U. 15
Gillard, J. 31
Goh, C. 40
Gonzales, E. 7, 28, 48, 57
Grabner, G. 21
Groen, A. 18
Groenen, C. 32
Guillot, A. 37
Guirao, R. 33, 34
Guo, G. 36, 46
Gurley, E. 64

Haas, J. 18
Haemmerle, G. 21
Hague, B. 12, 35
Havinga, R. 18, 43, 49
Hengstler, J. 58
Henry, P. 10
Henry, Z. 36, 46
Herrero, R. 33, 34
Heucke, N. 37
Heywood, L. 38
Hillman, E. 39
Himes, R. 44
Hoch, E. 62
Honda, A. 52
Horvatovich, P. 18
Hovingh, M. 18, 43, 49, 61
Hroch, M. 50
Hunziker, W. 40
Hylemon, P. 64

Ikegami, T. 52
Imperiale, M. 44
In het Panhuis, W. 41
Iwata, A. 13
Izcue, A. 27

Jacquemin, E. 7, 28, 48, 57
Janelidze, D. 42
Jansen, E. 51
Jarrett, K. 13
Jayadev, S. 44
Jo, Y. 62
Joseph, D. 48
Juarez, E. 34

Kahlfuss, S. 56
Kamaraj, R. 50
Karpen, S. 45
Ke, L. 43
Keely, S. 17
Keitel, V. 3, 22, 56
Keitel-Anselmino, V. 37
Kejeh, K. 2
Kempinska-Podhorodecka, A. 1, 47
Kennelly, J. 13
Kisanuki, Y. 44
Kleinlein, H. 23
Klindt, C. 45
Knopp, L. 3
Koehorst, M. 18, 61
Koelink, P. 53
Kohli, R. 10
Kolpak, A. 62
Kong, B. 36, 46
Kooijman, S. 41
Krawczyk, M. 30
Kremer, A. 23
Krutakova, M. 50
Kuipers, F. 18, 19, 43, 49, 61
Kunst, R. 41
Kydd-Sinclair, D. 8

Laba, A. 1, 47
Lai, R. 13
Lakli, M. 7, 28, 48, 57
Lancellotti, G. 9
Lanthier, N. 10
Lapalus, M. 7, 28, 48, 57
Larson, A. 44
Lastuvkova, H. 50
Leclercq, I. 14, 31
Leech, S. 20
Leibl, V. 23
Lenicek, M. 4, 58
Leroux, F. 28
Lexmond, W. 54
Li, J. 49
Lindstrom, E. 54
Linton, K. 15
Lopukh, I. 42
Luedde, T. 22
Luetjohann, D. 30
Lupo, M. 9

Magda, F. 26
Mahmoud, S. 24, 25
Maliha, S. 36
Manna, D. 50
Mareux, E. 28, 48, 57
Marin, J. 30
Marshall, H. 51
Martineau, M. 59
Mazza, C. 6
McCance, D. 59
Meijer, L. 7

Mejia, S. 33, 34
Micuda, S. 50
Milkiewicz, M. 1, 47
Milkiewicz, P. 1, 47
Mirela, P. 26
Mitchell, A. 51
Miyazaki, T. 52
Mlitz, V. 21
Monte, M. 30
Moore, J. 62
Mroz, M. 17
Muccioli, G. 31
Mulder, N. 18, 19, 43, 61
Murphy, E. 44
Mussi, C. 9
Myronovych, A. 10

Namer, B. 23
Nardo, A. 21
Neurath, M. 23
Nguyen, A. 41
Nguyen, T. 53
Nikolova, V. 51
Nobrega, P. 44
Nomden, M. 54

O'Rourke, R. 10
Olde Damink, S. 4, 58
Olotu, T. 29
Otersen, K. 46
Out, C. 18
Ovadia, C. 12, 63

Pachura, K. 45
Palmiotti, A. 49
Palumbo, M. 9
Papacleovoulou, G. 51
Papotti, B. 9
Paulusma, C. 32, 53
Pavek, P. 50
Pawlak, M. 18
Payne, T. 6
Pellicciari, R. 23
Perez, S. 44
Perse, B. 55
Petit, S. 48
Philippski, P. 56
Picalausa, C. 31
Pokhrel, S. 29
Porteiro, B. 41
Prakash, V. 44
Proot, V. 14

Ramdhani, R. 44
Rastegari, M. 56
Rau, M. 30
Reddy, B. 62
Reich, M. 37, 56
Reijngoud, D. 18
Remaggi, G. 9
Ren, Y. 62
Rensen, P. 41
Riahi, Y. 7, 28, 48, 57
Riva, A. 21
Roscam Abbing, R. 32, 53
Roumain, M. 31

