2nd International Workshop on Liver & Gut Fibrosis

26-27 October 2023
Valencia, Spain
Welcome 5
Scientific Committee 6
Speakers and Chairs 7
Programme 8
Oral communications 11
Posters. Basic research in Liver Fibrosis 37
Posters. Clinical research in Liver Fibrosis 69
Posters. Basic research in Gut Fibrosis 81
Poster. Fibrosis in other tissues and systems 89
Acknowledgements 94
WELCOME

2nd INTERNATIONAL WORKSHOP ON LIVER AND GUT FIBROSIS

Fibrosis - the excessive growth of fibrous tissue due to chronic inflammation and altered tissue remodelling and repair processes - is a global health problem. It occurs in various organs, including the lung, kidney, liver and intestine, and is a key component of important pathologies, including cystic fibrosis, Crohn’s Disease and Metabolic Dysfunction-Associated Steatotic Liver Disease. Despite the huge advances made by research in recent years, the molecular mechanisms involved in the development of fibrosis are still unclear; indeed, it would seem that different mechanisms are involved in each of the affected organs. Importantly, there are few effective therapeutic options, and only for some organs, such as the lung and kidney, with no anti-fibrotic pharmacological treatments currently available for the intestine or liver. The International Workshop on Liver and Gut Fibrosis is an initiative to bring together basic scientists and clinical experts working in the field to share perspectives about this condition. The workshop focuses on the molecular mechanisms involved in the different types of fibrosis, particularly that found in the gut and liver, with an emphasis on mechanisms that are common to different organs. In this way, the workshop sets out to explore avenues that might lead to an improvement of current therapeutic approaches and to identify new pharmacological targets that could relieve the burden of fibrosis for millions of patients.

The first edition of the workshop, held in October 2021 and endorsed by European and national scientific societies, was well-received by all those involved and has driven us to organise a second edition, which we are sure will be an equally stimulating and productive event. We invite those of you with an interest in this topic to join us in the beautiful Mediterranean city of Valencia, together with some of the most relevant international researchers in the field. We look forward to seeing you.

Juan V. Esplugues
Head of the Scientific Committee
SCIENTIFIC COMMITTEE

• Juan V. Esplugues Mota (University of Valencia)
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• Marta-Maia Boscá-Watts (Hospital Clínico Universitario, Valencia, Spain)
• Mario C. Manresa (University College Dublin, Ireland)
• Urko M. Marigorta (CICBioGUNE, Derio, Spain)
• Fabio Marra (University of Florence, Italy)
• Florian Rieder (Cleveland Clinic Foundation, Ohio, USA)
• Manuel Romero-Gómez (Hospital Virgen del Rocío-IBiS, Seville, Spain)
• Pau Sancho-Bru (IDIBAPS, Barcelona, Spain)
• Marianne Spalinger (University Hospital Zurich, Switzerland)
• Jacobo Sellares (Hospital Clinic Barcelona, Spain)
• Antonio Vidal-Puig (Wellcome Metabolic Diseases Unit, University of Cambridge, UK)
PROGRAMME

THURSDAY 26TH OCTOBER 2023

8:45 - 9:15 am Registration
9:15 am Welcome & overview of the workshop
9:30 - 10:50 am FIBROSIS: STATE-OF-THE-ART

Chairs: Jordi Gracia-Sancho (IDIBAPS, Barcelona) & Marta Maia Boscá-Watts (Hospital Clínico Universitario, Valencia)

9:30 - 10:05 am Mechanisms and clinical trial endpoints for structuring Crohn’s disease. Florian Rieder (Cleveland Clinic Foundation, Ohio)
10:05 - 10:40 am Liver fibrosis in ALD and NAFLD. Ramón Bataller (Hospital Clínico Barcelona)

10:45 - 11:30 am Coffee break
11:30 am - 1:10 pm FACTORS INFLUENCING THE DEVELOPMENT OF FIBROSIS

Chairs: Rubén Francés (Miguel Hernández University, Alicante) & Ana Bias-García (University of Valencia)

11:30 - 11:50 am Sinusoidal communication in liver fibrosis. Anabel Fernández-Iglesias (IDIBAPS, Barcelona)
11:50 am - 12:10 pm Epigenetic landscape in liver fibrosis. Maite García Fernández-Barrena (CIMA University of Navarra, Pamplona)

12:10 - 12:30 pm Regulation of fibroblast-monocyte interactions: how do we TWEAK the system? Mario C. Manresa (University College Dublin)
12:30 - 12:50 pm Lipotoxicity and fibrosis. Fabio Marra (University of Florence)
12:50 - 1:10 pm Autophagy in liver fibrosis: roles, mechanisms and targets. Nadezda Apostolova (University of Valencia)

1:10 - 2:30 pm Lunch
2:30 - 3:50 pm CUTTING-EDGE APPROACHES TO THE STUDY OF FIBROSIS

Chairs: Javier Cubero (Complutense University of Madrid) & Azucena Salas (Hospital Clínico Barcelona)

2:50 - 3:10 pm Multi-omics in IBD: overview of observations and take-aways. Urko M. Marigorta (CIC BioGUNE, Bilbao)
3:10 - 3:30 pm Using iPSCs to understand liver fibrosis. Pau Sancho-Bru (IDIBAPS, Barcelona)
3:30 - 3:50 pm Biomarkers in IBD. Marianne Spalinger (University Hospital Zurich)

3:50 - 4:45 pm Coffee break & Poster session
4:45 - 5:45 pm FIBROSIS IN OTHER ORGANS AND TISSUES

Chairs: Sara Calatayud & Elena Ortiz (University of Valencia)

4:45 - 5:05 pm Mechanisms of old fibrosis by impaired degradation. Antonio Vidal-Puig (Wellcome-MRC Institute of Metabolic Science and MRC Metabolic Diseases Unit, University of Cambridge)
5:05 - 5:25 pm Impact of diffuse myocardial fibrosis in heart failure. Arantxa González Miqueo (CIMA University of Navarra, Pamplona)
5:25 - 5:45 pm Role of cellular senescence in the development of lung fibrosis. Jacobo Sellares (Hospital Clínico Barcelona)

6:30 - 8:30 pm Tour of the city
8:30 pm Drinks and canapés. Only YOU Hotel (The City Lover Room)
FRIDAY 27TH OCTOBER 2023

9:00 - 11:00 am ORAL PRESENTATIONS

Chairs: David Martí-Aguado (Hospital Clínico Universitario, Valencia) & Jesús Cosín (University of Valencia)

9:00 – 9:10 AM: Protease-driven lysosomal activity is a core mechanism for macrophage driven collagen remodeling during liver and kidney fibrosis. María Fernández-Fernández (Institute of Biomedical Research of Barcelona)


9:20 – 9:30 AM: Microbiota dysbiosis, altered metabolomic profile and metabolite-sensing GPCRs expression is found in ileal resections from fibrotic-CD patients. Cristina Bauset (University of Valencia)

9:30 – 9:40 AM: Exploring the role of X-binding protein-1 (XBP1) in the gut–liver axis during alcoholic-related liver disease (AlLD). Carlos Sanz-Garcia (Complutense University)

9:40 – 9:50 AM: Peptidylprolyl isomerase C in chronic liver disease: pathogenic or protective mechanism. Isabel Fuster-Martínez (University of Valencia)

9:50 – 10:00 AM: Dissecting submucosal fibroblast populations of human colon Crohn's Disease patients through spatial transcriptomics. Victoria Gudiño (IDIBAPS)

10:00 – 10:10 AM: Circulating bone morphogenetic protein 8A is a novel biomarker to predict advanced liver fibrosis. Stephanie C. Izasa (Instituto de Investigación Sanitaria del Hospital Universitario de La Princesa)

10:10 – 10:20 AM: A novel senolytic formulation targeting the hepatic sinusoid improves hepatic function and fibrosis in experimental models of aging. David Sanfeliu-Redondo (IDIBAPS)

10:20 – 10:30 AM: The TNF superfamily factor TWEAK promotes colonic inflammatory fibroblast differentiation and fibroblast-monocyte interaction. Carlos Matellan (University College Dublin)

10:30 – 10:40 AM: Identification and assessment of circulating biomarkers for the evaluation of liver disease severity in NAFLD patients. Douglas Maya-Miles (Instituto de Biomedicina de Sevilla)

11:00 am - 12:00 pm Coffee break & Poster session

12:00 - 12:20 pm THERAPEUTIC APPROACHES TO FIBROSIS

Chairs: Jaume Bosch (University of Bern) & Ana Gutiérrez (Hospital General Universitario, Alicante)

12:00 - 12:20 pm Characterization of molecular targets in ileal fibrosis: a starting point for the development of new therapies. María Dolores Barrachina (University of Valencia)

12:20 - 12:40 pm Clinical trials in NAFLD-fibrosis. Manuel Romero-Gómez (Hospital Virgen del Rocío-IIBS, Seville)

12:40 - 1:00 pm Treating fibrosis in IBD: a challenge for the gastroenterologist. Marta-Maia Boscá-Watts (Hospital Clínico Universitario, Valencia)

1:00 - 1:20 pm Pathophysiology of advanced liver disease. Rafael Bañares (Hospital Gregorio Marañón, Madrid)

1:30 - 1:45 pm Best Communication Award and concluding remarks

2:00 - 3:30 pm Lunch
O01 - Protease-driven lysosomal activity is a core mechanism for macrophage driven collagen remodeling during liver and kidney fibrosis

Paloma Ruiz-Blázquez1,2,4*, Maria Fernández-Fernández1,2,4*, Valeria Pistoria1,3,4, Celia Martínez-Sanchez2,4, Michele Costanzo5,7, Paula Iruzubieta3, Ekaterina Zhuravleva3, Susana Núñez2, Jesper B. Andersen9, Margherita Ruoppolo5,7, Javier Crespo9, Carmen García-Ruiz2,8,9*, Luigi Michele Pavone2,3,4, Mar Coll2,3,11, José C. Fernandez-Checa1,2,4,10, Anna Moles1,2,4.

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Background and Aims: Fibrosis is caused by an excessive accumulation of extracellular matrix (ECM). Macrophages are important effectors of the ECM remodelling through recycling of the ECM within acidic compartments and can contribute to fibrosis resolution. Proteases, such as cathepsins, are essential for lysosomal proteolytic activity; however, their contribution to ECM remodelling within macrophages is unknown. Thus, the aim of this study was to investigate the proteolytic and degradative signalling pathways associated to macrophages during liver and kidney fibrosis.

Methods: A novel myeloid-cathepsin D KO mouse strain (CtsDΔMyel) was generated by breeding LysMCre (macrophages) with CtsD-floxed mice. Fibrosis was established by CCl4 administration and unilateral ureteric obstruction in CtsDΔMyel or CtsDflox/flox mice and determined by SR, α-SMA, ColIα1 and TGF-β RT-qPCR. Proteomic profile was determined by LC-MS/MS in fibrotic livers. Reversion was assessed 72h post-challenge in a 4-week CCl4 model by HP and R-CHP staining. Macrophage proteolytic secretome was assessed by protease array. In vitro collagen degradation and endocytosis was determined by FACS.

Results: High CtsD expression was observed in macrophages from cirrhotic and CKD patients. Next, CtsDΔMyel mouse was validated in isolated macrophages and tissue. CtsD deletion in macrophages enhanced fibrosis in both liver and kidney experimental models. Further analysis of fibrotic livers confirmed enriched matrisome proteomic signatures. CtsDΔMyel KC isolated from 72h-CCl4-treated livers demonstrated significantly lower expression of markers associated with resolutive macrophages and defective proteolytic secretory profile. CtsDΔMyel macrophages displayed defective proteolytic processing of collagen I without impairment of the endocytosis. Finally, CtsDΔMyel mouse was unable to remodel collagen in vivo when subjected to a liver fibrosis reversion model determined by percentage of HP and fluorescent intensity of R-CHP.

