Université catholique de Louvain

Faculté de médecine

Année académique 2017-2018

Promotrice : Professeur Isabelle Leclercq

Mémorant : Jean de Roissart

Functional role of the ductular reaction in the maintenance of the hepatobiliary bile flow

MÉMOIRE DE RECHERCHE CLINIQUE

JEAN DE ROISSART

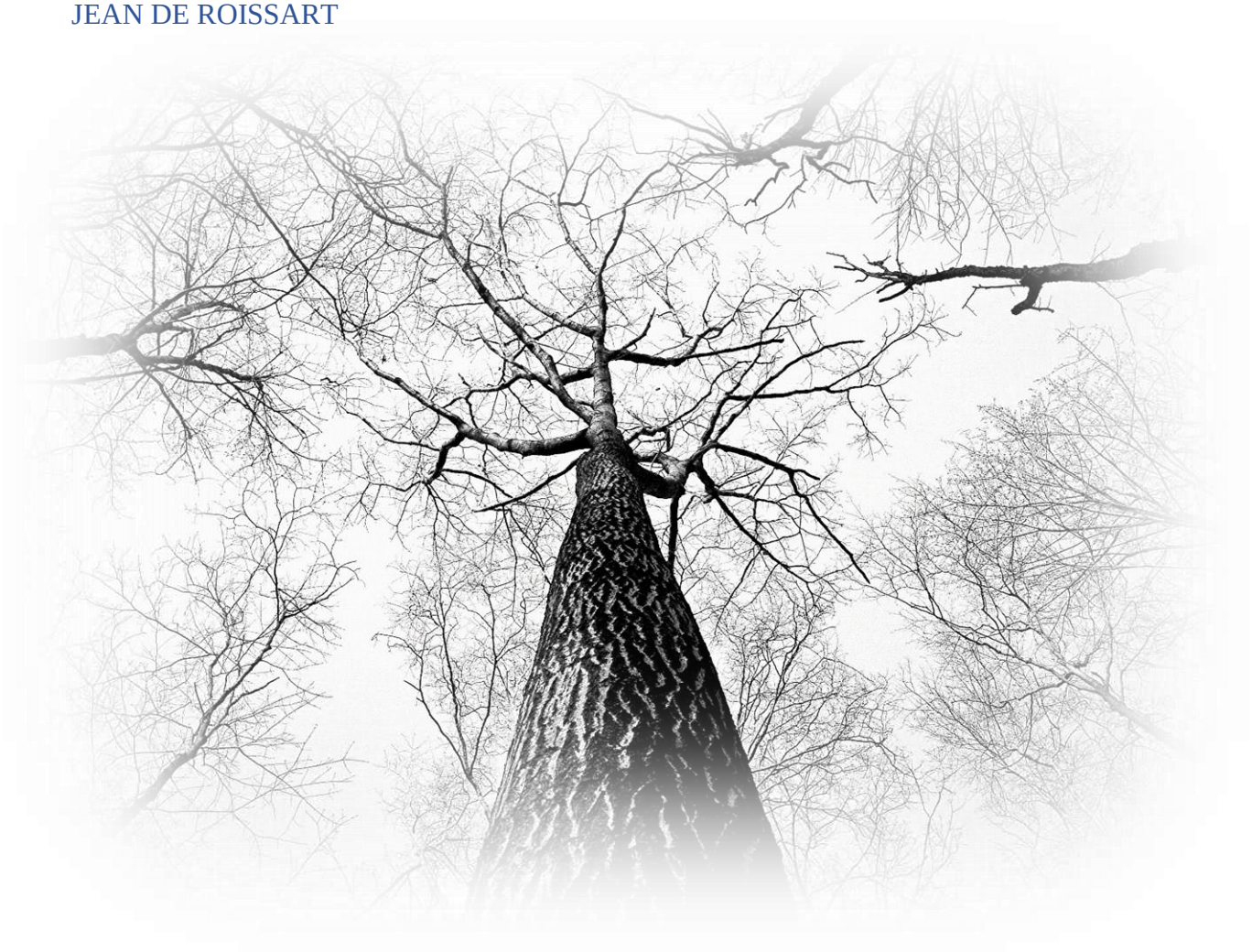


Table of contents

1. Abstract in English and French 2

2. List of abbreviations 4

3. Introduction 5

The liver 5

Liver microarchitecture and histology 5

Bile composition, flow and function 6

Bile acid production, secretion, cycle 9

Toxicity and regulation of bile acids 10

Cholestasis 14

Liver regeneration 15

Ductular reaction 15

The mouse model 16

4. Working hypothesis and current questions 17

5. Materials and methods 18

6. Results 19

In CDE mice, BA synthesis, efflux and secretion were unchanged, while uptake is profoundly down-regulated 19

Discussion 21

7. Literature review 23

Question 1: What is known over bile acid synthesis enzymes and transporters expression in other rodent models of ductular reaction? 23

Question 2: What is known over bile acid synthesis enzymes and transporters expression in human diseases involving ductular reaction? 27

Question 3: What are the strategies and outcomes of NTCP inhibition in the liver? 31

8. Conclusion and perspectives 35

Perspectives 37

9. Personal conclusions 39

10. Bibliography 40

1. Abstract

English abstract

In chronic liver injury when hepatocyte proliferation is impaired or overwhelmed, expansion of ductular structures from periportal regions of the hepatic lobule, called ductular reaction (DR) is observed. Previous data from the gastro-enterology (GAEN) laboratory of the Université catholique de Louvain (UCL) show that upon hepatocellular injury caused by a choline-deficient and ethionine-supplemented (CDE) diet in mice, DR is invasive, DR-cells scarcely differentiate into hepatocytes and canalicular bile flow is impaired. The first aim of this paper is to investigate canalicular bile flow in the CDE model by assessing modifications of hepatocellular bile acid (BA) synthesis enzymes and transporters expression. We analysed mRNA and protein levels of hepatocyte BA synthesis enzymes and secretion, uptake and efflux transporters in C57Bl/6J mice fed with chow diet or CDE during 3, 9, and 21 days. Our results show unchanged expression of BA synthesis enzymes, secretion transporters and efflux transporters; but we observe significantly decreased expression of uptake transporter sodium-taurocholate cotransporting polypeptide (NTCP). This does not fit with cholestasis-induced regulation of BA synthesis enzymes and transporters by farnesoid-X receptor (FXR), which leads us to suspect pre-hepatic cholestasis secondary to NTCP inhibition to be the trigger of the invasive DR observed in CDE model. To better understand interactions between chronic liver damage, BA trafficking and DR, three separate literature reviews were carried out. A comparison of BA synthesis enzymes and transporters expression in other rodent models of DR showed that in unlike models of hepatocellular damage, DR in models of biliary damage is confined to the periportal region and resembles small bile duct proliferation, furthermore BA trafficking adaptation in biliary-damage models complies with FXR-mediated regulation. A comparison of BA synthesis enzymes and transporters expression in human liver diseases involving DR was limited by lack of data in medical literature on this subject, but showed that hepatocyte BA secretion transporter bile salt export pump (BSEP) dysfunction has different consequences in humans and mice. Finally, the third literature review lays out various strategies of NTCP inhibition and highlights that unlike humans, mice can compensate for NTCP inhibition via other uptake transporters, which is crucial information for future NTCP inhibition studies on mice. To conclude, non-invasive methods of BA transporters function assessment are presented as perspectives for future data collection in human liver diseases and as means of evaluating at a dynamic level the effect of BA trafficking modifications on DR.

Résumé en français

La réaction ductulaire est caractérisée par l'expansion de structures ductulaires à partir de la région péri-portale du lobule hépatique, et s'observe dans les lésions hépatiques chroniques lorsque la capacité prolifération des hépatocytes est altérée ou débordée. Les données antérieures obtenues dans le laboratoire de gastro-entérologie de l'Université catholique de Louvain démontrent que lorsque des souris sont soumises à un régime pauvre en choline et enrichi en éthionine (CDE) causant des dégâts hépatocellulaires, la réaction ductulaire adopte un aspect invasif, les cellules formées par cette réaction ductulaire ne se différencient que rarement en hépatocytes, et l'écoulement de la bile dans les canalicules biliaires est perturbé. La première partie de ce mémoire de recherche clinique investigue des causes de perturbation d'écoulement de la bile dans les canalicules biliaires via une comparaison au niveau ARNm et protéique de l'expression hépatocytaire d'enzymes de synthèse et de transporteurs d'acides biliaires chez des souris C57Bl/6J soumises à un régime contrôle ou CDE durant 3, 9 et 21 jours. Nos résultats démontrent une expression inchangée des transporteurs de sécrétion, d'efflux et des enzymes de synthèse, mais une diminution significative de l'expression du transporteur de captation NTCP. Ces observations ne cadrent pas avec la régulation des enzymes de synthèse et transporteurs d'acides biliaires médiée par FXR en cas de cholestase, ce qui permet de supposer que la cholestase pré-hépatique secondaire à l'inhibition de l'expression de NTCP est à l'origine de la réaction ductulaire invasive du modèle CDE. Pour mieux comprendre les interactions entre hépatopathies chroniques, circulation d'acides biliaires et réaction ductulaire, nous avons effectué une revue de la littérature afin de comparer la régulation des enzymes de synthèse et transporteurs d'acides biliaires dans d'autres modèles de réaction ductulaire chez les rongeurs et dans diverses hépatopathies chroniques arborant une réaction ductulaire chez les humains. Chez les rongeurs, nous démontrons que dans les modèles de réactions ductulaires secondaires à une lésion biliaire (et non pas hépatocellulaire), la réaction ductulaire est confinée dans la région péri-portale et ressemble à une prolifération de petits canaux biliaire, de plus la régulation des enzymes de synthèse et des transporteurs d'acides biliaires dans les modèles de lésion biliaire est similaire à celle médiée par FXR. Chez les humains, le manque de données à ce sujet dans la littérature médicale ne nous a pas permis de tirer de conclusion autre qu'une différence évidente entre humains et souris face au dysfonctionnement du principal transporteur de sécrétion BSEP. Enfin, nous avons investigué diverses stratégies d'inhibition de NTCP, ce qui nous a permis d'identifier que les souris sont capables compenser l'inhibition de NTCP grâce à des transporteurs de captation secondaires, contrairement aux humains. Ceci est une information cruciale pour de futures études sur l'effet de l'inhibition de NTCP chez les souris. Afin d'ouvrir la voie pour de futures recherches, des méthodes non-invasives d'évaluation de la circulation d'acides biliaires sont présentées comme alternatives à la biopsie hépatique, et comme moyens de caractérisation dynamique du transport hépatocytaire des acides biliaires.