Sahin, E. 27
Saute, J. 44
Schaap, F. 4, 27, 58
Schanze, D. 3, 22
Schmelz, M. 23
Schmitt, T. 30
Schnarnagl, H. 21
Schoeler, D. 22
Schoeler, M. 22
Schoonejans, J. 59
Schoonjans, K. 32
Schreiberova, J. 50
Seeley, R. 10
Setchell, K. 60
Sgobbi, P. 44
Sgodda, M. 22
Sharaf, S. 24, 25
Shen, W. 61
Shin, J. 10
Sillje, H. 19
Silva, J. 21
Singaraja, R. 16
Smutna, L. 50
Staels, B. 18, 31
Staender, S. 23
Starkel, P. 14
Steel, M. 13
Stein, K. 3
Stoiber, P. 62
Stojakovic, T. 21
Su, B. 13
Sueer, A. 23
Szydlowska- Jakimiuk, M. 1

Tacke, F. 37
Tai, Y. 64
Tailleux, A. 31
Tan, Z. 40
Tarling, E. 13
Taveras, M. 13
Taylor, R. 46
Thibaut, M. 31
Tondelli, M. 9
Trauner, M. 21
Truong, J. 62

Ueda, H. 52
Valcheva, V. 54
Van Eijk, H. 4, 58

Van Zutphen, T. 19
Van de Graaf, S. 32, 41, 53
Vauthier, V. 28
Verkade, E. 61
Verkade, H. 21, 43, 49, 54
Vetter, M. 23
Vig, P. 44

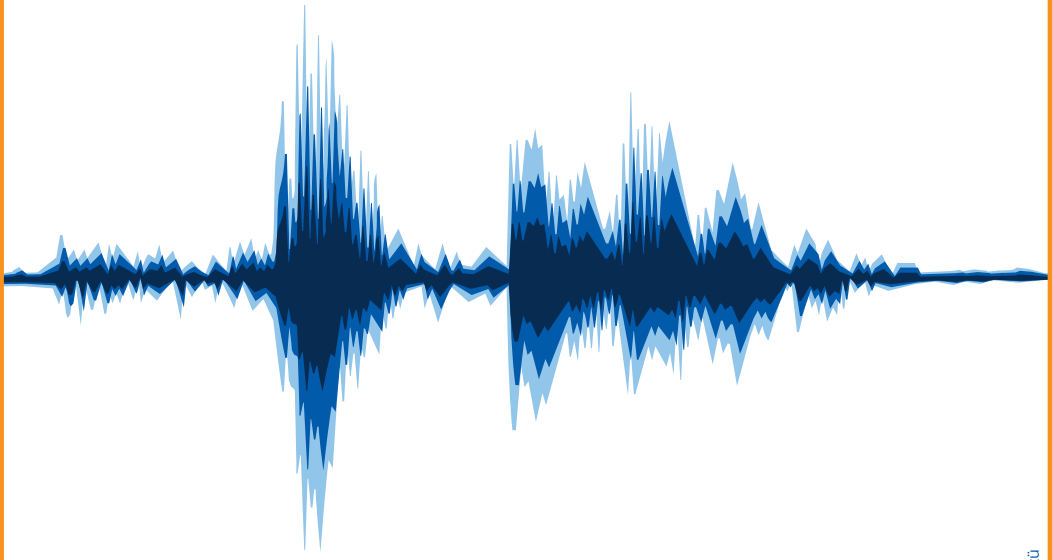
Wahlstrom, A. 51
Walters, J. 39
Wang, J. 60
Watson, K. 8
Weymouth-Wilson, A. 8
Wildenberg, M. 32, 53
Williams, K. 13
Williamson, C. 11, 12, 15, 51, 59, 63
Wolters, J. 18
Wong Po Foo, C. 44

Xu, J. 40

Yang, X. 63
Yu, Q. 54

Zeng, J. 64
Zenker, M. 3, 22
Zhang, We. 18
Zhang, Wu. 60
Zhang, Y. 18
Zhao, D. 64
Zhou, H. 64
Zimetti, F. 9
Zimmermann, R. 21
Zollner, J. 11, 15, 63

Registration via www.falkfoundation.org or simply scan and register.



Together we know more. Together we do more.
Falk Foundation e.V. | Leinenweberstr. 5 | 79108 Freiburg | Germany
T: +49 761 1514-400 | F: +49 761 1514-460 | E-Mail: meeting@falkfoundation.org
www.falkfoundation.org

P237E/2024/600 Stü