Conclusions: CtsD is essential in regulating the collagenolytic activity of macrophages during fibrosis and is part of a novel and currently unknown degradome landscape of restorative macrophages.
Oral communications

O02 - Mg2+ Homeostasis in Cholestatic Liver Diseases: Potential Therapeutic Target

Naroa Goikoetxea-Usandizaga1, Irene González-Recio1, Jorge Simón1,2, Marina Serrano-Maciá1, Marta R. Romero1, Beatriz Sanchez de Blas1, María Andrés-Rozas1, Ana Martínez-Alcocer1, Úte Schaepfer2, Małgorzata Milkiewicz2, Piotr Milkiewicz2, Rubén Nogueiras3, Jordi Gracia-Sancho4, Jose Juan García-Marín2,3, María L Martínez-Chantar1.

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Background and Aims: Primary biliary cholangitis (PBC) and primary sclerosing cholangitis (PSC) are common chronic cholestatic liver diseases (CLDs) characterized by bile duct inflammation-induced cholestasis and profibrotic processes. However, the underlying cause remains unknown. Perturbations in magnesium (Mg2+) homeostasis, a crucial cofactor in numerous enzymatic reactions and a regulator of mitochondrial and endoplasmic reticulum (ER) functions, have been observed in acute and chronic liver diseases. In this study, we aimed to characterize Mg2+ homeostasis in clinical and preclinical cholestasis models, with the goal of identifying a potential therapeutic target.

Methods: We studied Mg2+ homeostasis in liver biopsies from patients with PBC and PSC. Bile duct ligation (BDL) and mice lacking Mdr2 (Mdr2/-) were used as animal models of cholestasis. Following GalNAc-siCnnm4 therapy (3mg/kg) hepatic injury, inflammation, fibrosis and bile acid metabolism were assessed in vivo. Liver samples were studied using proteomics analysis. The effect of Cnnm4 silencing was also evaluated in precision cut human liver slices.

Results: Screening hepatic Mg2+ transporters showed upregulated levels of Cyclin M4 (CNNM4), a Mg2+ extruder, in PBC and PSC patients, and in preclinical models. Targeting CNNM4 with GalNAc-based approach ameliorated cholestasis in vivo, as it prevented CNNM4 overexpression, restored Mg2+ homeostasis, and reversed inflammation and fibrosis in BDL and Mdr2/- mice. Mechanistically, CNNM4 targeting modulates bile acid (BA) homeostasis. NIRBAD confirmed that GalNAc-siRNA therapy reduces liver BA emptying time by one-third in Mdr2/- mice, similar to wild-type mice. Proteome analysis of cholestatic models unveiled an antifibrotic and anti-inflammatory response upon restoration of Mg2+ homeostasis CNNM4-dependent. Moreover, the efficacy of GalNAc-siCnnm4 therapy was tested in precision cut human liver slices, and the treatment significantly reduced fibrosis.

Conclusions: Altered Mg2+ homeostasis is crucial in cholestatic liver disease. Restoring hepatic Mg2+ levels by targeting CNNM4 overexpression reverses cholestatic hallmarks, reducing liver fibrosis development and offering a novel therapeutic approach.
Microbiota dysbiosis, altered metabolomic profile and metabolite-sensing GPCRs expression is found in ileal resections from fibrotic-CD patients

Bauset, C.1, Carda-Diéquez, M.2, Buetas, E.2, Seco-Cervera, M.2, Macías-CEja, DC.1, Ortiz-Masiá, D.1,2, Calatayud, S.1,2, Barrachina, MD.1,3, Mira, A.2, Cosín-Roger, J.1,3.

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Background and Aims: Crohn’s Disease (CD) is a subtype of IBD characterized by chronic transmural inflammation of the gastrointestinal tract associated with several complications being intestinal fibrosis the most frequent. CD patients present microbiota dysbiosis and altered metabolomic profiles. GPCRs constitute a family of receptors which could be involved in inflammatory and fibrotic processes associated to CD. We aim to characterize microbiota composition, tissue metabolomic profile and metabolite-sensing GPCRs expression in ileal resections from fibrotic CD patients.

Methods: Ileal resections from B2CD (n=21) and non-IBD (n=13) patients were obtained. Microbiota characterization was performed by 16S rRNA gene Illumina Miseq sequencing and non-parametric Wilcoxon test was used to compare species proportions. Metabolomic analysis was performed by NMR. Results are expressed as μg metabolite/g tissue. Murine intestinal fibrosis was induced in C67BL/6 mice by: a) the heterotopic transplant model and b) chronic DSS-colitis model. Gene expression of GPCRs was analyzed by qPCR. Data were expressed as fold induction (mean±SEM) and compared by a t-test. Correlations were analyzed with the Spearman coefficient.

Results: First, microbiota analysis revealed a reduction in bacterial diversity and load in fibrotic CD patients. Then, in B2CD samples we found at genus level Enterococcus genera significantly decreased and at species level Ruminococcus bromii and Faecalibacterium prausnitzii also reduced compared to controls.

From the metabolomic analysis, altered levels of metabolites were found in ileal resections from fibrotic CD patients. Besides, B2CD patients exhibited differential expression of metabolite-sensing GPCRs vs non-IBD. Results are summarized in Table 1.

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>Non-IBD</th>
<th>B2CD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Propionic acid</td>
<td>4.09±0.45</td>
<td>7.82±0.97</td>
</tr>
<tr>
<td>Succinic acid</td>
<td>7.17±0.83</td>
<td>14.06±1.70</td>
</tr>
<tr>
<td>β-hydroxybutyric acid</td>
<td>9.05±0.88</td>
<td>13.13±1.07</td>
</tr>
<tr>
<td>Acetic acid</td>
<td>10.81±1.33</td>
<td>16.01±1.82</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>12.09±1.59</td>
<td>25.09±3.21</td>
</tr>
<tr>
<td>GPR91</td>
<td>1.66±0.34</td>
<td>21.86±8.24</td>
</tr>
<tr>
<td>GPR109A</td>
<td>4.12±2.01</td>
<td>57.89±18.87</td>
</tr>
<tr>
<td>GPR132</td>
<td>1.47±0.34</td>
<td>6.01±1.95</td>
</tr>
<tr>
<td>GPR40</td>
<td>2.00±0.50</td>
<td>17.33±4.07</td>
</tr>
<tr>
<td>GPR84</td>
<td>1.73±0.43</td>
<td>26.53±10.91</td>
</tr>
<tr>
<td>GPR120</td>
<td>2.09±0.57</td>
<td>0.40±0.06</td>
</tr>
<tr>
<td>GPR119</td>
<td>2.15±0.48</td>
<td>120.9±43.65</td>
</tr>
</tbody>
</table>

Moreover, gene expression of fibrotic markers was analyzed in B2CD patients and significantly increased levels of COL1A1 (13.22±4.38), COL3A1 (1.84±0.52), and COL4A1 (7.75±2.19), were found vs controls. Of interest, GPR81, GPR84, GPR4 and GPR68 positively correlated with pro-fibrotic markers, specifically with COL1A1 and COL4A1.

Finally, expression of metabolite-sensing GPCRs in the two different murine colitis models was also altered.

Conclusions: Fibrotic CD patients exhibit microbiota dysbiosis, joined with altered metabolomic profile and gene expression of metabolite-sensing GPCRs, which are also affected in murine colitis models. Their correlation with pro-fibrotic markers suggest their involvement in intestinal fibrosis.
Oral communications

004 - Exploring the role of x-binding protein-1 (XBP1) in the gut-liver axis during alcohol-related liver disease (ArLD)

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Background & Aims: In the context of alcohol-related liver disease (ArLD), changes in the homeostasis of the gut-liver axis have become a focus of major attention in the past few years. The transcription factor X-Binding protein-1 (XBP1) is a major regulator of UPR, mediating adaptation to ER stress. In the present study, we aimed to analyze the function of XBP1 in intestinal epithelial cells (IECs), and in the liver (hepatocytes) in promoting ArLD.

Methods: Eight- to 13-week-old female and male mice with specific deletion of XBP1 in IECs (XBP1ΔIEC), XBP1 in Hepatocytes (XBP1ΔHEPA), and XBP1-floxed wildtype (XBP1f/f) mice were subjected different experimental models of ArLD: (i) Lieber-DeCarli control and ethanol diet for 8 weeks plus a multiplePBS or EtOH gavage, respectively, and (ii) DUAL diet, a preclinical model of non-alcoholic fatty liver disease/non-alcoholic steatohepatitis (NAFLD/NASH) characterized by the development of metabolic syndrome. Upon sacrifice, organs were extracted, and markers of liver damage, histopathological examination and techniques of Biochemistry and Molecular Biology were performed. Finally, antibiotic treatment (Abx) was performed as a therapeutic approach.

Results: Serum markers of liver damage (e.g.: AST, ALT) were statistically increased in XBP1ΔIEC compared with XBP1f/f after both preclinical models, and associated with significantly higher cell death. Concomitantly, H&E staining of XBP1ΔIEC livers displayed macrovesicular ballooning accompanied by significantly elevated markers of inflammation including TNFα, IL-6, MCP-1, TLR2/4, liver fibrosis (Sirius red and collagen I deposition), and signs of bacterial translocation into the liver (LPS, LTA and bacterial DNA). Furthermore, the content of intrahepatic triglycerides revealed significantly increased lipid deposition in XBP1ΔIEC compared with XBP1f/f after both types of preclinical models. Presence of autophagic vacuoles, decreased lysozyme granules and dilation of the Golgi cisterns associated with loss of Paneth cells and increased gut permeability (Mucin-2, ZO-1) was characteristic of XBP1ΔIEC compared with XBP1f/f ilea, after both models of ArLD. Microbiota analysis revealed significantly increased abundance of Lachnospiraceae, Muribaculaceae and Romboutsia in in XBP1ΔIEC mice. Abx therapeutics reversed the inflammatory phenotype of DUAL-ArLD with reduced liver injury, steatosis and fibrosis.

Conclusion: Our results clearly suggest that loss of XBP1 in IECs trigger significant inflammation in the gut-liver axis, opening a novel therapeutic avenue for research in the context of ER stress and ArLD.

This work was supported by the MICINN Retos PID2020-11782RB-100, PID2020-117941RB-100 and PID2020-115299RA-100, all of which were co-funded with FEDER funds and Comunidad de Madrid S2022/BMD-7409. This project has received funding from the European Horizon’s research and innovation programme HORIZON-2022-STAYHLTH-02 under agreement No 101095679. The research group belongs to the validated Research Groups Ref. 970955 Liver Pathophysiology. CSG is a Atracción de Talento researcher (2019-T1/BMD-13313) and RBU is a UCM Ph.D. student – Banco Santander (CT65/19)
Peptidyl prolyl isomerase C in chronic liver disease: pathogenic or protective mechanism?

Isabel Fuster-Martínez1, Ana M. Benedicto1,2, José F. Català-Senent3, Marta R. Hidalgo1, Francisco García-Garcia2, Juan V. Esplugues1,2,4, Nadezda Apostolova1,2,4, Ana Blas-García2,4,5.

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Background and Aims: Pan-cyclophilin inhibitors have been proposed as a strategy to treat metabolic dysfunction-associated steatotic liver disease (MASLD). Although some members of this family are well known, little is described about Peptidylprolyl isomerase C (PPIC), whose expression has been reported to increase in human MASLD. We aimed to characterize its role in chronic liver diseases.