2. List of abbreviations

2-AAF	2-acetylaminofluorene
^{99m}Tc	99m-technetium
^{11}C-CSar	[N-methyl-^{11}C]cholylsarcosine
ALT	alanine aminotransferase
ASBT	apical sodium-dependent bile salt transporter
ATP	adenosine triphosphate
BA	bile acid
BDL	bile duct ligation
BSEP	bile salt export pump
CA	cholic acid
CDCA	chenodeoxycholic acid
CCK	Cholecystokinin
CCL_4	carbon tetrachloride
CDE	choline-deficient and ethionine-supplemented
CFTR	cystic fibrosis transmembrane conductance regulator
CLF	cholyl-lysyl-fluorescein
CYP27a1	cholestersol 27-hydroxylase
CYP7a1	cholesterol 7α-hydroxylase
CYP8b1	sterol 12α-hydroxylase
DCA	deoxycholic acid
DDC	3,5-diethoxycarbonyl-1,4-dihydrocollidine
DNA	deoxyribonucleic acid
DR	ductular reaction
E2	17β-estradiol
ECM	extracellular matrix
EHC	entero-hepatic cycle
Fgf15	fibroblast growth factor 15
FGF19	fibroblast growth factor 19
FXR	farnesoid-X receptor
GAEN	gastro-enterology
Gdx	gadoxetate dimeglumine
GLP-1	glucagon-like peptide 1
HBV	hepatitis B virus
HCC	hepatocellular carcinoma
HCV	hepatitis C virus
HDV	hepatitis D virus
HNF-4α	hepatocyte nuclear factor 4α
HSCs	hepatic stellated cells
IL-1β	interleukin-1β
IL-6	interleukin-6
KO	knock-out
LPCs	liver progenitor cells
LPS	lipopolysaccharide
MCD	methionine-choline-deficient
Mdr2	multidrug resistance transporter 2
MDR3	multidrug resistance transporter 3
MRI	magnetic resonance imaging
mRNA	messenger ribonucleic acid
MRP2	multidrug resistance associated protein 2
MRP3	multidrug resistance associated protein 3
MRP4	multidrug resistance associated protein 4
NASH	non-alcoholic steato-hepatitis
NTCP	sodium taurocholate cotransporting polypeptide
OATP	organic anion transporting polypeptide
OCT	optimal cutting temperature
OSTα/β	organic solute transporter α/β
PBC	primary biliary cholangitis
PET	positron emission tomography
PFIC	progressive familiar intrahepatic cholestasis
PHx	partial hepatectomy
PCR	polymerase chain reaction
RNA	ribonucleic acid
RXR	retinoid-X receptor
SAE	S-adenosyl ethionine
SAM	S-adenosyl methionine
sIgA	soluble immunoglobulin A
siRNA	small interfering ribonucleic acid
TGR5	G-protein coupled membrane receptor
UCL	Université catholique de Louvain
UDCA	ursodeoxycholic acid

3. Introduction

The liver

The liver fulfils numerous vital functions such as bile secretion, plasma protein synthesis, endogenous and exogenous substance detoxification, glucose and lipid homeostasis regulation, storage of various vitamins and minerals and production of glutathione. All these functions are accomplished by a single cell type: hepatocytes, occupying about 80% of the mammalian liver volume[1].

Liver microarchitecture and histology

The functional unit and building block of the liver is the hepatic lobule. Histologically, the liver lobule bears a hexagonal shape, with cords of hepatocytes radiating from its center. The diameter of a hepatic lobule is roughly 0,25mm. Between the cords, channels named hepatic sinusoids receive mixed blood from both the portal vein and the hepatic artery, flowing in direction of the center of the hepatic lobule wherein lies the centrilobular vein, draining blood processed by the liver to the hepatic vein. The hepatic lobule can be imaginatively divided in 3 concentric zones, differing in blood oxygenation levels and metabolism specialization: zone 1 is known as the perilobular zone, lying at the outermost region of the hepatic lobule, thus receiving the most oxygenated blood. Zone 2 is intermediary between zone 1 and 3, known as the mediolobular zone. The third zone lies at the center of the hepatic lobule and is called the centrolobular zone; it specializes in drug detoxification and biotransformation. Zone 3 is therefore generally the first zone to suffer from toxic injuries. In between hepatic lobules rests connective tissue, and a misnomer called the portal triad. The portal triad includes five structures: a branch of the portal vein, a branch of the hepatic artery, a branch of the vagus nerve, one or two bile ducts, and lymphatic vessels[2].

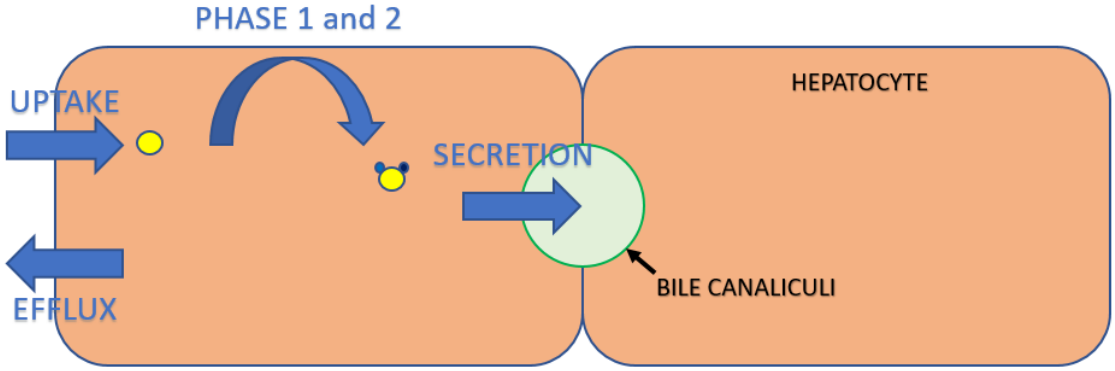
The hepatocyte is the main cell type in the liver. It is a polyhedric cell of 20-30µm diameter, with a vascular or basal pole facing the blood vessels, and a biliary or apical pole facing the bile canaliculi. The hepatocyte's cell membrane possesses microvilli on both poles.

To illustrate the complex liver microarchitecture, we will follow the fate of a freshly absorbed hydrophobic xenobiotic. Brought from the gastro-intestinal tract via the hepatic portal vein, the xenobiotic reaches the hepatic sinusoids where blood flow significantly slows down, giving necessary time for hepatocytes to carry out molecular exchanges. After having avoided resident liver macrophages lining the walls of the sinusoids, the xenobiotic will have to cross the hepatic sinusoid endothelium to be processed by the hepatocyte. This crossing is enabled by two characteristics of the hepatic sinusoid endothelium: its fenestrations and lack of basement membrane. Fenestrations are pores through the endothelium, easing molecule exchange. The basement membrane, which is absent in hepatic sinusoid endothelium, is a mechanical barrier made up of extracellular matrix, whose main function is to act as an anchor to epithelial cells.

Once the xenobiotic has crossed the hepatic sinusoid's porous endothelium, it finds itself in the perisinusoidal space, or space of Disse. This space allows exchanges to take place between molecules coming the blood and from the basal pole of hepatocytes. It is also the location of hepatic stellated cells (HSCs), a pericyte with three main functions: sinusoidal blood flow regulation, vitamin A storage, and extracellular matrix production (ECM)[3].

The hydrophobic xenobiotic will be taken up by specific pumps at the basal pole the hepatocyte, modified by phase I enzymes and conjugated by phase II enzymes to be secreted through specific pumps at the hepatocyte's apical pole into bile canaliculi (illustrated in Figure 1). More soluble metabolites will be secreted back into the blood by specific efflux pumps, and eventually eliminated by the kidney. Much like xenobiotics, endogenous substances such as estrogen, bilirubin, and BAs are also processed in such way by hepatocytes. BA production, secretion, and cycle will be detailed further in this paper.

Figure 1: General pathway by which molecules are metabolized by hepatocytes



A molecule metabolized by the hepatocyte is represented in yellow. The molecule is taken up by specific transporters, which corresponds to phase 0, then modified by phase I (oxidation or hydroxylation) and phase II enzymes (conjugation) to be finally secreted into bile canaliculi (phase III) or transported back to the space of Disse by efflux transporters (phase III).

Bile composition, flow and function

Bile composition

Canalicular bile is made up of numerous elements. BA and salts are the principal constituents of bile: they are amphipathic molecules, meaning they are both hydrophobic ("water-fearing") and hydrophilic ("water-loving"). BA are synthetized by hepatocytes from cholesterol, and play an important role in lipid, cholesterol and fat-soluble vitamin (vitamin A, D, E and K) digestion and absorption.

Lecithin, a phospholipid also included in bile forms micelles with BA in bile canaliculi to solubilize hydrophobic cholesterol secreted in bile, decrease BA toxicity against cells lining the bile ducts, and help form micelles to absorb lipids in the digestive tract[4,5]. Under physiological conditions, biliary phospholipids are transported into bile via the canalicular phospholipid flippase multidrug resistance transporter (MDR3 in humans, and its mouse ortholog, Mdr2)[6].

Bilirubin is a hydrophobic yellow biliary pigment produced by the degradation of red blood cells' heme. Red blood cells spend 120 days in the bloodstream. Senescent red blood cells are physiologically eliminated by resident macrophages, mostly in the spleen but also by the liver's Kupffer cells. Macrophages process heme into biliverdin then bilirubin, and secrete bilirubin back to the bloodstream. Bilirubin is transported to the liver by albumin, and taken up by hepatocytes via multi-specific uptake transporters named OATP (organic anion transporting polypeptide). For bilirubin to be eliminated in bile by the liver, it must be conjugated by an enzyme called glycuronosyl-transferase to facilitate its solubilization and elimination via a multi-specific secretion transporter named MRP2 (multidrug resistance associated protein 2). In the digestive tract, conjugated bilirubin is transformed by bacteria into urobilinogen then stercobilin. Urobilinogen is reabsorbed from the digestive tract and secreted in the urine. Stercobilin is the pigment responsible for feces' brown color.

Bile also contains cholesterol, and serves as the only way for the human body to get rid of excess cholesterol. Bicarbonate and ions in bile are secreted by cholangiocytes. These ions alkalinize and hydrate bile. The last component of bile, soluble immunoglobulin A (sIgA), is an antibody produced by portal tract and biliary tract submucosal plasma cells (a differentiated type B lymphocyte) and secreted into bile through cholangiocytes by a mechanism of transcytosis. It is the only component of bile that is not secreted by hepatocytes. Soluble IgA play a role in gut microbiota regulation as well as bacteria neutralization[7].

Bile flow and function

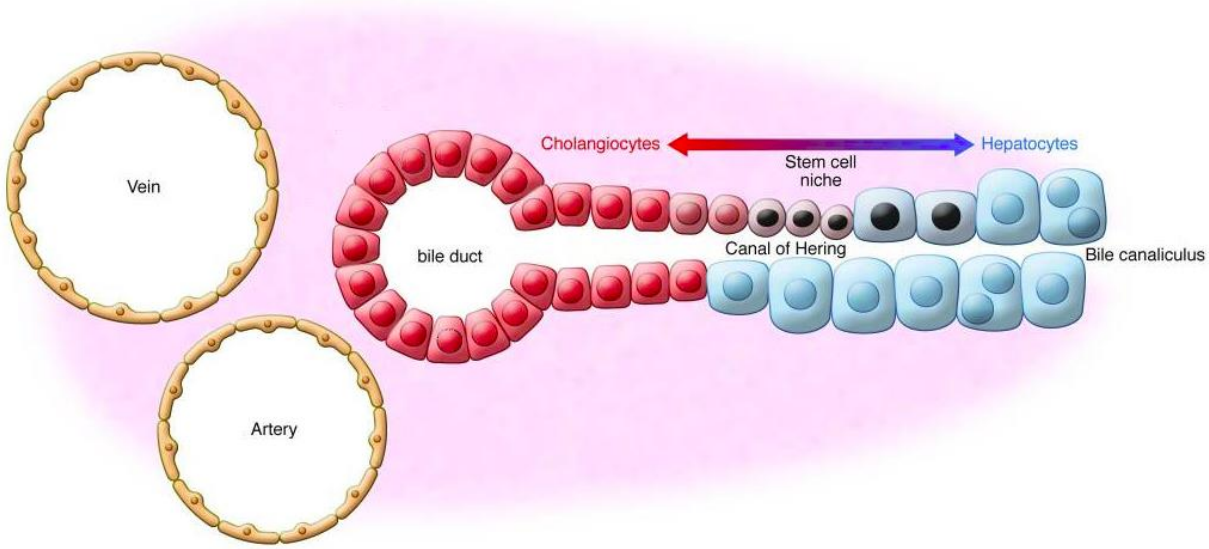
Bile is a green-looking liquid, and its secretion is continuous. All the constituents of bile except for sIgA are secreted by hepatocytes at their apical pole into bile canaliculi, which are the small canals circumscribed by hepatocytes. Bile flows away from the center of the hepatic lobule, towards the bile ducts of the portal triad. The narrow link between the intralobular, hepatocyte-lined bile canaliculi and the extralobular, cholangiocyte-lined bile ducts is called the canal of Hering (illustrated in figure 2 and 3 below)[8].

Small bile ducts from each liver lobe converge into larger ducts, forming the left and right hepatic duct, who in turn merge at the bottom of the liver into the unique hepatic common duct. From there on, bile will either flow down the common biliary duct to the duodenum or when the sphincter of Oddi prevents bile from entering the duodenum in interprandial state, bile will be stored, acidified and concentrated in the gallbladder.