Methods: Gene expression of PPIC was analysed using different experimental approaches: a meta-analysis from published transcriptomic studies of liver samples of murine models of high fat diet (HFD)-induced MASLD, with and without anti-steatotic treatments, murine models of chronic liver injury induced by CCl4 and Bile Duct Ligation (BDL), and in vitro, using fatty acid (FA)-overloaded human hepatoma cells (Hep3B) and TGFβ1-stimulated human hepatic stellate cells (LX-2). In these cell lines, key experiments were performed silencing PPIC, both in basal and the above mentioned experimental conditions.

Results: Hepatic expression of Ppic was significantly increased in the models of liver injury studied (HFD, CCI4, and BDL), and reversed by the anti-steatotic treatment explored. Moreover, it was increased in FA-overloaded Hep3B cells and TGFβ1-stimulated LX-2 cells. When PPIC was silenced in Hep3B cells, cell viability was not altered, but there was an activation of IRE1α-XBP1 pathway (involved in the endoplasmic reticulum-ER- stress response), a decrease of NF-κB activation and a down regulated expression of chemokines CXCL9 and CXCL10. In LX-2 cells, PPIC silencing triggered pathways related to the ER stress response (IRE1α-XBP1 and eIF2α) and the stress-induced JNK pathway, and exerted a pro-fibrotic effect (enhanced collagen and α-SMA expression). It also decreased gene expression of NLRP3-inflammasome components in these cells.

Conclusions: Increased hepatic PPIC expression is a potential marker of chronic liver injury. These data suggest a pleiotropic role of PPIC in chronic liver diseases, therefore further studies are needed to clarify its potential as a therapeutic target.
O06 - Dissecting submucosal fibroblast populations of human colon Crohn’s Disease patients through spatial transcriptomics

Victoria Gudiño1, Mario Acera2, Ana Mª Corraliza1, Alba Garrido1, Ángela Sanzo1, Holger Heyn3, Elisabetta Mereu2 and Azucena Salas1.

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3Single cell genomics, National Centre for Genomic Analysis (CNAG), Barcelona, Spain

Background and Aims: Recent studies using single-cell RNA sequencing (scRNA-seq) estimate in >80 the number of cell types that reside within the human intestine. Given this complexity, unraveling the interactions and tissue organization of all these cellular types, particularly in the context of chronic intestinal inflammation, remains an important challenge. Here, we apply spatial transcriptomics to human healthy and Crohn’s disease (CD) intestine and provide a map of cell types across healthy and diseased colon.

Methods: Fresh frozen colonic sections from healthy and CD patients were captured onto 10X Visium slides containing barcoded spots. For each tissue section, total RNA from 55µm diameter spots was barcoded and sequenced. Data was deconvoluted using scRNAseq datasets with Cell2location, and ligand-receptor interactions were analyzed using NICHES. For validation and further interrogation of tissue structure and cellular dynamics at single-cell resolution, a panel of 100 genes were measured using single molecule fluorescent in situ hybridization (smFISH, Molecular Cartography, Resolve Biosciences).

Results: Spatial transcriptomics analysis reveals 13 different clusters of spots differentially distributed across tissue areas and samples. CD-specific clusters that were widely abundant across sections included inflammatory fibroblasts and IgG plasma cells, while others like neutrophils and M1 macrophages were limited to inflammatory foci. Within the submucosa, inflamed samples presented an increase in GREM1+ fibroblasts, which were annotated as S3 fibroblasts after scRNA-seq deconvolution. As shown by smFISH, GREM1 was the main fibroblast-specific transcript expressed in the submucosa. While NICHES cell interaction analysis revealed KDR as the main and unique GREM1 interacting partner, we only observed partial co-localization of GREM1 and KDR by smFISH, highlighting the importance of validation when conducting computational analysis.

Conclusions: Using two different spatial transcriptomics technologies, we resolved the tissue distribution of cell populations previously described by scRNAseq and characterized inflammatory-associated cell interactions.
O07 - Circulating bone morphogenetic protein 8A is a novel biomarker to predict advanced liver fibrosis

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Background and Aims: Advanced hepatic fibrosis is the main risk factor of liver-related morbidity and mortality in patients with chronic liver disease. In this study, we assessed the potential role of bone morphogenetic protein 8A (BMP8A) as a novel target involved in liver fibrosis progression.

Methods: Histological assessment and BMP8A expression were determined in different murine models of hepatic fibrosis. Furthermore, serum BMP8A was measured in mice with bile duct ligation (BDL), in 36 subjects with histologically normal liver (NL) and in 85 patients with biopsy-proven non-alcoholic steatohepatitis (NASH): 52 with non- or mild fibrosis (F0-F2) and 33 with advanced fibrosis (F3-F4). BMP8A expression and secretion was also determined in cultured human hepatocyte-derived (Huh7) and human hepatic stellate (LX2) cells stimulated with transforming growth factor β (TGFβ).

Results: Bmp8a mRNA levels were significantly upregulated in livers from fibrotic mice compared to control animals. Notably, serum BMP8A levels were also elevated in BDL mice. In addition, in vitro experiments showed increased expression and secretion to the culture supernatant of BMP8A in both Huh7 and LX2 cells treated with TGFβ. Noteworthy, we found that serum BMP8A levels were significantly higher in NASH patients with advanced fibrosis than in those with non- or mild fibrosis. In fact, the AUROC of circulating BMP8A concentrations to identify patients with advanced fibrosis (F3-F4) was 0.74 (p<0.0001). Moreover, we developed an algorithm based on serum BMP8A levels that showed an AUROC of 0.818 (p<0.0001) to predict advanced fibrosis in NASH patients.

Conclusions: This study provides experimental and clinical evidence indicating that BMP8A is a novel molecular target linked to liver fibrosis and introduces an efficient algorithm based on serum BMP8A levels to screen patients at risk for advanced hepatic fibrosis.
**Background and Aims:** The aged liver exhibits hepatic microcirculatory dysfunction and sinusoidal fibrosis, which may be partly due to cell senescence. The senolytic drug Navitoclax, which interferes with the pro-survival pathways of senescent cells, ameliorates pathological effects of senescence. However, senolytic treatment to the clinic is still limited due to low cell specificity and toxic effects. Here, we aimed at developing a nanoformulation containing navitoclax as an efficient strategy to target the hepatic sinusoid in preclinical models of healthy aging.

**Methods:** Nanoformulation: A library of ~40 nanoparticles (NP) containing navitoclax was designed to be disassembled only in cells exhibiting increased SA-B-galactosidase activity. Nanoformulation sbioactivity was screened to identify the best one targeting senescent endothelial cells. Hepatic effects: 20 months old Wistar rats were injected with either vehicle, soluble navitoclax (50mg/kg/day) or navitoclax-containing nanoparticles (NPs; 0.2mg/kg/day) (n=8 per group). After a four-week resting period, in vivo hepatic and systemic hemodynamic, biochemical parameters, as well as molecular markers of liver fibrosis, senescence, and hepatic phenotype were assessed.

**Results:** The NP library screening showed a nanoformulation with an optimized senolytic index of over 40x when compared with soluble navitoclax. Upon in vivo administration, selected NPs were mostly accumulated in the liver. Aged animals treated with NPs exhibited a reduction in portal pressure compared to vehicle (8.6±0.2vs. 9.3±0.3, p=0.059), which was not observed with oral navitoclax treatment. Beneficial effects of novel NPs were associated with reduction in senescence markers (IL6-72% and MMP13-57%), improvement in hepatocyte phenotype (+52% hnf4; +51% slc22a, +5% albumin production) and, importantly, amelioration of hepatic fibrosis (-20% Sirius red staining).

**Conclusions:** Specifically targeting liver senescent cells in aging is possible using novel formulation of senolytic nanoparticles. This technology rejuvenates liver function & architecture, thus representing a unique therapeutic opportunity for diseases coursing with cellular senescence.
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**Background and Aims:** Colonic fibroblasts (FBLs) have emerged as key players in inflammatory bowel disease (IBD) for their potential to acquire a pro-inflammatory phenotype, but the factors driving this process and the interaction between these fibroblasts and the immune system are incompletely understood. The TNF superfamily factor 12 (TNFSF12/TWEAK) has gained interest as a potential mediator of chronic inflammation and tissue remodelling in IBD. Here, we explore its role as a driver of FBL inflammatory differentiation and a modulator of fibroblast-monocyte interaction.

**Methods:** Human primary colonic fibroblasts (FBLs) were stimulated with TWEAK for 24 hours. Subsequently, FBLs were co-cultured with THP1 cells or peripheral blood mononuclear cells (PBMCs) for an additional 24 hours to investigate their interaction. RNA sequencing, ELISA and flow cytometry were used for analysis.

**Results:** TWEAK induced a pro-inflammatory programme in FBLs characterised by the upregulation of inflammatory cytokines, chemokines and immune receptors, which resembled an inflammatory stroma subpopulation previously identified in ulcerative colitis. At protein level, TWEAK provoked changes in the expression of stromal markers (PDPN, PDGFRα); stimulated the secretion of IL-6, CCL2 and CXCL10; and enhanced the expression of surface adhesion molecules (ICAM-1, VCAM-1).

Co-culture of monocytes with FBLs induced monocyte adhesion and promoted a CD14\textsuperscript{high}/ICAM-1\textsuperscript{high} phenotype in THP1 cells, both of which were further enhanced when fibroblasts were pre-stimulated with TWEAK. Moreover, TWEAK-treated FBL-monocyte co-cultures secreted elevated levels of CXCL1 and IL-8 compared to vehicle-treated co-cultures, THP1 mono-cultures or FBL mono-cultures. Co-culture of PBMCs with FBLs also altered their polarisation state, characterised by the loss of CCR2 and CD16, and promoted CXCL1 and CXCL10 secretion, a phenotype further enriched by co-culture with TWEAK pre-treated FBLs.

**Conclusions:** Our results indicate that TWEAK can promote pro-inflammatory polarisation in colonic fibroblast and positions TWEAK as a potential mediator in the pro-inflammatory interaction between fibroblasts and monocytes.
O10 - Identification and assessment of circulating biomarkers for the evaluation of liver disease severity in NAFLD patients

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Background and aims: Practice Guidelines recommend two-step evaluations of liver disease severity using sequential algorithms combining NITs like FIB4, NFS or HFS to confirmatory tests like TE or ELF. First line NITs are extremely useful to detect, specially exclude, advanced liver disease, but offer modest sensitivity and specificity leading to many undiagnosed cases in addition to many unnecessarily referrals to hospitals owe to a lack of confirmatory tests like TE at relevant screening sites like primary and endocrine units. Finding new biomarkers that could be easily assessed in these units to improve the selection of patients referred to hepatology units is a must.

Methods: Candidates were identified by two-step metanalysis analyzing gene expression in biopsies from NAFLD patients [Identification cohort, 3 cohorts analyzed by Microarray, F0-F1 (139) vs. F2-F4 (47)] [Validation cohort, 4 cohorts analyzed by RNA-seq, F0-F1 (234) vs. F2-F4 (197)] Average ranking together with additional filters to retain bloodstream circulating secreted proteins were used to select 5 FICEs (Fibrosis Inducible Circulating Elements) which were explored in serum samples from a cohort of NAFLD biopsy-proven patients coming from three different hospitals in Spain (N=205).

Results: FICE4 diagnostic accuracy is superior to all routinary NITs (FIB-4, NFS, HFS, APRI) for the detection of significant and advanced fibrosis (F2+ or F3+), and similar to the one obtained by TE or ELF (Fig1a-b). Interestingly, FICE-4 circulating levels were also able to identify at-risk patients with similar efficacy to the recently developed FAST algorithm. Combination of FICE4 with some routinary clinical variables (FICE4C) significantly improved its diagnostic accuracy (Fig 1c-d).

Conclusion: FICE4 can be easily assessed by ELISA and constitutes a useful tool to evaluate liver disease severity that could be useful for screening sites in which confirmatory tests like TE or ELF are not easily accessible.
O11 - Oral administration *B. longum* sub. *Infantis* 16p1 approximates gut microbiota towards eubiosis and reduces fibrosis in the experimental model of CCl₄-induced liver injury

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**Background and Aims:** Microbiota dysbiosis is a key feature of advanced chronic liver disease, favoring the progression of the inflammatory and fibrotic processes. We evaluate the effect of a symbiotic bifidobacterial strain administration on fibrosis progression and on gut microbiota composition in the experimental model of CCl₄-induced cirrhosis.

**Methods:** Cirrhosis was induced by administration of CCl₄ in Sprague Dawley rats. A subgroup of animals received *B. longum* sub. *Infantis* 16p1 (10⁹ CFU/day/ig) or vehicle two weeks prior to laparotomy. A group of untreated animals were used as controls. Liver fibrosis was quantified by expression of profibrogenic genes and sirius red staining. We sequenced the bacterial ribosomal RNA 16S to determine microbiota composition on intestinal content.

**Results:** The administration of the bifidobacterial strain significantly reduced the hepatic fibrotic area induced by CCl₄, as observed by sirius red staining and its quantification in tissue samples (Figure 1A). Profibrogenic gene expression was also significantly higher in cirrhotic vs bifidobacteria-treated animals (Figure 1A). The distribution of taxa in cirrhotic rats treated with the bifidobacterial strain was closer to controls than to cirrhotic animals (Figure 1B). The Principal Coordinates Analysis revealed more similarity between cirrhotic rats treated with the bifidobacterial strain and controls, whereas untreated cirrhotic animals showed a higher distance to the rest of groups (Figure 1C). We performed a differential abundance analysis to know the most representative taxa rising from the comparison between groups. Volcano-plots show the amplicon variant sequences...
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(ASVs) that are more significant to each group in a two-group comparison. As observed, ASVs were significantly lower between cirrhotic rats treated with the bifidobacterial strain and controls than cirrhotic animals (Figure 1D).

Conclusions: The use of the bifidobacterial strain may help restore homeostasis in advance chronic liver disease, as it shifts microbiota composition towards eubiosis and reduces fibrosis in experimental CCl4-induced cirrhosis.
**P01 - COX-2: the new promising target for MAFLD treatment**

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**Background and Aims:** Our previous studies have shown that constitutive expression of Cyclooxygenase-2 (COX-2) in hepatocytes protects against hyperglycemia-induced liver damage, insulin resistance and lipid accumulation in mice fed with high-fat diet. However, to perform a more translational approach, we evaluated the role of COX-2 as a potential therapeutic target in MAFLD once that the liver pathology was already established.

**Methods:** Steatosis and fibrosis were prompted through a high-fat diet feeding for 6 weeks. COX-2 expression was induced in an inducible transgenic murine model (ihCOX-2-Tg) by intravenous administration of $10^{11}$ copies of adenovirus associated serotype 8 (AAV8)-CRE after 1 or 3 weeks with diet. Mice were sacrificed after 6 weeks of diet. Severe fibrosis was provoked through intraperitoneal administration of carbon tetrachloride (CCL$_4$), twice a week, during 9 weeks. COX-2 expression was induced by AAV8-CRE after 4 weeks of CCL$_4$ treatment. Mice were sacrificed after 9 weeks of CCL$_4$ treatment. COX-2 expression was evaluated by western blot and its activity through prostaglandins (PGs) production. Liver damage was assessed by biochemical and histological parameters as well as molecular biology assays.

**Results:** Our ihCOX-2-Tg model presented hepatic COX-2 expression after AAV8-CRE inoculation as well as an increase in hepatic PGs. In addition, the AAV serotype 8 showed hepatic tropism. Our results indicated that mice expressing COX-2 displayed a decrease in liver lipid accumulation, along with lower levels of plasma cholesterol. COX-2 expressing mice also displayed less hepatic fibrosis, accompanied by a reduction in protein levels related to fibrosis (STAT-1, ERK, JNK). These findings were further supported by the data observed in flow cytometry analysis, where mice expressing COX-2 displayed a lower inflammatory profile.

**Conclusions:** Our results point out the promising role of COX-2 as a therapeutic target in MAFLD through the reduction of hepatic steatosis and fibrosis in our murine model.
P02 - The antiretroviral drug Rilpivirine downregulates hepatic stellate cell migration and proliferation: A potential candidate for drug repurposing in liver fibrosis therapy


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Background and Aims: Liver fibrosis, a common feature of liver diseases, currently lacks targeted therapy. Our research showed that the anti-HIV drug Rilpivirine (RPV), exhibits anti-fibrotic effects in various experimental models of liver injury. It diminishes the activation hepatic stellate cells (HSCs) and induces their cell death, however the mechanisms implicated remain unclear.

Methods: Primary human HSCs (from liver resections) were activated with TGF-β or with PDGF-ββ and co-treated with clinically relevant concentrations of RPV (48h). For RNA sequencing analysis, mRNAs libraries were obtained with total RNA. Their size was assessed, and sequencing was performed using a single read of 75 cycles. After differential gene expression, over-representation analysis was done using the databases Gene Ontology (GO), KEGG pathways and Reactome. To validate the impact of RPV on PDGF-ββ-induced chemotaxis, a transwell chemotraction assay was performed (Boyden chamber) and cells were counted (trypan blue).

Results: In the RNA seq analysis, treatment with RPV resulted in differential expression of 2309 genes that impacted various pathways and processes aligning with previous studies (downregulation of collagen biosynthesis and upregulation of apoptosis). Comparing RPV 8µM+TGF-β vs TGF-β alone, novel pathways were revealed. The top downregulated Reactome pathways were “DNA unwinding”, “smooth muscle contraction”, “activation of chaperone genes by XBP1(S)”, “signaling with PDGF”, and “collagen biosynthesis and modifying enzymes”. Conversely, the top upregulated included “OAS antiviral response”, “keratan sulfate degradation”, “STING mediated induction of host immune responses”, “interferon α/β signaling”, and “stabilization of p53”. Similar results with GO terms. The downregulation of activated cell migration capacity and PDGF signaling pathways were observed in different gene set enrichment analysis. These findings were validated in experiments using PDGF-treated HSCs, where RPV effectively inhibited PDGF-ββ-induced chemotaxis and proliferation. Additionally, RPV downregulated PDGF downstream signaling pathways involving AKT and JNK, while not impacting p38 and ERK1/2.

Conclusions: RPV reduces migration and proliferation of HSCs and downregulates downstream signaling pathways in response to PDGF-ββ. These findings may contribute in the search for novel targets or repurposed drugs for treatment of liver fibrosis.
P03 - Comprehensive analysis of epigenetic and epitranscriptomic genes expression in human NAFLD

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Background and Aims: Non-alcoholic fatty liver disease (NAFLD) is a multifactorial condition with a complex etiology. Its incidence is increasing globally in parallel with the obesity epidemic, and it is now considered as the most common liver disease in Western countries. The precise mechanisms underlying the development and progression of NAFLD are complex and still poorly understood. The dysregulation of epigenetic and epitranscriptomic mechanisms is increasingly recognized to play pathogenic roles in multiple conditions, including chronic liver diseases. Epigenetics refer to the heritable modification of gene expression that does not involve changes in the underlying DNA sequence. Recently discovered post-transcriptional RNA modifications, known as epitranscriptomics, have been found to have a profound impact also on gene expression by regulating RNA stability, localization and decoding efficiency.

Methods: Here we have performed a comprehensive analysis of the expression of epigenetic and epitranscriptomic genes in a total of 905 liver tissue samples corresponding to patients with normal liver, obese patients and patients with non-alcoholic fatty liver (NAFL) and non-alcoholic steatohepatitis (NASH), advancing stages in NAFLD progression. We integrated ten transcriptomic datasets in an unbiased manner, enabling their robust analysis and comparison. We describe the complete landscape of epigenetic and epitranscriptomic genes expression along the course of the disease. The epigenetic and epitranscriptomic genes were selected from the literature, Modomics, EpiFactors, and ChromoHub databases to generate a manually curated list. The function of each selected gene was confirmed by the availability in reliable databases (GeneCards, PubMed and Uniprot) of experimental evidence demonstrating their purported biochemical activity. All the genes with no experimental evidence of functional activity were discarded.

Results: We identify signatures of genes significantly dysregulated in association with disease progression, particularly with liver fibrosis development. Most of these epigenetic and epitranscriptomic effectors have not been previously described in human NAFLD, and their altered expression may have pathogenic implications. We also performed a comprehensive analysis of the expression of enzymes involved in the metabolism of the substrates and cofactors of epigenetic and epitranscriptomic effectors.

Conclusions: This study provides novel information on NAFLD pathogenesis, and may also guide the identification of drug targets to treat this condition and its progression towards hepatocellular carcinoma.
**Methods:** C57Bl6 male mice received 10% of alcohol in the drinking water and western diet (WD) for 10 and 23 weeks (DUAL model). Liver and gut histology, as well as inflammation, intestinal permeability and 16s rRNA microbiome profiling were analyzed. Short- and long-term DUAL feeding was used to evaluate the effects of the microbiome through a) Use of broad spectrum of antibiotics (ABX); b) Fecal microbiome transplantation (FMT) from healthy murine donors. Results were compared to classical clinical recommendation of dietary modifications (DUAL withdrawal).

**Results:** DUAL mice exhibited strong damage in gut, intestinal inflammation, increased gut permeability and dysbiosis characterized by increase of Gram-negative bacteria and simultaneous decrease of commensal bacteria. Dysbiosis found in DUAL animals was comparable to results in MetALD patients. Microbiome depletion (by ABX) proved that dysbiosis is a target player in MetALD pathophysiology in terms of steatosis, inflammation, and fibrosis, therefore microbiome could be used as a therapeutic target. Microbiome modulation via FMT induced mild improvement in liver and gut physiology, but only in short-term DUAL feeding. DUAL withdrawal was the best therapeutic option for early CLD induced by short-term feeding of 10 weeks. Nevertheless, the diet withdrawal after advanced 23 weeks DUAL feeding had only minor beneficial effect on the liver.

**Conclusions:** Intestinal dysbiosis is a critical factor for MetALD. Gut-based early therapeutic interventions could be beneficial to halt the progression of disease.
Conclusions: LSEC derived EK1 up regulation in advanced CLD promotes paracrine activation of HSCs through EK1R. Inhibition of EK1 EK1R axis is a promising therapeutic approach for liver cirrhosis. Finally, future analysis will clarify the usefulness of EK1 as biomarker of advanced CLD.

Background and aims: Liver sinusoidal endothelial cells (LSECs) play a pivotal role maintaining the hepatic microcirculation through paracrine interactions with hepatic stellate cells (HSCs). In chronic liver disease (CLD), LSECs de differentiate altering their angiocrine function and leading to liver cirrhosis complications. We previously identified Endokyne1 (EK1) as a highly up regulated cytokine in cirrhotic LSECs. This study aimed to characterize the role of LSEC derived EK1 and its receptor EK1R in CLD.