In response to the presence of lipids in the intestinal lumen, cholecystokinin (CCK) is secreted by intestinal cells. CCK induces secretion of pancreatic enzymes, gallbladder contraction and relaxation of Oddi's sphincter. Gallbladder contractions propel bile down the common bile duct, through the pancreas where bile mixes with pancreatic enzymes, and finally through Oddi's sphincter into the duodenum. With the help of intestinal mixing movement, BA act as detergents and bury their hydrophobic part into the emulsified fat droplet, while presenting their hydrosoluble, negatively-charged

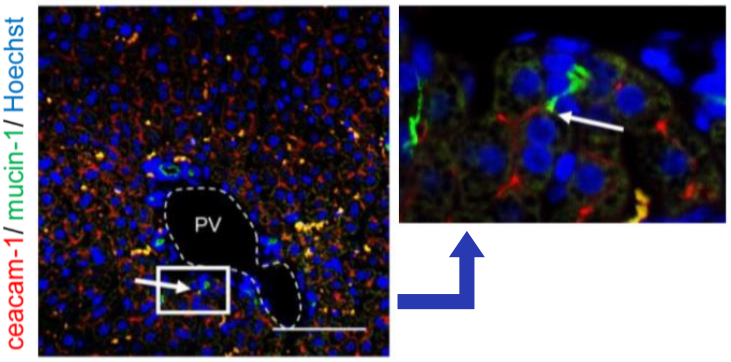
surface on the outer edge. This repels the small fat droplet from other negatively-charged, similarly formed small fat droplets, and prevents them to collapse back into large fat droplets. The division of large fat droplet into numerous small fat droplets increases the total surface accessible to pancreatic lipid-digesting enzymes (named lipases), hence accelerating fat digestion. This process goes on until lipid droplets reach a nanometric size. A nanometric lipid droplet is called a micelle. Micelles containing fat hydrolysed by lipases can collide and join with our intestinal epithelial cell membrane. This is the way digested lipids are absorbed in most vertebrates[9].

Figure 2: Schematic illustration of a canal of Hering



Localized in the periportal zone outside of the hepatic lobule, the canal of Hering constitutes the narrow link between bile canaliculi lined by hepatocytes (light blue), and bile ducts lined by cholangiocytes (red)[8]. Ductular reaction (DR) is known to originate from the canal of Hering, and DR-cells have shown to be bipotent progenitors for both hepatocytes and cholangiocytes (see "Liver regeneration" page 15)[10,11]. The "Stem cell niche" depiction sits above DR-cells and DR-yielded hepatocytes (with black nuclei). Portal structures included in this figure are a branch of the portal vein and of the hepatic artery, and a bile duct. Other portal structures not illustrated in this figure include a branch of the vagus nerve and a lymphatic vessel. From Kordes et al, 2013[12].

Figure 3: Histological illustration of the canal of Hering



Liver section from wild type mouse, co-stained with ceacam-1 (red) for hepatocytes apical pole; mucin-1 (green) for cholangiocytes; Hoechst (blue) for cell nucleus. The region of interest is periportal, as shown by its proximity to the portal vein (PV). The canal of Hering (white arrow) is the junction between green-stained cholangiocytes and red-stained apical pole of hepatocytes. From Clerbaux et al, unpublished.

Bile acid production, secretion, cycle

Production of bile acids

BA are synthetized by hepatocytes from cholesterol. Cholesterol may be absorbed in the intestines as pre-described or synthetized by hepatocytes themselves from acetylcoenzyme A, an intermediary in multiple biochemical pathways. As any lipid, cholesterol possesses hydrophobic properties. The reaction pathways leading to primary BA synthesis aims to increase cholesterol's solubility in water by hydroxylating the cholesterol molecules. Increasing hydrosolubility of cholesterol has two positive effects: first, it reduces passive paracellular re-absorption from the intestinal lumen to the blood. Secondly, because hydroxylation reactions always take place on one side of the cholesterol molecule, it polarises the synthetized BA with a hydrophobic pole and hydrophilic pole, making BAs capable of interacting both with water and lipids.

In humans, the most abundant primary BA species synthetized are cholic acid (CA) and chenodeoxycholic acid (CDCA). The initial and rate-limiting reaction of this multi-step process is the 7α hydroxylation of cholesterol. It is done by the cytochrome P450 monooxygenase, also known as CYP7a1[13]. Another important enzyme of this primary pathway is CYP8b1, which plays a critical role in CA synthesis[14]. It relevant to note the existence of a second pathway for BA synthesis from cholesterol, known as the 'alternative' or 'acidic' pathway. This pathway accounts for less than 50% of BA synthesis in both humans and rodents[15]. It does not operate with CYP7a1, and the rate-limiting enzyme of the alternative pathway is cholesterol 27-hydroxylase or CYP27a1[16].

Primary BAs can be conjugated with either glycine, taurine, glucuronide or sulphate. Conjugation of primary BAs also aims to increase hydrosolubility. Molecules called bile salts are deprotonated BAs: bile acids which have given away a hydrogen atom to another molecule. Deprotonation also increases hydrosolubility.

Causes of bile production fluctuations are numerous: variation of hepatocyte metabolism, variation of biliary acid pool, and variation of cholangiocyte ion and water secretion. Ursodoexycholic acid (UDCA), a naturally occurring BA, is known to increase bile flow and is used as a medication in pathologies like primary biliary cholangitis (PBC) to increase bile flow as to slow disease progression[17]. Cholangiocyte's secretion is inducible by secretin, an intestinal hormone.

Secretion of bile acids

BAs and salts are secreted at the apical pole of hepatocytes, into the bile canaliculi, via membrane transport proteins. These transporters help intracellular BAs and salts to cross the cell membrane. There are various transporters, each having a different affinity for different BAs. BA and salt secretion is adenosine triphosphate (ATP) -dependant, meaning it needs energy to work; and saturable, meaning the transporters have limited number of occupiable sites for their ligands. BSEP (bile salt export pump) is the main transporter involved in BA and salt secretion[18]. Another transporter that

has been shown to transport BAs is MRP2, but unlike BSEP is not specific to BA transport (see "Bile composition" section on bilirubin)[16].

Cycle of bile acids

Some of bile's components follow a cycle, called the entero-hepatic cycle (EHC), in which these components secreted by the liver are reabsorbed by enterocytes, and brought back to the liver by the portal vein, to be re-secreted. The components of bile following the EHC include bile salts, bilirubin, and some drugs. We will only address bile salt EHC in this description.

In the small intestines, bacteria deconjugate and transform BAs such as CA and CDCA into deoxycholic acid and lithocholic acid, respectively. Biotransformed BAs and UDCA are named secondary BAs, whether conjugated or not. Conjugated bile salts are reabsorbed by terminal ileum enterocytes via an active process involving an apical transporter called apical sodium-dependent bile salt transporter (ASBT). At the basal pole of terminal ileum enterocytes, conjugated bile salts are secreted into portal blood via a heterodimeric transporter named OSTα/β (organic solute transporter α/β). Unconjugated bile salts however are mostly eliminated in feces: only a minor portion of unconjugated bile salts undergo passive paracellular reabsorption[19]. Overall, 95%[20] of the bile salts are reabsorbed from the intestinal lumen.

Reabsorbed bile salts are transported in the bloodstream mainly by serum albumin[21]. They are bought from the terminal ileum via the portal vein to the liver, and are extracted by hepatocytes' uptake transporter. The main conjugated bile salt uptake transporter from portal blood is NTCP (sodium taurocholate cotransporting polypeptide)[16]. Another bile salt uptake transporter is OATP, but this transporter is not specific to BA transport (see "Bile composition" section on bilirubin)[22].

Intracellular bile salt will be conjugated and secreted in bile via BSEP and MRP2. In some situations (see "Regulation of bile acids"), bile salts may be exported back to the bloodstream via efflux pumps called MRP3 and MRP4 (multidrug resistance associated proteins 3 and 4)[23].

Toxicity and regulation of bile acids

The main BA synthesis enzymes and transporters and their regulation by FXR are listed in Table 1 below.

Toxicity of bile acids

BAs are detergents, capable to interact with lipids in the intestinal lumen, just as much as with intracellular lipids. BA toxicity is mostly determined by its hydrophobicity: in increasing order of hydrophobicity, BAs can be arranged as follows: UDCA < CA < CDCA[24]. Accumulation of cytoplasmic BAs has shown to be cytotoxic, because of their interaction with mitochondria[25] and intracellular side of the cell membrane[26]. BAs have also been shown to induce inflammatory genes inside hepatocytes

without causing cell death[27]. Secreted BAs can also interact with the extracellular side of the cell membrane: the phospholipids secreted in bile canaliculi help decrease this toxicity[4,5]. BA leakage outside bile canaliculi possess chemotactic properties and attract neutrophils and other inflammatory cells, triggering the activation of HSCs[28,29,30,31].

Regulation of bile acids

The hepatocyte's defence mechanism against intracellular BA toxic accumulation is managed by a BA receptor, the farnesoid X-activated receptor (FXR). FXR is found in liver, kidney and intestines, and acts as an intracellular BA sensor[16,32]. When FXR senses BA, it becomes a transcription factor for multiple genes, mainly BA synthesis enzymes and BA transporters, resulting in reduction of intracellular BA concentration. Other roles of FXR include regulation of cholesterol and lipoprotein synthesis, regulation of lipogenesis, and recently FXR has been found to play a role in the process of liver regeneration, and to act as a protective agent against the development of HCC[33].

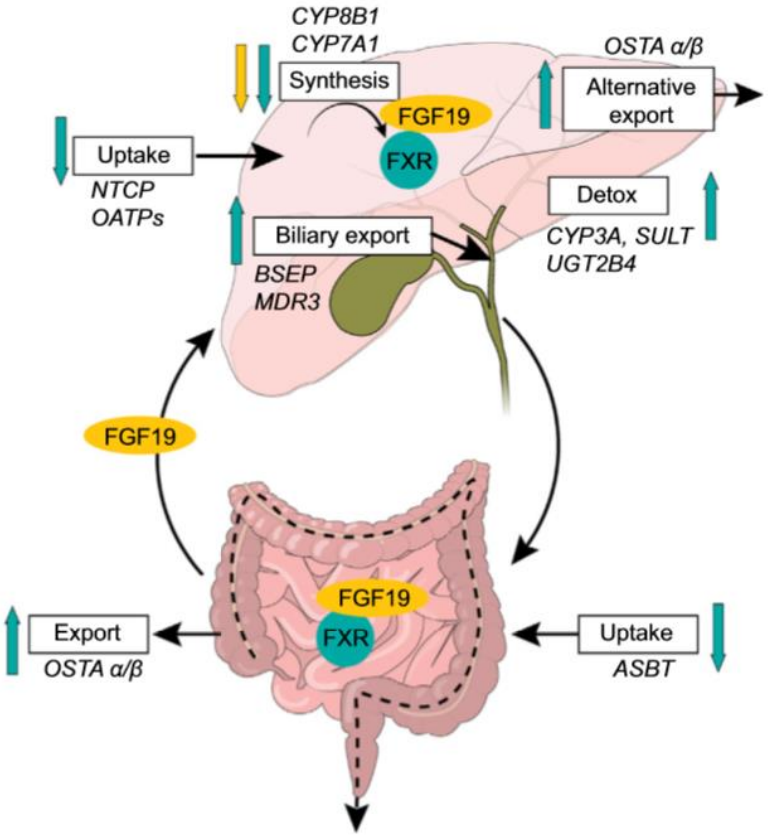
FXR plays a crucial role in BA homeostasis by its action in hepatocytes and in terminal ileum enterocytes. In hepatocytes, FXR activation increases BA secretion by up-regulation of BSEP and MRP2 expression, decreases BA uptake by down-regulation of NTCP and OATP expression, and decreases BA synthesis by down-regulation of CYP7a1 and CYP8b1 expression[33,34]. This complex regulation reduces BA intracellular concentration, sparing hepatocytes from toxic intracellular levels of BA, especially useful in cholestasis. Furthermore, FXR has been shown to up-regulate Mdr2/MDR3 (phospholipid flippase) expression in hepatocytes, thus decreasing BA toxicity in bile canaliculi[35]. In terminal ileum enterocyte, FXR down-regulates ASBT and up-regulates OSTα/β expression to protect those enterocytes from toxic intracellular BA concentrations[36,37]. Moreover, BA activation of FXR induces secretion of FGF19 (fibroblast growth factor 19, or its ortholog Fgf15 in mice) by terminal ileum enterocytes, which also down-regulates CYP7a1 expression in hepatocytes[38,39].