Methods: Healthy or cirrhotic human liver tissue were used for gene and protein assays. LSECs and HSCs were isolated from rats with different stages of liver disease and were used for EK1 gain and loss of function and subsequent molecular analysis.

Results: Analysis of transcriptomic data from five patient cohorts showed significant increased EK1 expression in advanced CLD. This upregulation was confirmed in liver tissue and serum samples from patients with advanced CLD and portal hypertension. Immunofluorescence analysis revealed specific localization of EK1 in cirrhotic LSECs. Hepatic EK1 up regulation was validated at the protein and mRNA levels in a preclinical model of liver disease progression, being LSECs the main source of EK1 by specific cellular expression and secretion. Moreover, serum EK1 levels were increased in cirrhotic rats. Additionally, treatment of quiescent HSCs with recombinant EK1 resulted in EK1R up regulation together with increased expression of αSMA and collagen1α1, suggesting HSCs activation. Silencing EK1 in cirrhotic LSECs prevented HSCs activation through EK1R expression reduction. Moreover, when cirrhotic HSCs were treated with an EK1R antagonist, exogenous EK1 did not maintain their activated phenotype showing down regulation in αSMA and collagen1α1 expression.
P06 - Efficacy of dietary and physical activity intervention in MetALD

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Background and Aims: Nowadays, the lifestyle in the prosperous parts of the world often lead to MetALD (Metabolic Dysfunction Associated Steatotic Liver Disease (MASLD) plus increase alcohol intake). However, the mechanisms by which high fat/sugar diet and alcohol together trigger the liver damage remain unclear, moreover, the treatment options are limited. In the present study, we used a synergistic DUAL murine model of MASLD and alcohol consumption. We aimed to analyse if: 1. MetALD is reversible, 2. lifestyle modifications including increased physical activity, and dietary changes can be the treatment of choice for MetALD.

Methods: C57BL/6 male mice received 10% alcohol in sweetened drinking water together with a Western diet (DUAL model) for short (10 weeks) and long (23 weeks) period followed by either (A) the replacement with chow diet and normal water for 20 days (WTD); or (B) chow diet/normal water plus treadmill sessions for 20 days (WTD+EXER). Metabolic syndrome (MS), serum parameters, liver, e-WAT and intestine histology were analysed in detail.

Results: After the short-term DUAL feeding: diet WTD alone or WTD in combination with treadmill running was able to decrease obesity, adiposity, hepatomegaly and steatohepatitis, enhance b-oxidation in the liver, improve intestinal permeability. After the long term feeding only the combination of diet withdrawal and treadmill running (WTD+EXER) reduced obesity and adiposity, normalized levels of glucose, cholesterol and transaminases in blood, decreased hepatomegaly and, liver damage, hepatic inflammation, attenuated activation of HSC, improved gut permeability.

Conclusions: Dietary modification, alone or combination with physical exercisers in patients with the initial stages of MetALD might be considered as an efficient non-pharmacological therapy. However, only a combination of dietary changes and physical activity can lead to the clinical improvement at the advance stages of steatohepatitis.

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P07 - Sphingolipid metabolism is dysregulated in liver fibrosis: insights from spatial lipidomics

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Background and Aims: During fibrogenesis, specific lipid pathways are re-wired in the liver and this is especially relevant for steatosis-associated liver fibrosis (LF) where increased hepatic storage of potentially lipotoxic species occurs. The aim of this study is to uncover the complex metabolic adaptations and identify key lipid markers of LF.

Methods: We studied 3 animal models of LF: bile duct ligation, chronic CCl4 treatment and western diet + CCl4 (all in C57BL/6 male mice, n=5). Cirrhotic liver tissue from patients with fatty liver-associated chronic liver disease (n=21) and background liver from patients with metastatic colorectal carcinoma (n=36) were collected. RNA sequencing was performed on mouse liver samples. Hepatic lipids were extracted from mouse and human tissue and analysed using liquid chromatography mass spectrometry (LC-MS) to study metabolic changes. Matrix-assisted laser desorption/ionization (MALDI) - MS imaging (MSI) on cryosectioned human liver tissue were used to explore the spatial distribution of altered lipids.

Results: RNA sequencing revealed upregulation of glycosphingolipid metabolism in the fibrotic groups compared to their control groups in the animal models. Differential gene expression showed an increased gene expression of the enzymes implicated in ceramide production and these results were confirmed using LC-MS analysis, demonstrating higher amounts of hexose ceramides and sphingomyelins (SM) in the fibrotic groups. In the human samples, LC-MS analysis showed that phospholipids with short carbon chains and SM(34:1) were relatively increased in cirrhosis. In addition, spatial lipidomics by MALDI-MSI revealed a dramatic increase of SM(34:1) in the fibrotic regions of the tissue (Figure).

Conclusions: Overall, these results have identified key lipid markers for LF and suggest that altered sphingolipid metabolism is a major process occurring during fibrogenesis. Targeting these metabolic pathways could be a novel strategy to resolve LF.
P08 - Isolation and characterization of extracellular vesicles (EVs) in faecal matter: Implications for future biomarker discovery

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Background & Aims: Extracellular vesicles (EVs) are microscopic particles (~30 nm to 10 μm) abundantly released into body fluids by all types of cells. EVs are of growing interest due to their potential diagnostic, disease surveillance, and therapeutic applications. While several studies have evaluated EV isolation methods in various biofluids, there are only few data on these techniques when applied to stool. However, the feces are an ideal biospecimen for studying intestinal inflammation and damage. In this study, we aimed to assess EVs from murine faeces (fEVs) for reproducibility, purity, and protein expression in stool supernatant.

Methods: We used stool from 33 weeks old C57BL/6J mice. fEVs were isolated by ultracentrifugation (UC), and fully characterized by FACS, nanoparticle tracking analysis (NTA), Western blotting and Transmission Electron Microscopy (TEM).

Results: The isolated fEVs confirmed size homogeneity in the nanoparticle population at the range of 50-100 nm, together with high fEV concentration and high protein yield in the isolated samples. Vesicle-associated markers CD63, CD81 and Alix were present in fEV fractions. Importantly we showed, the fEVs are released by two main domains - eukaryotes and bacteria. The presence of fEVs from Gram-negative and Gram-positive bacteria was detected by Western blot using anti-lipid A and anti-lipoteichoic acid antibodies, whereas Western blot using anti-beta-actin antibody was employed to detect host-derived EVs. Further, fEVs were administered into mice by intraperitoneal injection, and inflammatory responses were investigated in the peritoneum by FACS. Bacteria-free fEVs from healthy C57BL/6J mice introduced into the peritoneum did not induce local and systemic inflammation.

Conclusions: These findings serve as the groundwork for future studies in order to investigate the potential of fEVs as a source for novel biomarkers for diagnosis of intestinal homeostasis.

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P09 - Role of Fbln7 in the dedifferentiation of liver sinusoidal endothelial cells in cirrhotic portal hypertension

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Background and Aims: Liver sinusoidal endothelial cells (LSECs) play crucial roles in liver function. Hepatic microcirculatory dysfunction, which includes LSECs dedifferentiation, leads to portal hypertension (PH) in chronic liver disease (CLD). Recently, we demonstrated that PH itself contributes to LSECs deregulation. This study aimed to identify and characterize drivers of LSECs dysregulation under pathological pressure with potential for therapeutic modulation.

Methods: Primary LSECs were cultured under normal (1mmHg) or pathologic (12mmHg) hydrodynamic pressure using a microfluidic liver-on-a-chip. Gene discovery was performed by RNAseq in these settings and in LSECs from three preclinical models of cirrhosis. Validations were performed in liver biopsies from a cohort of well-characterized patients with CLD and PH (n=14) and healthy individuals (n=8). Gain/loss of function experiments were conducted with siRNA and adenoviral constructs in primary LSECs from CCl4-induced cirrhotic (CH) or healthy (CT) rats, respectively.

Results: Transcriptomic analysis of LSECs under pathological pressure and three preclinical models of CLD revealed Fbln7 as a pressure-modulated molecule markedly upregulated in CLD. Increased hepatic Fbln7 protein levels were validated in CH patients (fc=3.2), which was specifically expressed by LSECs in the sinusoid and localized in the plasma membrane. Fbln7 plasma levels significantly correlated with HVPG.

In vitro, Fbln7 rapidly decreased during culture without pathological pressure stimulation. siRNA-mediated inhibition of Fbln7 in primary LSECs isolated from CH rats modified key pathways, including ROS production and plasma lipoprotein clearance, mainly affecting cell surface and extracellular proteins, suggesting paracrine effects. Indeed, supernatant of endothelial cells overexpressing Fbln7 promoted primary human HSC deactivation, reducing ACTA2 (~38%) and PDGFRB (~33%) levels. All changes p>0.05.

Conclusions: Fbln7 is overexpressed in the cirrhotic sinusoidal endothelium in response to increased hydrodynamic pressure, showing compensatory protective effects on HSCs. Ongoing in vivo studies will determine its therapeutic potential for liver disease.
P10 - Evaluation of the effects of a JNK1 Inhibitor on the progression of Non-Alcoholic fatty liver disease

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**Background and Aims:** Non-alcoholic fatty liver disease (NAFLD) is considered the hepatic manifestation of the Metabolic Syndrome and, in the most advanced stages, can lead to fibrosis, cirrhosis or even hepatocellular carcinoma. Interleukin 1 beta (IL1beta) is a key factor in the progression of this disease, pointing it out as a promising therapeutic target for the treatment of both NAFLD and other chronic inflammatory pathologies. The aim of the study was to assess the impact of a new inhibitor which blocks IL1beta synthesis on the progression of NAFLD.

**Methods:** The compound AIK3aXXX, developed by Allinky Biopharma, is an allosteric inhibitor of JNK1 kinase, which selectively inhibits IL1beta synthesis mediated by the activation of this kinase. For this purpose, male mice fed with standard diet or high-fat diet (HFD) were treated with AIK3aXXX or its corresponding vehicle. At the end of the experiment, different histological and molecular analysis of the livers were performed. In addition, in vitro assays were conducted on different cell lines to further elucidate the molecular mechanisms involved in AIK3aXXX-induced effects.

**Results:** The results revealed that AIK3aXXX slowed down the progression of NAFLD in HFD mice. Specifically, mice treated with the compound exhibited a lower degree of lobular inflammation and hepatocellular degeneration compared to mice treated with the vehicle. Moreover, the inhibitor reduced the development of fibrosis in HFD mice. In fact, a lower hepatic expression of fibrogenic markers was observed in HFD mice treated with AIK3aXXX. In vitro experiments showed that this compound selectively inhibited IL1beta expression in macrophages activated by both LPS and palmitate. Furthermore, AIK3aXXX reduced the activation of hepatic stellate cells.

**Conclusions:** In conclusion, these results indicate the therapeutic potential of the compound AIK3aXXX to confront the progression of NAFLD.
Conclusion: This study shows, for the first time, the antifibrotic effects of GTX-011m in liver human tissue, confirming previous results in a preclinical model of steatohepatitis. These results encourage its clinical evaluation as a possible new treatment for this disease.

Figure:
Posters. Basic Research in Liver Fibrosis

P12 - Presence of urine metabolomic biomarkers of liver fibrosis in early stages of liver disease

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Background and Aims: Liver fibrosis is one of the major complications of the development of non-alcoholic fatty liver disease (NAFLD). Some authors proposed urine metabolomic biomarkers of liver fibrosis [Wu,F et al. 2017; Biliotti, E et al. 2021; Qin, J et al. 2023]. However, these studies reflect late-stage fibrosis and their value for early detection has not been demonstrated. To estimate the predictive or early detection value of these biomarkers, we study them in a model of early subclinical liver disease. The main objective of our research was to analyze the variation of these urine metabolomic biomarkers in the early stages of liver disease in a longitudinal study.