BAs act as signaling molecules, being ligands of various gene-regulating intracellular receptors such as FXR. BA are also ligands of transmembrane receptors such as the G protein-coupled membrane receptor (TGR5) which has numerous roles: basal metabolism regulation, GLP-1 (glucagon-like peptide 1, an incretin which plays a role in glucose homeostasis) secretion, regulation of bile composition in the gallbladder, and even reduction of hepatic and intestinal inflammation[40,41,42,43,44]. Altogether, with the help of their gene-regulating receptors, BAs can regulate their own intracellular concentration, as well as lipid metabolism, glucose metabolism, hepatic and intestinal inflammation, gallbladder BA concentration, and much more. This makes BAs and their receptors very attractive targets for multiple reasons which we chose not to discuss in this essay.

Table 1: Main bile acid synthesis enzymes and transporters, and their regulation by FXR

Abbreviations	Full name	Function and FXR-regulation
Bile acid synthesis enzymes		
CYP7a1	cholesterol 7α-hydroxylase	Rate-limiting enzyme in primary bile acid synthesis pathway[13] Down-regulated by FXR[33]
CYP8b1	sterol 12α-hydroxylase	Enzyme of the primary bile acid synthesis pathway[13] Down-regulated by FXR[33]
Bile acid & salt secretion transporters		
BSEP (ABCB11)	bile salt export pump	ATP-dependant main bile salt secretion transporter[18] Up-regulated by FXR[33]
MRP2 (ABCC2)	Multidrug resistance associated protein 2	ATP-dependant multispecific organic anion transporter[16] Up-regulated by FXR[34]
Bile acid & salt uptake transporters		
NTCP (SLC10A1)	Sodium taurocholate cotransporting polypeptide	Main conjugated bile acid uptake transporter[16] Down-regulated by FXR[33]
OATPs (SLCOs)	Organic anion transporting polypeptides	Multispecific organic anion transporter[22] Down-regulated by FXR[33]
Bile acid & salt efflux transporters		
MRP3 (ABCC3)	Multidrug resistance associated protein 3	ATP-dependant bile acid efflux[23] FXR-independant[45]
MRP4 (ABCC4)	Multidrug resistance associated protein 4	ATP-dependant sulfated bile acid efflux[23] FXR-independant[45]
Bile salt transporters of the terminal ileum enterocyte		
ASBT (SLC10A2)	Apical sodium-dependent bile salt transporter	Terminal ileum sodium-coupled conjugated bile salt reabsorption Down-regulated by FXR[36]
OSTα/β (SLC51A/B)	Organic solute transporter α/β	Bile salt secretion transporter of terminal ileum enterocyte Up-regulated by FXR[37]

Figure 4: Illustration of the bile acid cycle and FXR-mediated regulation (Fickert et al, 2017)[46].



Bile acids, synthetized in the liver with the help of CYP7a1 and CYP8b1, are secreted in bile via BSEP and MDR3. Bile flows to the digestive tract, and helps with digestion and absorption of lipophilic molecules. Uptake of bile acids occurs at the terminal ileum, with the help of ASBT. Non-recaptured bile acids are wasted in faeces. BA in terminal ileum enterocytes are secreted into the bloodstream at the vascular pole of terminal ileum enterocytes via OST. Brought back to the liver by portal blood, BA are up-taken into hepatocytes by NTCP and OATPs. FXR is activated in cholestatic situations by the excessive presence of intracellular BA. FXR-regulation in hepatocytes induces secretion up-regulation, and uptake and synthesis down-regulation. In terminal ileum enterocytes, FXR down-regulates uptake from the digestive tract, and increases export to the portal blood. FGF19 is secreted by terminal ileum enterocytes, and down-regulates CYP7a1 in hepatocytes. Abbreviations: ASBT, apical sodium-dependent bile salt transporter; BSEP, bile salt export pump; CYP7A1, cholesterol 7α-hydroxylase; CYP8b1, sterol 12-α hydroxylase; FGF19, fibroblast growth factor 19; FXR, farnesoid X receptor; MRP2, 3 and 4, multidrug resistance associated protein 2, 3 and 4; NTCP, sodium-taurocholate cotransporting polypeptide; OATPs, organic anion transporting polypeptides; OST, organic solute transporter.

Cholestasis

Cholestasis is a pathological condition where bile secretion and/or flow is reduced or completely blocked. Bile accumulation inside hepatocytes and in bile ducts causes damage, lessened by protective mechanisms such as those related to FXR regulation.

Clinically, intrahepatic and extrahepatic cholestasis are distinguished. Intrahepatic cholestasis may either be due to bile secretion deficit originating from hepatocyte dysfunction, or from intrahepatic bile duct obstruction. Secretion deficit may originate from genetic alterations of protein associated with bile flow (e.g. bile acid secretion transporter MRP2 mutation in Dubin-Johnson syndrome, BSEP mutation in type 2 progressive familial intrahepatic cholestasis, ion transporter CFTR mutation in cystic fibrosis), by drug interaction (e.g. inhibition of CYP450 by antiretroviral medication, inhibition of NTCP by cyclosporin A, drug competition and saturation of transport or phase II and II enzymes,…), or by both acute and chronic liver insufficiency (e.g. viral hepatitis, consumption of glutathione by paracetamol metabolite, alcoholic and non-alcoholic hepatitis,…). Intrahepatic bile duct obstruction may originate from cirrhosis, intrahepatic malignancies, amyloidosis, granulomatous diseases (tuberculosis, sarcoidosis), primary biliary cholangitis and primitive sclerosing cholangitis.

Extrahepatic cholestasis is due to common biliary tract obstruction. The main causes being gallstones and malignancies (pancreas cancer, cholangiocarcinoma, cancer of the Ampulla of Vater, invasive malignancies from the gallbladder, stomach, duodenum). Other less frequent extrahepatic obstruction causes include acute or chronic pancreatitis, postoperative scarring stenosis, Mirizzi syndrome, primitive sclerosing cholangitis, secondary sclerosis due to duodenal ulcer, ascariasis, and granulomatous or neoplastic lymphadenopathy.

In consequence, products normally excreted into bile such as cholesterol, BAs and bilirubin accumulate in the hepatocyte, triggering FXR-led defence mechanisms. Accumulation of cholesterol, BAs and bilirubin clinically lead to hypercholesterolemia, prurit and jaundice, respectively. Furthermore, reduced concentration of BAs reaching the intestines causes lipid malabsorption and vitamin A, D, E and K deficiency. Long-term cholestasis induces portal fibrosis, also called secondary biliary cirrhosis. To this date, the most accepted etiology of cholestatic secondary biliary cirrhosis is direct toxicity of BA[47]. As mentioned previously, BA have cytotoxic and pro-inflammatory properties. Pathways by which BA could cause inflammation include extra-biliary pro-inflammatory BA leakage, and hepatocyte exposure to toxic concentrations of BAs, causing apoptosis[48,49]. But the exact molecular pathway by which cholestasis induces inflammation is still discussed.

Liver regeneration

The liver is an organ that fulfils vital functions. However, sources of liver damage are numerous and frequent, cholestasis only being one of them. Fortunately, the liver has a unique regenerative capacity. Other organs such as the epidermis, the intestine mucosa and the hematopoietic system share a similar regenerative capacity but unlike in the liver, the role of tissue-specific stem cells in the replenishment of mature cells in these organs has been extensively characterised[50,51].

Liver mass homeostasis and liver regeneration after acute injury is achieved by mature hepatocyte hypertrophy and proliferation[52,53]. If the acute damage is severe it may lead to liver failure, a state in which the liver is unable fulfil its functions. Untreated, acute liver failure has a poor prognosis. Acute liver injury in humans may result from a wide variety of causes, such as hepatitis A, acetaminophen toxicity, or liver hypoperfusion. In mice, models used to study regeneration in acute liver injury include partial hepatectomy (PHx) and bile duct ligation (BDL).

Chronic liver injury is a more complex scenario, histologically characterized by hepatic fibrosis. Hepatic fibrosis arises from excessive ECM production by HSCs, activated by cytokines subsequent to chronic liver injury. The activation of HSCs induces their transformation into myofibroblasts, which are extracellular matrix secreting cells. This is the turning point for liver fibrosis, since excessive ECM in the space of Disse impairs exchange between hepatocytes and blood, leading to liver dysfunction[54,55]. From then on hepatic fibrosis eventually evolves to cirrhosis, representing the end-stage of any chronic liver disease. Cirrhosis is characterized by disruption of hepatic architecture, regenerative nodules, hepatocyte dysfunction and is associated with life-threatening complications such as hepatocellular insufficiency (i.e. liver failure) and hepatocellular carcinoma (HCC)[56]. So far, liver transplantation represents the only curative therapeutic solution for many chronic liver diseases, but because of organ shortage and the drawbacks of adult living donor liver transplant, new therapies are needed. This situation is the main drive behind research in liver cell therapy.

Ductular reaction

In chronic liver injury states when hepatocytic proliferation is impaired or overwhelmed, liver failure is inexorable. Ductular reaction (DR, or oval cell proliferation in rodents), a periportal proliferation of small immature cells forming ductular structures is observed histologically in all chronic liver injury models[57,58,59,60]. DR originates from the canals of Hering, a strategic periportal location where hepatocyte-lined bile canaliculi join with cholangiocyte-lined bile ducts. DR-cells, also referred as oval cells in rodents or liver progenitor cells (LPCs), possess proteins also expressed by both hepatocytes and cholangiocytes, and have the potential to differentiate into either of these two lineages, as proven by both in vitro culture and in vivo LPC lineage-tracing[10,11]. Other than in human, LPCs have also been located in the liver of rodents, dogs and cats[61,62,63].

Ductular reaction phenotype is variable, and differs accordingly to the injury type[64]. In cholestatic conditions such as bile duct ligation, DR resembles small bile duct proliferation. This proliferation is confined in portal mesenchyma, and characterized by an increase in bile duct branching, ductular length and surface area[65]. Studies using the 3,5-diethoxycarbonyl-1,4-dihydrocollidine (DDC) diet in mice as model of cholestasis chronic liver injury, failed to demonstrate DR contribution to the hepatocyte pool[66,67]. Furthermore, cholestatic injury has not been shown to perturb hepato-biliary junctions. We therefore hypothesize that the goal of this duct proliferation is to accommodate excess bile.

In chronic liver injury targeting hepatocytes, ductular reaction exhibits a much different phenotype than in cholestatic conditions. A study by the gastro-enterology (GAEN) laboratory of the Université Catholique de Louvain (UCL), in which the fate of DR-cells was followed upon hepatocellular injury caused by a choline-deficient and ethionine-supplemented (CDE) diet in mice, observed invasive ductular reaction from the periportal region deep into the hepatic lobule, but only very scarce DR-cell differentiation into hepatocytes (total hepatocyte proportion originating from DR-cells representing <2.5%)[68]. This raises the question of the etiology and utility of ductular reaction in the CDE model. Our hypothesis is that DR in the CDE model invades the hepatic lobule in order to establish new connections between bile canaliculi and periportal cholangiocyte-lined bile ducts to reduce intralobular cholestasis caused by hepatocyte damage from CDE-diet.

The mouse model

The mouse has long been used as an organism model to study various human diseases such as non-alcoholic steato-hepatitis, alcoholic steato-hepatitis, portal hypertension, cholestatic cirrhosis, sclerosing cholangitis, parasitic infectious disease, hepatectomy, as well as liver tumors and metastases. Other than practical and ethical advantages that mouse models have over human or ape, mouse liver disease models are numerous, and can be modeled for specific needs: these models may be genetically induced like CFTR-/- models to study cystic fibrosis transmembrane conductance regulator mutation, surgically induced like BDL or PHx models, drug-induced like intravenous CCl_4 (carbon tetrachloride) or diets such as the CDE or DDC diets. Compared with the study of in vitro human hepatocytes, mouse models have the advantage that the whole organ and organism is intact[69]. There are however limitations to mouse liver models, such as chronic alcohol injury, or the study of liver infection with hepatitis C and B viruses, which can only infect humans and chimpanzees. Such diseases could be studied in humanized mouse models in the future[70].