Methods: Male and female Wistar rats were randomly divided into different groups fed with a control diet (CTL group) or with a 45% high-fat diet (HFD group). Animals were fed for 21 weeks, and urine samples were obtained every three weeks. Samples of urine were measured with a Nuclear Magnetic Resonance (NMR) spectrometer. Animals were sacrificed at week 21 and the liver was analyzed histologically and biochemically. Statistical analysis was carried out with SPSS and statistical significance was established at 95%.

Results: Urine metabolomic biomarkers of fibrosis were analyzed throughout the 21 weeks. In the early stage of liver disease without fibrosis, all these biomarkers are already altered. However, some of them oscillated throughout the 21 weeks and their value as predictive biomarkers was therefore decreased. Others, on the other hand, were altered in early stages and remained stable during the whole study suggesting a predictive and early detection value. Interestingly, differences between males and females exhibit different trends in these biomarkers, which suggest that a stratified analysis may be required in the future.

Conclusions: Metabolomics is a technique that may allow early detection of fibrosis but studies in subclinical disease models are needed for validation.

P13 - The role of sex in preclinical research on portal hypertension and cirrhosis

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Background and Aims: Cirrhosis is a major health concern, nevertheless the sex-specific differences in its pathophysiology remain unclear. Indeed, preclinical studies predominantly focused on male animals, limiting the applicability of findings to both sexes. This research project explored cirrhosis and portal hypertension in rats, aiming to understand the gender complexities and implications.

Methods: Male and female 3 months-old Sprague-Dawley rats received chronic thioacetamide (TAA; 250mg/kg; 12 weeks) as model of cirrhosis, in addition to healthy control rats (n=11-18/group). Parameters of hepatic and systemic hemodynamics were assessed in vivo, including mean arterial pressure, portal pressure (PP), portal blood flow (PBF), and intrahepatic vascular resistance (IHVR). Hepatic microvascular function in response to incremental acetylcholine and hepatic sinusoid fenestrae through scanning electron microscopy were also evaluated. Furthermore, a transcriptomic analysis of hepatic tissue was performed (n=5/group).

Results: Both sexes exhibited similar PP (14.2 vs 14.1 mmHg), with no differences in PBF, IHVR, or hepatic microvascular dysfunction, yet female TAA rats showed a trend towards higher number of fenestrae and porosity than male TAA (p=0.1). Transcriptional analysis revealed a similar degree of dysregulation in cirrhotic male and female livers, with 325/3697 differentially expressed genes compared to healthy rats. Among dysregulated genes in TAA rats, 2063 were common in both sexes, mostly associated with fibrogenesis and metabolism pathways. However, 1208 genes were exclusively dysregulated in males, related to inflammation and immune response pathways. In contrast, women showed 1646 exclusively dysregulated genes, associated to a more balanced involvement of disease-related pathways, including genes involved in growth hormone signaling.

Conclusions: This study evidences the molecular differences of sex in the pathophysiology of cirrhosis in a commonly used model for preclinical research.
Further studies in human liver disease are essential to accelerate the development of safe and effective treatments for chronic liver disease in both sexes.

**P14 - Gelatin hydrogels of controlled stiffness for the in vitro 3D modelling of liver fibrosis**

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**Background and Aims:** Chronic liver damage can cause liver fibrosis, characterized by the gradual increase of extracellular matrix stiffness. Different grades of liver fibrosis are described: non-hepatic fibrosis (F0), mild (F1), moderate (F2) and severe (F3) fibrosis and cirrhosis (F4). No effective treatment for liver fibrosis exists, and a better understanding of the mechanisms underlying the disease is needed. 3D in vitro models have been proposed to replace animal tests. Hydrogel-based biomaterials are promising, as they recreate the in vivo microenvironment. In them, the mechanical properties play a crucial role in modulating cell function and disease development. The aim of this study was the design of new in vitro 3D culture platforms based on gelatin hydrogels to model different stages of liver fibrosis.

**Methods:** Gelatin hydrogels were enzymatically crosslinked (Gel) and the stiffness was increased by the addition of glyoxal (GlyO) (Gel-GlyO). Mechanical properties of the hydrogels were analysed by rheology. HepG2 cell line and freshly isolated primary porcine hepatocytes (PHePs) were cultured within the hydrogels. Cell viability was tested by live/dead assay.
Results: Gel resulted with a stiffness of 2.0 ± 0.3 kPa, higher than F0 fibrosis. GlyO addition allowed an increase in the Gel-GlyO stiffness up to 5.0 ± 0.3 kPa, characteristic of F1 fibrosis. HepG2 cells’ viability in Gel hydrogels after 5 days of culture was 70%, while in the Gel-GlyO was 86%. In the case of PHeps, cell viability after 3 days of culture, resulted in 70% and 56% for Gel and Gel-GlyO hydrogels, respectively.

Conclusions: Hydrogels obtained in this study match the mechanical properties of stage F1 liver fibrosis, and have the potential to achieve higher stiffness, mimicking more advanced liver fibrosis. Pilot short-term cell cultures suggested good cell viability of hepatic cells.

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P15 - Specific profibrogenic chemokine gene expression dynamics in aged liver sinusoidal endothelial cells during progression of cirrhosis

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Background and Aims: Liver Sinusoidal Endothelial Cells (LSECs) are considered promoters of hepaticc tolerance modulating immune response through the expression of relevant chemokines and innate receptors. During cirrhosis, the tolerantogenic hepatic state turns into a dysregulated immune response leading to a chronic inflammatory environment. As cirrhosis is more prevalent on aged population, our aim was to study fibrosis progression in an aged model of advanced chronic liver disease and to characterize the dynamics of chemokines related to fibrosis in aged LSECs.

Methods: Progressive liver damage was induced in mice by oral administration of carbon tetrachloride (CCl4) during 6 or 12 weeks (w) on Young (Y, 16 w) and aged mice (O, 40 w). Liver and gut damage were assessed by IHC and qPCR. Relevant chemokines were analyzed by using custom RT² Profiler PCR Array on FACS-isolated hepatic LSECs from the animals included in the protocol.

Results: Liver fibrosis was more severe in aged than in young mice subjected to experimental cirrhosis, as observed by the % of positive area in IHCs for the evaluated markers (Figure 1A-1B). Timp1 profibrogenic gene was significantly increased in old compared to young mice at 12 weeks, and Tgfb1 and Mmp2 showed the same tendency (Figure 1C). LSECs show an age-dependent profibrogenic chemokines’ gene expression profile. While CCL3, CCL5 and CCL20 exhibited a significant increase during early stages of experimental cirrhosis induction in young cirrhotic mice, old cirrhotic mice displayed a delayed increment of these chemokines at advanced stages of cirrhosis (Figure 1D). CXCL10 and CXCL16 behaved similarly for both experimental models, with a sustained gene expression at 6 and 12 weeks of protocol.

Conclusions: CCl4 induced an earlier and increased expression of liver fibrosis markers in aged mice. An aged-related specific pattern of chemokines gene expression is observed in LSECs during progression of fibrosis.
P16 - Single-cell RNA sequencing in DIAMOND mice reveals differentially regulated pathways specifically associated with the transition from simple steatosis towards NASH

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**Background and Aims:** To use single-cell RNAseq (sc-RNAseq) technology in DIAMOND mice to unveil the differential changes specifically associated with the progression from simple steatosis to NASH.

**Methods:** sc-RNAseq of parenchymal and non-parenchymal cells (NPCs) isolated from 8 DIAMOND™ mice fed HF-HFD vs. controls at 19 and 32 weeks were performed using 10x Genomics. Ingenuity pathway analyses (IPA) tools were used to find altered pathways, upstream regulators and toxic molecules specifically associated to NASH transition (ΔΔn-19w activation-z-score>|2|). Clustering was carried out with Seurat at different resolutions and validated using published markers and human protein atlas. Similarity to human NASH progression was measured using a robust NASH signature from previously published data.
**Results:** NASH, according to SAF score, was present exclusively at 32w despite significant inflammation after 19w of diet. Mild fibrosis was observed in all animals fed HF-HFD. We identified six clusters of hepatocytes at 19w and five at 32w. NPCs clustering identified 21 clusters at week 19 and 24 at week 32. 12/21 and 10/24 were assigned to known cell types. Preliminary IPA analyses on hepatocytes indicate an association between NASH and mitochondrial dysfunction (Mitochondrial dysfunction $\Delta z$-score=3.78; Oxidative phosphorylation $\Delta z$-score=-5; Sirtuin signaling pathway $\Delta z$-score=5.74). Interestingly, our data also indicate a significant downregulation of several pathways linked to caspase-dependent apoptosis (Apoptosis of hepatocytes $\Delta z$-score=-2.178; Cell death of hepatocytes $\Delta z$-score=-2.662) despite higher presence of ballooned hepatocytes in NASH tissue together with the activation of caspase-independent apoptosis pathway (Granzyme A signaling $\Delta z$-score=3.74). Gene expression analyses highlighted the induction of several proteins proven to play hepatoprotective roles during oxidative stress.

**Conclusions:** A single-cell model to analyze the transition towards NASH independently of fibrosis stage using DIAMOND™ mice has been established. Preliminary results link mitochondrial dysfunction in hepatocytes to NASH and ballooning, and highlight some noteworthy deregulations in pathways mediating apoptosis.
P17 - Characterization of relevant hepatic sinusoidal cell populations in human liver disease: from single-cell data to personalized medicine

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Background and Aims: Single-cell sequencing allows for fine characterization of tissue phenotypes, but its implementation for routine patient care is nowadays unrealistic. The aim of this study is to propose an unbiased gene signature that could reliably define the state of the liver sinusoid in health and liver disease (LD) with a similar accuracy to single-cell analyses.

Methods: From published liver sc-RNAseq data, we generated signature matrices with specific genes for each sinusoidal cell population (gene deconvolution). Using these matrices, we estimated changes in sinusoidal cell subpopulations (healthy vs activated/dedifferentiated populations) due to chronic LD in human liver samples. The expression of relevant genes was fitted into a generalized linear model for predicting the hepatic phenotype and patient stratification. Validations were performed with standard RT-PCR.

Results: Gene deconvolution from decompensated cirrhotic livers (ethanol, n=12) showed significant increments in capillarized LSEC (FC=5.7), activated HSC (FC=1.8), and fiber-associated macrophages (FC=4.9) vs control tissues, which were validated in an external cohort of patients with MASH (n=39, GSE139602). The most specific genes per cell type (LSEC, HSC, macrophages) were validated in an internal cohort (n=19 control, n=36 cirrhosis, p<0.05) and in data from an external cohort of 216 patients with SLD (GSE135251) with different METAVIR stages (control, MASLD and MASH F0-F4). Importantly, our panel discriminated samples from early vs advanced chronic LD patients with an accuracy of 96% and 80%, respectively, and predicted endothelial capillarization (r=0.90, p<0.001), HSC activation (r=0.77, p<0.001) and macrophage polarization (r=0.79, p<0.001).

Conclusions: This novel unbiased gene panel can be easily assessed by RT-PCR and allows the characterization of sinusoidal cells phenotype in human liver tissue. Our gene signature could be a useful tool for personalized clinical decision making, aiding in assessment of drug response or in choosing the most relevant cell target for therapy for an individual patient.