In whole morphology, the mice's biliary tract is largely identical to humans. A few anatomical differences exist, and should be noted when using mice as an animal model, such as the constant existence of the *arteria gastrica sinistra accessoria*, the continuous junction between the duodenal muscle and Oddi's sphincter muscle, and the absence of a sphincter muscle at the junction between the pancreatic duct and the bile duct[71]. Histologically, cellular organization in mouse and human liver is

similar[72]. In physiological conditions, the most abundant serum BAs in humans are CA and CDCA. Because CDCA is converted into β-muricholic acid (βMCA) in mice, the most physiologically abundant serum BA in mice are CA and βMCA[73,74]. By convention, human proteins are stated in capital letters (eg. NTCP) and mouse proteins are stated with only the first letter in uppercase (eg.Ntcp). Most human BA synthesis enzymes and transporters have their mice ortholog: this includes the BA synthesis enzymes and transporters studied in this essay.

4. Working hypothesis and current questions

DR is consistently present in chronic liver injury, but differentiation of DR-cells into hepatocytes only occurs in rare situations where hepatocyte proliferation is totally impaired[75]. We propose that a function of the invasive DR observed in the CDE model is to connect hepatocyte bile canaliculi with periportal bile ducts to ensure bile drainage and reduce intralobular cholestasis during damage-healing response.

Previously obtained data in the GAEN laboratory conclude that LPCs at the canal of Hering border bile flow: this has been proven histologically by highlighting bile flow with cholyl-lysyl-fluorescein (CLF) and tagging LPCs using mice expressing tamoxifen-inducible Cre recombinase under control of osteopontin regulatory region crossed with yellow fluorescent protein to follow LPCs[68]. The GAEN laboratory also showed that hepatocyte proliferation in the CDE model is not fully impaired, as assessed by staining of the proliferation marker Ki-67. Furthermore, in the CDE model, bile flow rate and CLF uptake is significantly decreased, and a high increase in serum BA levels and a decrease in total bile flow rate was observed[68]. CDE-induced hepatocellular damage therefore impairs canalicular bile flow at a hepatocellular level. This could either be due to decreased BA uptake from blood to hepatocytes, increased basolateral efflux, decreased BA secretion, or decreased BA synthesis.

In agreement with the current GAEN laboratory hypothesis that DR's function in the CDE model is to connect to bile canaliculi to improve bile drainage, the current aim of this paper is to inquire over modification in BA flow as being a possible trigger for DR in the CDE model. To answer this question, we will first analyse mRNA and protein expression levels of hepatocyte BA synthesis enzymes and transporters in control mice versus CDE-fed mice. From these results, three questions will be addressed through a medical literature review:

1. What is known over bile acid synthesis enzymes and transporters expression in other rodent models of ductular reaction?
2. What is known over bile acid synthesis enzymes and transporters expression in human diseases involving ductular reaction?
3. What are the strategies and outcomes of NTCP inhibition in the liver?

5. Material & methods

The CDE model of hepatocellular injury: pathophysiological mechanisms and DR phenotype (Clerbaux et al, 2017)[76]

The CDE model consists of *ad libitum* administration of a choline deficient diet together with procurement of ethionine in the drinking water. Choline is normally provided by food intake and participates to the structural integrity and signaling function of cell membranes. A choline withdrawal leads to a decreased synthesis of phosphatidylcholine, a phospholipid crucial for cell membrane and a major building stone of the very low-density lipoprotein particles produced by hepatocytes to export triglycerides. Choline deficiency causes thus intracytoplasmic fat accumulation, hepatocyte dysfunction and damage[77,78]. This extensive hepatocellular damage results into high hepatocyte replication ratio, causing their exhaustion and restraining the production of hepatic drug metabolism-related enzymes[79]. Ethionine, a synthetic amino acid, specifically targets the hepatocytes in which, when provided in large excess, competes with its naturally occurring analogue methionine. Competition of ethionine with methionine favors to the synthesis of S-adenosyl ethionine (SAE) instead of S-adenosyl methionine (SAM). Consequently, an ethyl group is transferred instead of a methyl group in methylation reactions hereby generating abnormal proteins, lipids, RNA (ribonucleic acid) and DNA (deoxyribonucleic acid) molecules, which results in hepatocytic cell damage[80]. Administration of ethionine in supplement to a choline-deficient diet greatly shortens the time required for LPC proliferation[81]. Although, the exact mechanism of action of CDE-induced injury is not well known, it appears that the combined administration of ethionine with choline deficient chow induces a liver injury in which the hepatocytes are specifically targeted and the replication of the surviving hepatocytes is inhibited[82]. Hepatocyte proliferation to replace damaged liver cells is prevented and activation of the LPC compartment ensues. Briefly, short-term CDE feeding results in steatosis, inflammation, LPC expansion (DR) and fibrosis that progress in parallel. Cirrhosis and hepatocellular carcinoma may be observed in long-term studies.

Animals and diets

All animal experiments were performed with approval of the University Animal Welfare Committee (2012UCLMD026; 2016UCLMD003). Five-week old male C57Bl/6J mice obtained from Janvier-Breeding Center (Le Genest St. Isle, France) were housed in a pathogen-free facility following a 12-hour light/dark cycle. After 1 week of acclimatization, mice received either chow diet (control group) or a diet deficient in choline (MP Biomedicals, Irvine, CA) together with drinking water supplemented with 0.15% (wt/vol) ethionine (Sigma, Bornem, Belgium) during 3, 9 or 21 days. Mice were fasted overnight before sacrifice. Part of the liver was fixed in 4% formalin or frozen in optimal cutting temperature (OCT) medium for histological analyses. The remaining liver lobes were immediately snap-frozen in liquid nitrogen and kept at -80°C until use.

RNA extraction and real time PCR (RT PCR)

Total RNA was extracted using TRIzol (Invitrogen). Quantitative real-time polymerase chain reaction (qRT-PCR) was performed by AB StepOne Plus (Applied Biosystems Foster City, CA) using SYBR Green PCR Master Mix (Applied Biosystems, Foster City, CA). 36B4 was used as an internal standard. Results are expressed relative to expression in control (value set at 1) using the ΔΔCt method.

Protein extraction and Western blots

Frozen liver tissues were homogenized in ERK buffer, then centrifuged at 100,000 × g for 10min at 4 °C. The supernatant was then divided into aliquots stored at -80°C until use. Protein concentration was determined by bicinchoninic acid (BCA) protein assay (Pierce Chemical, Rockford, Illinois, USA). A total of 50 µg of protein was resolved on a sodium dodecyl sulfate-polyacrylamide gel electrophoresis gel and transferred to a nitrocellulose membrane (Hybond C-extra; Amersham International plc, Little Chalfont, UK). Membranes were incubated with anti-NTCP antibody (1/1000) (LSBio, LS-C407957) or with anti-BSEP antibody (1/500) (Santa Cruz Biotechnology, sc-74500) and peroxidase conjugated mouse anti-rabbit (1/30 000) and goat anti-mouse (1/20 000) IgGs (Jackson ImmunoResearch, West Grove, Pennsylvania, USA) and revealed with the Western Lightning detection system (PerkinElmer, Waltham, Massachusetts). Quantification was performed with the Molecular Imager ChemiDoc XRS System (Bio-Rad Laboratories, Nazareth, Belgium). Hsp90 was used as a loading control.

Statistical analysis:

All data are presented as means ± standard deviation and were compared using the unpaired two-tailed Student t test or one-way analysis of variance.

6. Results

In CDE mice, BA synthesis, efflux and secretion were unchanged, while uptake was profoundly down-regulated

In control mice and CDE-fed mice, we tested hepatic mRNA expression of two main BA synthesis enzymes (Cyp7a1 and Cyp8b1), two main BA secretion transporters (Bsep and Mrp2), two main BA efflux transporters (Mrp3 and Mrp4) and two main BA uptake transporters (Ntcp and Oatp1a1). The results are detailed in Table 2. Our results show that in CDE-fed mice, BA synthesis enzymes mRNA of Cyp7a1 remained static. Cyp8b1 mRNA levels showed a very slight decrease at 21 days ($p<0.05$). BA secretion transporter mRNA of Bsep and Mrp2 were both up-regulated at 9 days of CDE-feeding ($p<0.001$), and Bsep mRNA levels remained up-regulated at 21 days ($p<0.001$), unlike Mrp2 whose mRNA levels fell back to normal after 21 days. BA efflux transporter Mrp3 mRNA were very slightly increased after 21 days ($p<0.05$), while Mrp4 showed an increase in mRNA transcription

at 9 days ($p<0.05$), which persisted until 21 days ($p<0.05$). BA uptake transporters Ntcp and Oatp1a1 both showed progressive transcription down-regulation at 9 days ($p<0.05$), which was even more profound after 21 days ($p<0.001$).

To confirm that the mRNA variations which we obtained result in protein level modifications, we tested protein expression of Bsep and Ntcp in pre-described specimens with Western Blot analysis, detailed figure 5 and 6, respectively. Our results show that despite an mRNA up-regulation of Bsep, protein levels remained unchanged. However, decreased Ntcp mRNA level resulted in a decreased Ntcp protein expression ($p<0.05$).

In summary, in the CDE model BA uptake transporter expression was decreased in the absence of BA synthesis, secretion and efflux transporter expression modification.

Table 2: Hepatic relative mRNA expression of several BA synthesis enzymes and transporters genes in control (CTL) and CDE-fed mice.

Gene	CTL	CDE 3d	CDE 9d	CDE 21d
Synthesis				
Cyp7a1	1.0 ± 0.5	0.6 ± 0.2	1.1 ± 0.6	1.0 ± 0.7
Cyp8b1	1.0 ± 0.2	1.4 ± 0.5	0.6 ± 0.2	0.4 ± 0.2*
Secretion				
Bsep	1.0 ± 0.1	1.4 ± 0.3	2.2 ± 0.4***	1.8 ± 0.5***
Mrp2	1.0 ± 0.3	1.2 ± 0.3	2.0 ± 0.4***	1.4 ± 0.2
Efflux				
Mrp3	1.0 ± 0.1	0.9 ± 0.1	1.0 ± 0.1	0.8 ± 0.1*
Mrp4	1.0 ± 0.3	1.1 ± 0.6	3.6 ± 2.4*	3.5 ± 2.1*
Uptake				
Ntcp	1.0 ± 0.2	1.0 ± 0.4	0.5 ± 0.1*	0.4 ± 0.1***
Oatp1a1	1.0 ± 0.2	0.9 ± 0.2	0.7 ± 0.1*	0.3 ± 0.1***

*Hepatic mRNA expression levels of several BA synthesis enzymes and transporter genes involved in bile acids circulation in mice fed a chow diet (CTL) and 3 days, 9 days and 21 days of CDE regimen (CDE). *p<0.05, ***p<0.001. Results show no change in mRNA levels of CYP7a1 and CYP8b1, a slight significant increase of BSEP and MRP2 mRNA expression peaking at day 9, static MRP3 mRNA expression, increase in MRP4 mRNA expression, and significant decrease in NTCP and OATP1a1 mRNA expression.*

Figure 5: Western blot of Bsep

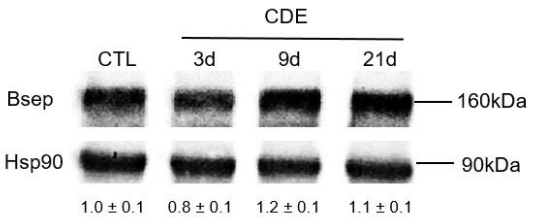
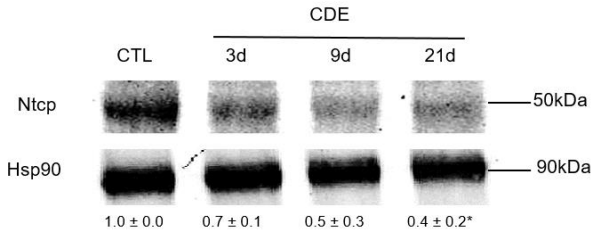


Figure 6: Western blot of Ntcp



*Western blot analysis of Bsep, Ntcp and Hsp90 (used as a loading control) in mice fed a chow diet (CTL) and 3 days, 9 days and 21 days of CDE regimen (CDE). Figure 5: we observe static Bsep protein expression ; Figure 6: we observe a decrease in Ntcp protein expression. *p<0.05.*

Discussion

Ductular reaction (DR) arises from the canals of Hering, and yield biliary cells with an immature phenotype. This phenomenon is observed in virtually all chronic liver injury diseases. However, DR's etiology and utility is still unknown. In the CDE model, DR is extensive and invasive, but only very scarcely yield hepatocytes. In previous studies, we have observed that ductular reaction expands as a biliary polarized epithelium and establish new hepatobiliary junctions. We therefore think that ductular reaction's function is to connect hepatocyte bile canaliculi with periportal bile ducts to ensure bile drainage and prevent superimposed cholestasis in CDE model. The aim of this paper was to inquire over modification in BA transport as being a possible trigger for DR in the CDE model.