Graphical abstract. A) From signle-cell RNAseq data we define a gene signature that defines the non-parenchymal phenotype in chronic liver diseases. B) The specific gene signature is able to identify phenotypical alterations in each of the sinusoidal cell population in human liver biopsies.
Background and Aims: Metabolic dysfunction and alcohol consumption cause steatotic liver disease (SLD) and both factors frequently co-exist. New nomenclature on metabolic dysfunction-associated SLD (MASLD) allows 20-30 g/day of alcohol intake. We aimed to assess the impact of different levels of alcohol consumption on MASLD fibrosis severity.

Methods: Multicentric, cross-sectional, population-based study, including transient elastography (TE). A controlled attenuation parameter CAP ≥275 dB/m was used to identify SLD in the general population, and the FAST score was used to identify significant fibrosis in SLD subjects (FAST ≥0.35). Within SLD, presence of any cardiometabolic criteria defined MASLD. Alcohol consumption was assessed with clinical interview, considering pure MASLD as 0-1 drink/day and dual MASLD as reported 2-3 drinks/day. Under-reporting of alcohol intake was presumed with the ANI score. Multivariable analysis adjusted by sociodemographic and metabolic factors, was performed to assess the independent contribution of different number of alcoholic drinks/day in liver fibrosis. Higher alcohol intake (>20-30 g/day) and other causes of SLD were excluded.

Results: A total of 6,999 adults with valid TE were included, of whom 33.1% (n=2,319) had SLD. Among them, 14.1% had dual MASLD. In the remaining 85.9% with pure MASLD, 10.7% had an ANI score >0 suggesting alcohol intake under-reporting. The prevalence of significant fibrosis was 13.7%. Compared to pure MASLD, subjects with dual MASLD had higher rates of significant fibrosis (OR=4.05 [95%CI 3.08-5.32]). Obesity, diabetes, and number of alcoholic drinks/day were independently associated with significant fibrosis (P<0.001). Reporting only one alcoholic drink/day on average was independently associated with significant fibrosis (OR=1.73 [95%CI 1.11-2.70]). A dose-dependent supra-additive interaction was seen between number of alcoholic drinks and both BMI and diabetes, with detrimental effect at any level of alcohol consumption (Figure).

Conclusions: Alcohol intake is prevalent in MASLD subjects. This patients have a higher risk of advanced disease than pure MASLD.

Figure:
Methods: Candidates were identified by two-step metaanalysis analyzing gene expression in biopsies from NAFLD patients [Identification cohort, 3 cohorts analyzed by Microarray, F0-F1 (139) vs. F2-F4 (47)] [Validation cohort, 4 cohorts analyzed by RNA-seq, F0-F1 (234) vs. F2-F4 (197)] Average ranking together with additional filters to retain bloodstream circulating secreted proteins were used to select 5 FICEs (Fibrosis Inducible Circulating Elements) which were explored in serum samples from a cohort of NAFLD biopsy-proven patients coming from three different hospitals in Spain (N=203).

Results: FICE4 diagnostic accuracy is superior to all routinary NITs (FIB-4, NFS, HFS, APRI) for the detection of significant and advanced fibrosis (F2+ or F3+) and similar to the one obtained by TE or ELF (Fig1a-b). Interestingly, FICE-4 circulating levels were also able to identify at-risk patients with similar efficacy to the recently developed FAST algorithm. Combination of FICE4 with some routinary clinical variables (FICE4C) significantly improved its diagnostic accuracy (Fig 1c-d).

Conclusion: FICE4 can be easily assessed by ELISA and constitutes a useful tool to evaluate liver disease severity that could be useful for screening sites in which confirmatory tests like TE or ELF are not easily accessible.
P20 - Biomarkers of endothelial dysfunction in patients with Non-Alcoholic Fatty Liver Disease

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Background and Aims: To assess a panel of endothelial dysfunction biomarkers in serum and liver tissue in biopsy-proven NAFLD patients.

Methods: 148 NAFLD patients were included with different stages of the disease (control (n=8), simple steatosis (n=22), NASH F0 (n=27), NASH F1-F2 (n=46) and NASH F3-F4 (n=45)). ELISA techniques were performed for the selected endothelial dysfunction markers: ICAM-1, VCAM-1, VEGF and Serpin E1. We analysed liver fibrosis and features that compose the NAS Score. In addition, immunohistochemistry of hepatic sections was performed in 20 patients following the same groups as described below (n=4 patients/group) for CAV-1 and ICAM-1.

Results: Mean age was 55±10 years old, 53.4% were women. Circulating levels of ICAM-1 were associated with liver fibrosis (C 17.8±9.7 vs. SS 18.1±5.2 vs. NASH F0 20.6±7.7 vs. NASH F1-F2 22.5±11.6 vs. NASH F3-F4 25.5±15.5 ng/mL; p=0.021; n=145; Figure 1A). Similarly, ICAM-1 levels were associated with ballooning (none 16.0±6.1 vs. mild 20.5±6.9 vs. seveve 28.6±18.1 ng/mL; p=0.001; n=145; Figure 1B). Circulating levels of VCAM-1 were also associated with fibrosis (F0 44.4±26.4 vs. F1 47.4±25 vs. F2 60.3±23.9 vs. F3 65.4±37.1 vs. F4 65.7±25.1 ng/mL; p=0.012; n=145; Figure 1C). Serpin E1 were found to be increased with the degree of hepatic steatosis (0-5% 4.6±3.3 vs. 6-33% 7.3±5.7 vs. 34-66% 9.9±6.1 vs. >66% 10.5±7.9: p=0.008; n=145; Figure 1D) and correlated with the Castelli index (r:0.248; p=0.007; n=117). VEGF levels were increased in patients with NAS Score>5 vs. NAS Score≤5 (265.2±103.8 vs. 235.6±161.1 pg/mL; p=0.018; n=138; Figure 1E). Moreover, the hepatic...
expression of CAV-1 and ICAM-1 were found to be increased following the hepatic injury (Fig 1F and 1G) and were correlated with liver fibrosis (r=0.730; n=20; p<0.001 and r=0.689; n=19; p<0.001, respectively).

Conclusion: Several markers of endothelial dysfunction are related to the main histopathological features of NAFLD. These biomarkers would be used as non-invasive approaches to diagnose NAFLD.
POSTERS.
BASIC RESEARCH IN GUT FIBROSIS
**P21 - Notch pathway: a new anti-fibrotic therapy in Crohn’s disease**

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**Background and Aims:** Studies have suggested that the Notch pathway may be involved in the regulation of senescence and in the development of fibrosis. The general aim of the present study is to determine the possible potential of Notch pathway as a therapeutic target in intestinal fibrosis associated with Crohn’s disease (CD). Specifically, we pretend: to analyze the localization and the expression of NOTCH markers in the intestinal tissue of patients with complicated CD, and to study the relevance of the NOTCH pathway in the senescence of intestinal fibroblasts.

**Methods:** Protein expression of senescence/apoptotic markers and NOTCH markers were analyzed in intestinal samples from CD. The protein expression of senescence/apoptotic proteins were analyzed in HSIF treated with DLL4 or DLL3.

**Results:** NOTCH3 was located preferentially in muscular areas. The expression of DLL4/3, NOTCH4 and HES1 were significantly higher in the affected tissue compared to the ileal samples of control patients. BCL2, MCL1, P16 and P53 showed significantly elevated levels in the affected CD tissue, compared to the ileal sample of control patients. P16, the main marker of senescence, correlates positively and significantly with DLL3/4, NOTCH3 and HES1 in intestinal tissue. DLL3, and not DLL4, produced in intestinal fibroblasts a significant increase in the protein expression levels of BCL2 and P53, compared to the vehicle.

**Conclusions:** NOTCH pathway is involved in the regulation of key cellular functions and processes essential for the pathogenesis of intestinal fibrosis in CD patients. DLL3 seems to have a relevant role in activation of senescence in fibroblasts.

**P22 - GPR109A is increased in IBD patients and exert a pro-inflammatory role in macrophages**

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**Background and Aims:** Inflammatory Bowel Disease (IBD) is a chronic inflammatory disease of the gastrointestinal tract associated with complications such as fibrosis, which cannot be pharmacologically reversed. G-protein coupled receptors (GPCRs) have been identified as promising pharmacological targets due to their key role in inflammatory and fibrotic processes. We aim to characterize the role of GPR109A in IBD.

**Methods:** Intestinal resections from UC (n=18), CD (n=21) patients and colon (n=20) and ileum (n=15) from controls were obtained. GPR109A protein expression was analysed by WesternBlot and immunohistochemistry. U937 were treated with IFN-γ 20 ng/ml and LPS 0.1μg/ml or β-hydroxybutyrate 10mM for 24h and transiently silenced with a GPR109A siRNA. HSIFs were treated with TGF-β 5ng/ml or β-hydroxybutyrate 10mM for 24h. Gene expression of GPR109A, pro-inflammatory and pro-fibrotic markers was analysed by qPCR. Results are expressed as fold induction (mean±SEM).

**Results:** Firstly, gene (19.05±5.30, 400.6±130.6) and protein (140.60±18.70, 163.7±41.48) expression of GPR109A was significantly increased in both UC and CD patients respectively. Moreover, immunohistochemistry showed GPR109A+ cells in epithelium and lamina propria of IBD patients. In HSIFs, TGF-β treatment did not modify GPR109A’s expression (1.09±0.32), while IFN-γ and LPS significantly increased its expression in macrophages (3.11±0.73). Of interest, transiently silencing of GPR109A significantly reduced the expression of IL1B (9.26±3.04) and TNFa (1.35±0.11) versus non-transfected macrophages (33.870±9.99), (2.97±0.45) respectively.

On the other hand, β-hydroxybutyrate did not modify the expression of GPR109A on HSIFs (0.68±0.21), but increased expression of COL1A1 (1.83±0.20) and COL3A1 (2.48±0.56). In macrophages, β-hydroxybutyrate increased expression of GPR109A.
(1.99±0.31), as well as IL1B (2.88±0.41) and CD86 (1.74±0.16).

Finally, supernatants from macrophages treated with β-hydroxybutyrate increased expression of IL1B (52.42±20.17) and COL4A1 (3.01±0.38) in HSIFs compared with HSIFs treated with macrophage's medium (2.083±1.42), (1.45±0.42) respectively.

Conclusions: GPR109A is increased in IBD patients. In addition, β-hydroxybutyrate and GPR109A exert a pro-inflammatory role in macrophages, modifying their cytokine profile triggering the activation of HSIFs.

P23 - Investigating the Effect of Hydroxylase Inhibitors on Inflammatory Fibroblasts

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Background and Aims: Intestinal fibroblast differentiation into an inflammatory phenotype likely plays a key role in Inflammatory Bowel Disease (IBD). However, the mechanisms that control this process are poorly understood. The cytokine TNFSF14 (LIGHT) drives inflammatory fibroblast responses in other diseases such as Eosinophilic esophagitis, giving it great potential as a mediator of the same response in IBD. Additionally, hypoxia, defined as low cellular or tissue levels of oxygen, is a feature of IBD progression. Here we test the role of LIGHT on intestinal fibroblast differentiation and how this may be affected by hypoxia mimetics.

Methods: Primary human colonic fibroblasts (18CO), the hepatic stellate cell line (LX2) and the epithelial cell line (T84) were treated with LIGHT in time course studies. 18COs were also treated with hydroxylase inhibitors Dimethyloxalylglycine (DMOG), JNJ42041935 and Roxadustat. Responses were analysed using qPCR, flow cytometry and Immunoblot-analysis.