Our results show unchanged expression of BA synthesis enzymes, and secretion and efflux pumps, but significantly decreased BA uptake pump expression both at mRNA and protein levels. Previous studies in the GAEN lab show that CDE model causes an increase in total BA and bilirubin blood levels (Figure 7A and 7B, respectively, *Clerbaux et al, unpublished*). Therefore, cholestasis is present in the CDE model. However, our findings do not fit with cholestasis-induced FXR regulation of BA synthesis enzymes and transporters, where BA uptake decrease is accompanied by a decrease in synthesis, and an increase in secretion. This suggests that cholestasis in CDE model is not caused by secretion deficit, since Bsep protein levels were unchanged after 21 days of CDE diet. Rather, since we observe a decrease in Ntcp protein expression, cholestasis must be pre-hepatic: in other words, BA only accumulate in blood because Ntcp expression is decreased.

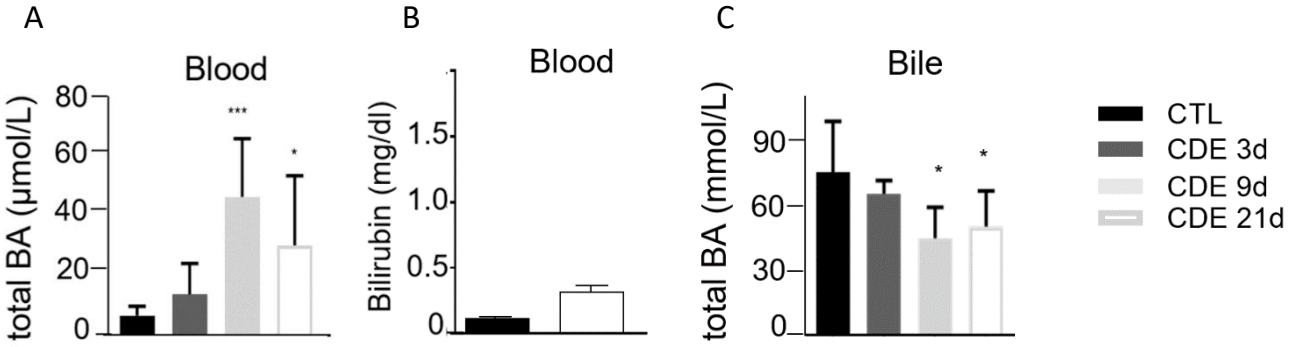
A possible bias to the interpretation of unchanged BA synthesis in this experiment is that the 'alternative' or 'acidic' synthesis pathway, which accounts for less than 50% of BA synthesis in both humans and rodents and does not operate with Cyp7a1 nor Cyp8b1, was not tested. However, BA are known to have minimal effect on mRNA regulation of cytochromes of this pathway[15,83]. Another possible bias is that decreased uptake through Ntcp could be compensated by overexpression of other transporters than Oatp1a1, this last transporter only being one of the three most abundant Oatp isoforms in the liver of rodents, together with Oatp1a4, Oatp1b2[84].

In my opinion, the main drawback to this experiment is that correlation between mRNA and protein levels is not absolute, as seen when comparing mRNA and protein levels of Bsep. Although FXR regulates mRNA transcription, knowledge over post-transcriptional regulation of BA synthesis enzyme and BA transporter mRNA is still scarce. A feasible solution would be to measure end-products only, for example by using immunostaining with anti-transporter antibodies[85]. But the utopic solution to alleviate this bias is to stain only functional pumps, or to test in a dynamic way BA trafficking.

From our results, we hypothesize that in the CDE model, increased blood BA levels secondary to pre-hepatic cholestasis secondary to Ntcp inhibition, associated with a decrease in BA bile levels (Figure 7C, *Clerbaux et al, unpublished*) is the trigger of DR. To better understand the links between chronic liver damage, BA trafficking and DR, three separate literature reviews will be carried out:

1. What is known over bile acid synthesis enzymes and transporters expression in other rodent models of ductular reaction?
2. What is known over bile acid synthesis enzymes and transporters expression in human diseases involving ductular reaction?
3. What are the strategies and outcomes of Ntcp inhibition in the liver?

Figure 7:



Results for total blood bile acid (BA) levels, serum bilirubin levels and total BA in bile are shown for control (CTL) and CDE-fed mice after 3, 9 and 21 days.
*(A) We observe an increase of total blood BA levels after 9 days, and after 21 days of CDE diet, signing the presence cholestasis. (B) We observe a slight increase in blood bilirubin of CDE-fed mice after 21 days, signing the presence of cholestasis. (C) We observe a non-statistical decrease in total bile acid levels in bile after 3 days of CDE diet, which becomes statistically significative after 9 days, and maintained at 21 days. *p<0.05, ***p<0.001. (Clerbaux et al, unpublished)*

7. Literature review

Question 1: What is known over bile acid synthesis enzymes and transporters expression in other rodent models of ductular reaction?

We analyzed six models of ductular reaction (DR) and selected thirteen articles relevant to the topic of this literature review. The DR models presented are divided into two categories, based on the ductular reaction phenotype. All observations in the following paragraphs of this first literature review and their respective articles are mentioned in Table 3.

The first category, named "duct proliferation", contains three models of DR consequent to cholestatic biliary injury. The models included in this category are BDL with observations at 7, 14 and 28 days ; the Mdr2 knock-out model (Mdr2-/-) ; and the DDC diet. Bile duct ligation leads to acute extrahepatic obstructive cholestasis, whereas the DDC and Mdr2-/- cause sclerosing cholangitis and biliary fibrosis. All three models showed increased serum BA levels, typical of cholestasis. The histological description of the DR in these models is quite similar, consisting of duct proliferation. The most precise description of the duct proliferation that is observed in these models is made by *Vartak et al, 2016* with 3D duct reconstruction 28 days after BDL, observing an increase in luminal surface area, consequent to an increase in duct length and not duct diameter[65]. This observation leads to think that the duct proliferation aims to "*optimize the interface of the duct lumen to the cholangiocyte apical membrane*", and not to maximize duct volume. In contrast, larger bile ducts showed an increase in diameter, thus reducing the ducts' surface to volume ratio. The hypothesis made by *Vartak et al, 2016* to explain the discrepancy observed between small and large bile duct's reaction to BDL is that cholangiocytes may alleviate biliary cholestasis, and that smaller bile duct cholangiocytes have greater resorption capacity for bile salts than larger bile ducts do.

Molecularly in duct proliferation models, BA synthesis and transporters profile showed Ntcp and Oatp down-regulation both at mRNA and protein levels, Mrp3 and Mrp4 efflux protein up-regulation in BDL and DDC models (no data for Mdr2-/-), and mitigated BA synthesis enzymes results (increase Cyp7a1 mRNA in BDL 7 days, and unchanged Cyp7a1 mRNA in BDL 14 days). Bsep observed an up-regulation in BDL and DDC (with a temporary decrease after 1 week of DDC), however in Mdr2-/-, the mRNA increase was met with a protein decrease.

Globally, in "duct proliferation" models, BA up-take was decreased, efflux was increased, secretion was increased (except for the Mdr2-/- model), and synthesis was mitigated. These observations overall comply with the FXR-mediated regulation of BA transporter in situations of cholestasis.

The second category was named “invasive ductular reaction”, because DR observed in these models tends to spread into the lobular parenchyma towards the centrilobular vein rather than stay confined in portal mesenchyma like models in the “duct proliferation” category. Furthermore, unlike in “duct proliferation” models where the damage is located in the biliary compartment, liver damage in “invasive DR” models was secondary to hepatocellular damage. The models presented in this category are the MCD diet (methionine-choline-deficient) which replicates non-alcoholic steato-hepatitis (NASH) ; the 2-AAF/PHx (2-acetylaminofluorene administration with partial hepatectomy) where 2-AAF temporarily blocks hepatocyte replication after PHx, causing an acute and irreplaceable shortage in hepatocytes ; and the CDE model that causes hepatocytic damage secondary to intracytoplasmic fat accumulation and DNA, RNA and protein damage. Hepatocytic injury in these models cause disruptions of hepato-biliary junctions, and the invasive ductular reaction observed in these models is directed towards the hepatic parenchyma. We previously hypothesized that the goal of this invasive ductular reaction was to reconnect hepatic canaliculi to bile ducts, to prevent superimposed cholestasis. Correlating with our observations in the CDE model, the AAF/PHx model possesses invasive DR without contribution to the hepatocytic pool. The MCD model however only shows a small invasive ductular reaction, and no studies to its contribution to the hepatocytic pool have been realized. At a molecular level, data in medical literature is unfortunately scarce, making interpretation unwise.

My literature review for this question was impeded by the lack of articles studying both DR and BA synthesis enzymes and transport pumps at the same time. Most articles would only focus on one of the two, or very briefly describe the histological modifications. Usually, ductular reaction description would only consist of a few sentences. A lot of articles identified trends in BA synthesis enzymes and transport pumps regulation, without being statistically significant. Larger mice groups could be useful to distinguish trends from actual regulation mechanisms. Furthermore, some articles would only focus on either mRNA or protein modifications, and others would not study the whole spectrum of BA synthesis enzymes and transport pumps. Correlation between mRNA and protein variations was frequent, but not absolute: the best counter-example being the Mdr2-/- model, where secretion pumps mRNA is increased, while protein levels of these same pumps is decreased. Possible explanations to this discrepancy in the Mdr2-/- model include post-transcriptional regulation of Bsep mRNA, and/or Bsep instability in altered canalicular membranes secondary to the absence of protective phospholipids in bile due to Mrd2 flippase knock-out. In my opinion, such situations shine a light on the unsuspected complexity and numerous variable factors at play between mRNA transcription and fully-functional membrane protein. We also note that the CDE model causes RNA and protein damage, which could also bias mRNA and protein levels. Overall, it is my opinion that we must view these mouse models as precious tools to study BA synthesis enzymes and transporters profiles while keeping in mind the complex and unexplored mechanisms at play when drawing conclusions.

Table 3: What is known over bile acid synthesis enzymes and transporters expression in other rodent models of ductular reaction?
Part 1 out of 2: "Duct proliferation" models

	Model description	Histological observations	Ductular reaction	Bile flow rate	Serum bile acid	UPTAKE	SECRETION	EFFLUX	SYNTHESIS	Reference
BDL 7 days	Acute extrahepatic obstructive cholestasis	Bile infarcts, neutrophil infiltration.	Bile duct corrugation and elongation.	Blocked	ND	mRNA: Ntcp: ↓ Protein: Ntcp: ↓	mRNA: Bsep: ↑ Mrp2: = Protein: Bsep: = Mrp2: =	mRNA: Mrp3: ↑ Mrp4: ↑ Protein: Mrp3: ↑ Mrp4: ↑	mRNA: CYP7a1: ↑ CYP8b1: =	Wagner et al, 2017[86] Concerning "Uptake" results only: Zollner et al, 2002[87]
BDL 14 days		ND	Bile duct branching, increase in duct length and loop formations.		Increased	mRNA: Ntcp: ↓ Oatp1a1: ↓	mRNA: Bsep: = Mrp2: =	mRNA: Mrp3: = Mrp4: ↑	mRNA: CYP7a1: = CYP8b1: ↓	Zhang et al, 2012[88]
BDL 28 days		Cholangiocyte proliferation, bile duct branching, elongation and self-joining. No increase in duct diameter, but increase in ductular length and total intraluminal surface area, confined in portal area.	Bile duct proliferation confined in portal area. No increase of links between bile canaliculi and bile ducts was observed.		ND	Protein: Ntcp: ↓ Oatp2b1: ↓	Protein: Bsep: ↑ Mrp2: ↓	Protein: Mrp4: =	ND	Eipel et al, 2013[89] Concerning "Histological observations" and "Ductular reaction" results only: Vartak et al, 2016[90]
Mdr2-/-	Sclerosing cholangitis and biliary fibrosis	Disrupted bile duct tight junctions, leading to bile acid leakage from bile ducts into portal tracts, leading to portal inflammation and periductal fibrosis, leading to separation between peribilary plexus and bile duct epithelium, leading to cholangiocyte death by lack of nutrition.	Bile duct proliferation	Unchanged	Increased	mRNA: Ntcp: ↓	mRNA: Bsep: ↑ Mrp2: ↑ Protein: Bsep: ↓ Mrp2: ↓	ND	ND	Cai et al, 2014[91] For "Histological observations": Fickert et al, 2004[92]
DDC 1 week	Sclerosing cholangitis and biliary fibrosis	Porphyrin pigment plugs, portal inflammation, portal fibrosis	Bile duct proliferation confined in portal area.	Unchanged	ND	Protein: Ntcp: ↓ Oatp: ↓	Protein: Bsep: ↓ Mrp2: =	Protein: Mrp3: =	ND	Fickert et al, 2009[93]
DDC 8 weeks					Increased	mRNA: Ntcp: ↓ Oatp4: ↓ Protein: Ntcp: ↓	mRNA: Bsep: = Mrp2: = Protein: Bsep: ↑ Mrp2: ↓	mRNA: Mrp3: ↑ Mrp4: ↑ Protein: Mrp3: ↑ Mrp4: ↑	ND	Fickert et al, 2007[94]

Table 3: What is known over bile acid synthesis enzymes and transporters expression in other rodent models of ductular reaction?
Part 2 out of 2: "Invasive ductular reaction" models

	Model description	Histological observations	Ductular reaction	Bile flow rate	Serum bile acid	UPTAKE	SECRETION	EFFLUX	SYNTHESIS	Reference
MCD	Non-alcoholic steato-hepatitis	Macrovesicular hepatic steatosis, branching fibrosis	Small invasive ductular reaction	ND	ND	mRNA: Oatp1a1: ↓	mRNA: Mrp2: ↑	mRNA: Mrp3: = Mrp4: ↑	ND	Canet et al, 2014[95]
MCD					Increased	mRNA: Ntcp: = Oatp1a1: ↓	mRNA: Bsep: = Mrp2: =	mRNA: Mrp3: = Mrp4: ↑	mRNA: CYP7a1: = CYP8b1: ↓	Gyamfi et al, 2009[96]
2-AAF/ PHx (rat model)	Hepatocyte proliferation block followed by acute injury	Large LPC expansion but few LPC-derived hepatocytes. Ductular reaction intensity was correlated with liver failure, whereas delayed hepatocyte proliferation was observed in some cases and was associated with good recovery.	Very invasive ductular reaction	ND	ND	ND	mRNA: Bsep: = Mrp2: =	mRNA: Mrp3: ↑ Mrp4: ↑	ND	Ros et al, 2003[97] For "Histological observations" and "Ductular reaction": Dusabineza et al, 2011[98]
CDE	Hepatocyte-specific chronic liver injury	Canaliculi morphologic distortion and dilatation, mainly in pericentral zone of hepatic lobules	Invasive ductular reaction	Decreased	Increased	mRNA: Ntcp: ↓ Oatp1a1: ↓ Protein: Ntcp: ↓ Oatp1a1: ↓	mRNA: Bsep: ↑ Mrp2: ↑ Protein: Bsep: = Mrp2: =	mRNA: Mrp4: = Mrp4: =	mRNA: CYP7a1: = CYP8b1: =	(my results)

All models included in this table are mice models, unless stated otherwise. Results are all statistically significative ($p<0.05$). Unchanged regulation is indicated by an equal symbol (=), up-regulation is indicated by upwards arrow (↑), down-regulation by downwards arrow (↓), no data is abbreviated with "ND".

Question 2: What is known over bile acid synthesis enzymes and transporters expression in human diseases involving ductular reaction?

As detailed previously, mouse and human histology are comparable. Similarly to mice, DR in human liver arise from the CoH, and yield intermediate hepatobiliary cells called DR-cells, capable of differentiating into both hepatocytes and cholangiocytes and possessing an immunophenotype combining stem cells, hepatocyte and cholangiocyte[99]. DR in human liver disease exhibit multiple histological phenotypes, and an official DR histological classification is still lacking[64]. In literature, very few articles compare BA synthesis and transport pumps with ductular reaction. However, BA synthesis enzymes and transport pumps have been studied in different types of liver disease, to understand drug metabolism and detoxification to prevent adverse drug reaction. Indeed, drug metabolism and detoxification is a saturable process, subject to genetic polymorphisms, rate-limiting enzymes and competition. Moreover, human liver diseases may cause variations of this process by disturbing liver architecture and/or hepatocytic function. Therefore, efflux pumps such as multidrug associated resistance proteins (MRPs) are the most widely studied transporters in human liver disease, because of their role in adverse drug reactions.

To answer my question, ductular reaction and BA transport pumps in two types of acute liver damage disease and five types of chronic liver damage disease in humans was analysed. In total, eleven literature articles were reviewed. Because all DR phenotypes in the seven featured diseases of this literature review could not strictly be divided into two categories as in the rodent models of literature review question 1, the diseases were divided into acute and chronic liver damage. All observations in the following paragraphs of this section and their respective articles are mentioned in Table 4.

The two acute liver damage diseases selected were acetaminophen intake which causes massive hepatic necrosis, and acute biliary obstruction by gallstones. Ductular reaction is different in the two models: in acetaminophen intake, where hepatocellular damage occurs, intermediate hepatobiliary cells with more prominent hepatocyte-like features are observed; in biliary obstruction, duct proliferation is observed, similarly to BDL-models in mice. Molecularly, both models show an increase in efflux proteins MRP3 and MRP4 and unchanged BA secretion. Synthesis enzyme CYP7a1 expression is decreased at both mRNA and protein levels in acute biliary injury, and no data is available over synthesis enzymes in acetaminophen intake.

The five chronic liver diseases presented in this literature review are late-stage primary biliary cholangitis (i.e. PBC, previously called primary biliary cirrhosis); hepatitis C virus (HCV) infection at cirrhotic stage; non-alcoholic steato-hepatitis (NASH); and progressive familiar intrahepatic cholestasis (PFIC) type 2 and 3. We note that the first three models usually occur after the fourth decade of life, whereas the last two models are present in children and young adults.

PBC and PFIC-3 are both bile-damage type injuries. PBC is an autoimmune disease, where progressive cholestasis is caused by destruction of small bile ducts. Cholangiocyte-phenotype cells are

involved in the DR. PFIC-3 is caused by loss of function of MDR3 protein, the phospholipid (PL) flippase. Without PL in bile, bile ducts are more prone to BA damage. This model can be compared to the Mdr2-/- model presented in question 1 since MDR3 is the human ortholog of mouse Mdr2. DR in this model may be categorized as "duct proliferation" since strong duct proliferation is observed within the portal tract. Molecularly, these models both present decreased uptake, and increased efflux. Secretion was unchanged, except in PFIC-3 model where MRP2 protein levels were decreased albeit unchanged mRNA levels.

HCV, NASH and PFIC-2 models originate from hepatocellular damage: in HCV, infected hepatocytes are killed by white blood cells, causing chronic hepatocellular damage. NASH model may be compared to the MCD model featured in literature review question 1, causing chronic hepatocytic damage by excessive accumulation of fat in hepatocytes, leading to cell death, inflammation and fibrosis. DR in both HCV and NASH occur in late-stage diseases, and intermediate hepatobiliary cells are observed, interfacing directly with hepatocytes. This kind of DR may have the same goal as DR in CDE model, where DR invades the parenchyma not to replenish hepatocytes, but to reconnect hepatocyte-lined bile canaliculi with cholangiocyte-lined bile ducts in order to prevent superimposed cholestasis. However, clear evidence of that is lacking. PFIC-2 model of liver damage is due to BSEP loss of function. In this disease, BSEP is produced, but it does not function correctly, as assessed by negative BSEP immunohistochemical canalicular membrane staining. The consequences of decreased BA secretion in this model causes hepatocellular necrosis, probably due to excessive intracellular BA accumulation, and cholestasis in bile canaliculi. PFIC-2 is histologically characterized by pronounced portal fibrosis, and there is an absence of ductular proliferation. Molecularly, BA transporter profile resemble PBC and PFIC-3's. Secretion pump mRNA and protein levels vary between the three models: in PFIC-2, BSEP mRNA level is unchanged, which is misleading since its function which is not evaluated is totally lost; in HCV model mRNA levels of BSEP and MRP2 are unchanged, while protein levels are decreased; in NASH secretion expression is unchanged although we only note a non-statistical trend for increased protein levels of MRP2. Uptake is decreased in HCV and PFIC-2, and no data is available for uptake in NASH. Efflux is increased in HCV, while efflux in PFIC-2 is unchanged, except an increase in MRP4 mRNA with unchanged protein levels. In NASH, we note a non-statistical up-regulation of MRP3 and MRP4 mRNA and protein levels.

Overall, the main conclusion that can be drawn to the results presented in this table is that there is a lack of data, concerning BA transporters other than efflux pumps, and especially in synthesis enzymes in the medical literature. This is partly explained by regulation to human liver biopsies access, as any invasive act requires proper justifications. Nevertheless, this literature review provided me with better understanding of various subjects. For instance, I discovered that members of the OATP family do not always show similar regulation: in HCV cirrhosis, OATP1B1 and OATP2B1 protein expression was unchanged, whereas OATP1B3 was decreased. Because in most studies only one OATP isoform expression was measured, usually OATP1B1, I decided to exclusively use OATP1B1 data in this table.