Results: LIGHT-treated 18COs upregulated CCL2 and IL34 at transcript level, and surface expression of ICAM1 and VCAM1 displaying distinct kinetics. This response was absent in LX2s and T84s. DMOG decreased CCL2 and IL-34 gene expression in 18COs when subsequently treated with LIGHT but did not affect other targets tested such as CSF1, CXCL5, VCAM1 and ICAM1. Immunoblot analysis of 18COs showed a decreased HIF-1α stabilization when treated with hydroxylase inhibitors in the presence of LIGHT compared to hydroxylase inhibitors alone.

Conclusions: Our current studies show that LIGHT is a potential mediator of colonic inflammatory fibroblast differentiation and a possible inhibitor of HIF-1α stabilization. The effects of LIGHT are either significantly weaker or not displayed in colonic epithelial cells or hepatic stellate cells, indicating LIGHT-specific effects on intestinal stroma. Hydroxylase inhibitors show selective effects on the LIGHT driven response. Collectively our data reveals a potential LIGHT-hypoxia network that may be amenable for therapeutic interventions.
P24 - Investigating the Effect of Tweak in Fibroblast WNT Signalling and Colonic Epithelial Cell Regeneration

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**Background and Aims:** A breakdown in communication between the intestinal stroma and epithelium may contribute to defects in intestinal epithelial barrier structure and function, which is a key part of inflammatory bowel disease (IBD) pathogenesis. However, the mediators and mechanism that control the interaction between fibroblasts and IECs in homeostasis and inflamed environments remains unknown. TWEAK and its receptor fibroblast inducible 14 (Fn14) may promote epithelial barrier breakdown and chronic inflammation. We hypothesized that TWEAK dysregulates the crosstalk between fibroblast and epithelial cells by impairing the homeostatic signals.

**Methods:** To assess the effect of TWEAK on WNT signalling in fibroblasts and epithelial cells, the expression of WNT ligands and receptors were assessed. Primary human colonic fibroblasts and human colonic epithelial cells were stimulated with TWEAK in a time course manner and analysed by qPCR, Western blot and Flow cytometry.

**Results:** Both Fibroblasts and epithelial cells displayed abundant Fn14 surface receptor, and this was upregulated in response to TWEAK. We identified a significant downregulation in the expression of WNT ligands RSPO2, BMP6, WNT5A and WNT5B mRNA level in fibroblasts stimulated with TWEAK at 24 hrs, but not in epithelial cells. Fibroblasts treated with TWEAK showed that Axin2, RSPO2 and B Catenin protein level were strongly elevated at 1 and 4 hr, and reduced at 24 and 48 hrs. Our results showed that TWEAK specifically impairs the regulation of WNT signalling in fibroblasts, but not epithelial cells.

**Conclusions:** TWEAK specifically alters the expression of WNT targets in colonic fibroblasts. The effect that this impairment of WNT signals in stroma I cells may have on their differentiation and communication with the epithelium will be subject for further study.

P25 - Role of MIR-378a-3P in Intestinal Fibrosis

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**Background and Aims:** Fibrosis constitute an important complication of CD. MicroRNAs (miRNAs), which are small RNA molecules that regulate gene expression, have been shown to participate in the molecular interactions of both inflammation and fibrosis. Lower levels of miR-378a-5p have been reported associated to murine liver fibrosis[1] and we analyse here the relevance of miR-378a-3p on intestinal fibrosis.

**Methods:** B57BL/6 mice were intravenously injected with 2,5mg/kg of negative control (NC) or mm-miR-378a-3p mimic, twice a week and received vehicle or Dextran Sulfate Sodium (DSS) for 2 cycles (7 days drinking DSS 2% in water solution followed by 10 days drinking water). Body weight and DAI score was obtained every day and the colon was collected after sacrifice. Sirius and hematoxylin-eosin dyes were employed to determine the fibrosis and structural state in 5µm slides of intestinal tissue. U937 were differentiated to M1 macrophages and then transfected 50nM of NC or hsa-miR-378a-3p mimic during 24h. Also, Human small intestinal fibroblasts (HSIF) were transfected with 20nM of NC or hsa-miR-378a-3p mimic during 24h. Gene expression and miRNA profiles were analysed by RT-qPCR.

**Results:** Colon of mice treated with DSS, exhibited a significant diminution in the mRNA expression of mm-miR-378a-3p compared with naïve samples. In DSS-treated mice, the iv administration of the mimic compared with the NC alter intestinal architecture and inflammatory infiltrates and significantly increased the mRNA expression of Tgfb1, Il1b, and Mmp2. M1 macrophages polarization showed a decreased levels of has-miR-378a-3p and increased gene expression levels of IL1B, CXCL3, CXCL5, and GZMB. After treatment of this cells with mmR-378a-3p mimic, IL6 and ARG1 gene expression was significantly increased. Finally, treatment of HSIF with this mimic did not significant modify the mRNA expression of markers of fibrosis but it significantly increased the mRNA expression of two antiapoptotic molecules BCL2 and MCL1.
Conclusions: Levels of miR-378a-3p are diminished in a murine model of intestinal fibrosis, and in polarized M1 macrophages; The exogenous administration of miR-378a-3p, increased the acute inflammatory response and the expression of fibrosis markers in murine fibrotic colon and in human M1 macrophages and the expression of antiapoptotic molecules in human intestinal fibroblasts.

References:

**P26 - Role of perlipin-1 in the differentiation of myofibroblasts in lung fibrosis**

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**Background and Aims:** Pulmonary fibrosis is a chronic progressive lung disorder in which the walls of the alveolus undergo inflammation and fibrotic thickening resulting in respiratory failure. The cells producing extracellular matrix components are myofibroblasts. Myofibroblasts are mainly derived from fibroblast activation but also from epithelial and endothelial cells (via EMT/endoMT), or pericyte fibrocyte differentiation. In recent years, lipofibroblasts, fibroblasts filled with lipid droplets, have been postulated as a precursor of myofibroblasts. Perilipin-1 (PLIN1) is a protein associated with intracellular lipid droplets and involved in lipid metabolism and storage. Our hypothesis is that PLIN1 has a main role in the differentiation of lipofibroblasts to myofibroblasts and the progression of fibrosis.

**Methods:** We have used human alveolar cell lines and both biopsies or primary lung fibroblasts from control patients of patients with Idiopathic Pulmonary Fibrosis (IPF). We have performed WB, confocal microscopy on cells and tissues, and qPCR to assess the expression and colocalization of PLIN1.

**Results:** We have seen that PLIN1 is overexpressed in human fibrotic lungs, where it colocalises with α-sma. PLIN1 inhibition in vitro increases the differentiation to myofibroblasts and the deposition of collagen, suggesting a protective role in the development of fibrosis. In primary lung fibroblasts, PLIN1 shuttles to the nucleus after a fibrotic stimulus, which could show an alternative nuclear function for PLIN1 repressing the differentiation to myofibroblasts and the expression of profibrotic genes.

**Conclusions:** We suggest that PLIN1 has a protective role in the development of fibrosis and that this molecule could be a potential link (and putative therapeutic target) between lipid metabolism and fibrosis.

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**P27 - Bronchoalveolar-Lavage derived 3D cultures to predict lung fibrosis evolution**

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**Background and Aims:** Interstitial lung diseases comprise a heterogeneous group of diseases that can result in a progressive and aberrant process of tissue repair, leading to destruction of the normal lung architecture, pulmonary fibrosis and, ultimately, organ failure. There is currently no way or any early biomarker to predict whether a patient diagnosed with interstitial lung disease (non-idiopathic pulmonary fibrosis) will progress to a process of progressive pulmonary fibrosis. The aim of our study is to predict whether a patient with interstitial disease will develop progressive fibrosis.

**Methods:** The bronchoalveolar lavage (BAL) will be taken from three different group of patients: a control group, patients with any type of non-fibrosing interstitial disease and a third group of patients with interstitial lung disease with established fibrosis. Cells in the lavage will be analysed using cytometry to analyse their cellular content and will be grown in 3D cell cultures. We intend to perform RNAseq, as well as proliferation, migration, and metabolic assays to compare the spheroids from the three different groups of patients.

**Results:** We have obtained 15 BAL samples. We have optimized the cytometry panel to distinguish between cells with epithelial or mesenchymal characteristics. Finally, we have grown the cells within the BAL both in plaques and in 3D spheroids.

**Conclusions:** We believe that the patient-derived BAL are a good sample for the characterization of early fibrosis biomarkers that will allow us to predict the evolution to fibrosis. Moreover, the culture in 3D of the patient’s cells could open avenues to explore new therapeutic targets in a personalized manner.
Sex 57.14% female
Age 71, 21 ± 9.16

<table>
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<tr>
<th>Clinical diagnosis</th>
<th>Group 1: Non ILD patients</th>
<th>Group 2: 60% NSIP, 20% uILD, 20% CTD-ILD</th>
<th>Group 3: 20% FPF, 20% IPF, 20% sarcoidosis, 20% NSIP, 20% CTD-ILD</th>
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**P28 - Study of the potential cardiac antifibrotic of losartan and nintedanib**

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**Background and Aims:** Cardiac fibrosis is characterized by the accumulation of extracellular matrix produced by fibroblasts leading to an impairment on heart contractility. It remains an unresolved pathology as some drugs used to treat heart failure such as Losartan, improve hemodynamics, but they do not directly modify fibrotic events. Currently, Nintedanib, a tyrosine kinase inhibitor approved for lung fibrosis, has been postulated as a drug to treat cardiac fibrosis. The aim of this study was to compare the cardiac anti-fibrotic effects of Losartan with that of Nintedanib.

**Methods:** Primary human cardiac fibroblast (HCF) cells were treated (24h) with Losartan (1.5 and 10μM) or Nintedanib (0.02 and 2μM). Cell viability was evaluated by an MTT assay while the expression of profibrotic gens (col3a1, fn-1, timp-1 and n-cadherin) by RT-qPCR. Results were analyzed by ANOVA Kruskal-Wallis by Dunn’s corrections. On the other hand, the effect of Losartan or Nintedanib (in the absence or presence of TGF-β₁ 5ng/mL) was studied on functional wound healing assay by quantifying (0-48h) the % of wounding area versus control (n=3) and employing the two-way ANOVA followed by Tukey test for the analysis.

**Results:** None of the drugs at any concentrations affected cell viability. Losartan significantly reduced the expression of col3a1, timp-1 and n-cadherin while Nintedanib ameliorated that of fn-1, timp-1 and n-cadherin (Figure 1). Losartan, neither by itself nor in the presence of TGF-β₁, affected the closure of the wound. In contrast, Nintedanib 2μM reduces wound closure by itself (34.1±10.6 versus vehicle, 9.2±5.0); parameter that was significant when inducing fibrosis with TGF-β₁ (Nintedanib 2μM + TGF-β₁, 41.2±3.3** versus TGF-β₁, 1.6±1.3) at 24h.

**Conclusions:** Losartan and Nintedanib have some positive actions on the expression of fibrotic gens on human cardiofibroblasts. Nintedanib provided a better anti-fibrotic profile reducing migration on cardiofibroblasts. This fact highlights the need for more research on this potential therapy to treat cardiac fibrosis.
**Posters. Fibrosis in other tissues and systems**

Figure 1: Effects of Losartan or Nintedanib on the expression of profibrotic genes. (A) Col3a1, (B) fn-1, (C) timp-1 and (D) n-cadherin were analyzed by RT-qPCR after the treatment of 24h of primary human cardiac fibroblasts (HCF) cells with Losartan (1.5 and 10μM) or Nintedanib (0.02 and 2μM). Results are represented as mean of fold-induction ±SEM versus vehicle (n=5). Anova Kruskal-Wallis by Dunn's corrections was used to analyzed the results. *p≤0.05; **p≤0.01; ***p≤0.001 versus vehicle.