Table 4: What is known over bile acid synthesis enzymes and transporters expression in human diseases involving ductular reaction?
Part 1 out of 2

	Histology	DR Phenotype	UPTAKE	SECRETION	EFFLUX	SYNTHESIS	Reference
ACUTE LIVER DAMAGE DISEASES							
Acetamino-phen intoxication	Massive hepatocyte necrosis	Intermediate hepatobiliary cells with more prominent hepatocyte-like features, surrounded by stroma.	ND	mRNA: MRP2: = Protein: MRP2: =	mRNA: MRP3: = MRP4: ↑ Protein: MRP3: = MRP4: ↑	ND	Gouw et al, 2011[100] For "Hepatocyte BA transporter expression": Barnes et al, 2007[101]
Biliary obstruction	Bile duct proliferation and elongation	Multiplication of small ductules at the periphery of portal stroma.	mRNA: NTCP: ↓ Protein: NTCP: ↓	mRNA: BSEP: = MRP2: = Protein: BSEP: = MRP2: ND	mRNA: MRP3: ↑ Protein: MRP3: ↑	mRNA: CYP7a1: ↓ Protein: CYP7a1: ↓	Gouw et al, 2011[100] For "Hepatocyte BA synthesis enzymes and transporter expression": Chai et al, 2012[102]
CHRONIC LIVER DAMAGE DISEASES							
HCV cirrhosis stage	Fibrosis develops in the periportal portal region, and spreads to form septal fibrosis then cirrhosis. Portal tract inflammatory infiltration.	DR predominantly found in later-stage disease with intermediate hepatobiliary cells interfacing directly with hepatocytes, located at the stroma-parenchymal interface.	Protein: NTCP: ↓ OATP1B1: =	mRNA: BSEP: = MRP2: = Protein: BSEP: ↓ MRP2: ↓	mRNA: MRP3: ↑	ND	Gouw et al, 2011[100] For "Hepatocyte BA transporter expression mRNA": Ros et al, 2003[103] For "Hepatocyte BA transporter expression protein": Wang et al, 2016[104]
NASH	Macrovesicular steatosis, inflammation, branching fibrosis that disrupt the hepatic architecture.	DRs occur in later stage disease and are associated with dense scar and relatively scant mononuclear infiltrates. Intermediate hepatobiliary cells interface directly with often steatotic hepatocytes.	ND	mRNA: MRP2: = Protein: MRP2: =	mRNA: MRP3: = MRP4: = Protein: MRP3: = MRP4: =	ND	Hardwick et al, 2011[105] For "Ductular Reaction" only: Gouw et al, 2011[100]
PFIC-2	Loss of BSEP function, causing hepatocellular necrosis and canalicular cholestasis. Progressive fibrosis, nodularity and cirrhosis ensues at later stages. Immunohistochemical staining of BSEP reveals undetectable presence on canalicular membrane.	Absence of true ductular proliferation.	mRNA: NTCP: = OATP1B1: ↓ Protein: NTCP: ↓ OATP1B1: ↓	mRNA: BSEP: = MRP2: = Protein: BSEP: ND MRP2: =	mRNA: MRP3: = MRP4: ↑ Protein: MRP3: = MRP4: =	ND	Davit-Spraul et al, 2009[106] For "Hepatocyte BA transporter expression": Keitel et al, 2005[107]

Table 4: What is known over bile acid synthesis enzymes and transporters expression in human diseases involving ductular reaction?
Part 2 out of 2 (CHRONIC LIVER DAMAGE continued)

Late-stage PBC	Inflammation and progressive destruction of small bile ducts, resulting in ductopenia and ductular reaction with formation of tortuous bile ducts.	DR with cholangiocyte-phenotype cells, forming tortuous, non-discernable lumen.	mRNA: NTCP: = OATP1B1: = Protein: NTCP: ↓ OATP1B1: ↓	mRNA: BSEP: = MRP2: = Protein: BSEP: = MRP2: =	mRNA: MRP3: = Protein: MRP3: ↑	ND	For "Histology": Nakanuma et al, 1993[108] For "Ductular reaction": Desmet, 2011[109] For "Hepatocyte BA transporter expression": Zollner et al, 2003[110]
PFIC-3	Loss of function of MDR3 (human ortholog of mouse Mdr2). Bile duct plugs and biliary obstruction is sometimes observed. Progressive fibrosis, nodularity and cirrhosis ensues at later stages.	Strong duct proliferation within the portal tract, confirmed by cytokeratin immunostaining.	mRNA: NTCP: = OATP1B1: ↓ Protein: NTCP: ↓ OATP1B1: ↓	mRNA: BSEP: = MRP2: = Protein: BSEP: ND MRP2: ↓	mRNA: MRP3: ↑ MRP4: ↑ Protein: MRP3: = MRP4: ↑	ND	Davit-Spraul et al, 2009[106] For "Hepatocyte BA transporter expression": Keitel et al, 2005[107]

Results presented in this table are statistically significative ($p<0.05$). Unchanged regulation is indicated by an equal symbol (=), up-regulation is indicated by upwards arrow (↑), down-regulation by downwards arrow (↓), no data is abbreviated with "ND".

Another observation is that once again, the correlation between mRNA variation and protein variation was not absolute. Furthermore, after supplementary research over PFIC-2 animal models, I learned that mouse Bsep knock-out models existed and had different consequences than BSEP knock-out in PFIC-2 disease. While PFIC-2 subjects suffer from fulminant liver failure and death around 4 years of age, Bsep knock-out mice were viable and fertile, and only suffered from non-progressive but persistent intrahepatic cholestasis[111,112].

Finally, this literature review made me take into account the difficulty to distinguish whether BA transporter modifications were an adaptive response, or the cause of the disease in the model. For example, BSEP down-regulation in intrahepatic cholestasis of pregnancy is thought to originate from 17β-estradiol (E2) increase, suggesting BSEP down-regulation by E2 as being the cause for the disease[113]. Another example is the HCV model of chronic liver damage, where HCV has been shown to modulate transcription of the retinoid X receptor (RXR) which in turn regulates NTCP and BSEP, causing a similar situation where it is complicated to distinguish if BA modifications is the disease's cause or an adaptive response[114,115]. Furthermore, systemic inflammation has been proven to interact with BA transporters: LPS (lipopolysaccharides, which are bacterial endotoxins), IL-1β and IL-6 (interleukins, which are inflammatory cytokines) have been proven to down-regulate NTCP, and are also suspected to modulate OATP and synthesis enzymes expression, adding to the complexity of mRNA and/or protein down-regulations interpretation[116,117,118].

Question 3: What are the strategies and outcomes of NTCP inhibition in the liver?

NTCP has recently been identified as a specific receptor for both hepatitis B virus (HBV) and hepatitis delta virus (HDV)[119]. By binding to NTCP, HBV has been proven to inhibit its function[120]. This discovery permitted to connect HBV infection with lipid metabolism dysregulation observed upon HBV infection such as hepatic steatosis[121,122]. Since then, research for NTCP inhibitors capable of inhibiting HBV infection begun to surge and various strategies have been developed, detailed in this literature review. However, articles studying NTCP inhibition to modulate DR are still lacking, which is why histological correlations of NTCP inhibition are scarce to find; the focus of most articles being on NTCP inhibition's effectiveness for HBV infection prevention/treatment.

Drugs such as Myrcludex-B, Cyclosporin-A, Ezetimibe, Irbesartan and Simvastatin have been shown to bind specifically with NTCP and inhibit its function[123,124,125]. A few of these drugs are subject to pharmacological studies, to establish the possibility to prevent/treat HBV infection by blocking NTCP-HBV binding, and Myrcludex-B has already been tested on healthy human volunteers to study its pharmacologic properties[126]. Myrcludex-B's properties were discovered in 2005. It is a synthetic specific ligand of NTCP, designed to bind on the same NTCP region as HBV[127]. Since then, *Ni et al, 2014* have also shown that Myrcludex-B inhibits NTCP uptake of bile salt *in vitro*, raising the questions over possible adverse reaction[128]. However, the same authors revealed that the concentration needed to

inhibit HBV and HDV entry was 100 times lower than the dose required to inhibit BA transport. Mouse models are not suited for studying HBV infection, because although HBV binds to mice Ntcp, it does not infect mice hepatocytes[128,129]. For this instance, chimeric mice with humanized liver have been produced, making it possible to study of HBV infection inhibitors in non-human models and produce transposable results to humans.

This literature review of NTCP inhibitors lays out multiple strategies to inhibit NTCP. These include loss of NTCP through Ntcp knock-out mouse model, and a case of naturally-occurring human NTCP deficiency; and NTCP knock-down through siRNA, mouse model of HNF-4α knock-down, HBV infection, and Myrcludex-B injection. All observations in the following paragraphs of this section and their respective articles are mentioned in Table 5.

The Ntcp knock-out mouse model was very efficient, with undetectable Ntcp mRNA and protein. But although total body weight was reduced, and BA entero-hepatic cycle slower than in wild type mice, majority of mice were normocholanemic, suggesting an up-regulation of other BA uptake transporters, namely Oatps. A subset (25%) of the mice population in this model was however hypercholanemic. The authors analysed various Oatp mRNA and protein levels in Ntcp knock-out normocholanemic mice, as well as in the smaller hypercholanemic subpopulation. Results differed between the normocholanemic and hypercholanemic populations. In normocholanemic mice, unchanged Oatp mRNA and protein levels were observed. In hypercholanemic mice, Oatp1b2 and Oatp1a1 mRNA and protein levels were decreased, while Oatp1a4 mRNA and protein levels were increased. The authors also observed gender-dependent Oatp regulation. The human NTCP deficiency by a nucleotide mutation in NTCP gene showed a growth retardation, similarly to Ntcp-/- mice, and elevated serum BA levels. Unfortunately, no histological data is available (normal abdominal ultrasonography results, excluding the necessity to perform a hepatic biopsy). The patient observed a catch-up growth after introduction of highly caloric diet, and at the time the article was published, was 5 years of age and showed normal development other than difficulty with fine motor skills.

The siRNA-mediated NTCP knock-down model was realized in mice with humanized liver. With the help of siRNA, NTCP mRNA levels were reduced by over 50%, and NTCP immunohistochemical staining was reduced. This model was conceived to study siRNA's effect on HBV infection, which is why mRNA levels of other transporters than NTCP were not studied, and no histological data is given.

The HNF-4α knock-down model is cited in this literature review for intellectual reasons, as an atypical method to down-regulate genes. HNF-4α has been shown to bind to over 40% of active promoters in the hepatocyte genome[130]. Its knock-down by *Geier et al, 2008* using siRNA was very efficient, resulting in a 95% drop of Ntcp mRNA. However, down-regulation of HNF-4α results in disruption in liver functions, rendering this method inappropriate to study DR[131].

HBV binding to NTCP is known to inhibits its function. *Oehler et al, 2014* studied the repercussions of HBV infection in mouse with humanized liver. They observed unchanged NTCP and

BSEP mRNA levels, but up-regulated CYP7a1 mRNA upon HBV infection. Myrcludex-B single injection to wild type mice and chimeric mice by those same authors caused unchanged Cyp7a1 mRNA levels in wild type mice, but significative up-regulation of CYP7a1 mRNA levels in chimeric mice. This intriguing discrepancy is clarified by a study lead by *Slijpcevic et al, 2017*: their research compared Myrcludex-B administration in wild type mouse and Oatp-KO (knock-out for Oatp1b2 and for Oatp1a1 to 5) 1.5 hours post-injection, and after 5 days of daily injections. In wild type mice, Myrcludex-B efficiently inhibited Ntcp function, but plasma BA levels were unchanged. Cyp7a1 mRNA levels in wild type mice were unchanged after injection and after 5 days of daily injections. Results in Oatp KO mice were strikingly different: authors observed complete inhibition of conjugated BA transport, and elevated plasma BA levels. Cyp7a1 mRNA levels were up-regulated after single injection, and down-regulated after 5 days of daily injections. Both wild type mice and Oatp KO mice showed no histological abnormalities after 5 days of Myrcludex-B injection. This article from *Slijpcevic et al, 2017* therefore attests that mouse Oatps can palliate to NTCP inhibition by Myrcludex.

The main conclusion to this literature review is that unlike human OATPs, mice Oatps seem capable of compensating NTCP inhibition. *Slijepcevic et al, 2015* showed that Ntcp KO mice were normocholanemic, except for a minor subpopulation of hypercholanemic mice in which Oatp1b2 and Oatp1a1 was severely down-regulated. This led the same team to produce Oatp-KO mice in 2017, and highlight that Oatp-KO mice administered Myrcludex-B had complete inhibition of conjugated BA transport and elevated plasma BA levels, unlike their wild-type counterparts which only received Myrclydex-B. Naturally-occurring cases of NTCP KO in humans had pathological BA levels, whereas mouse Ntcp KO or Ntcp knock-down models by Myrcludex-B had physiological BA levels. The first-in-human trial of Myrcludex-B showed elevated plasma BA after single dose injection, without complications even at high doses[126]. Data for long-term administration are still lacking.

For the pre-cited reasons, Oatp-knockout mouse models appear as the most promising way to obtain transposable results to humans when experimentally inhibiting NTCP. Furthermore, I once again come to observe that mouse knock-out models and naturally-occurring human knock-out models have different consequences. Similarly as to when comparing mouse and human BSEP knock-out where Bsep knock-out mice were viable and only suffered from non-progressive but persistent intrahepatic cholestasis while BSEP knock-out in PFIC-2 leads to fulminant liver failure and death usually around 4 years old in humans; mouse and human NTCP knock-out also have different consequences. Mice were unaffected by Ntcp KO other than a delayed BA clearance, and human NTCP KO showed increased BA levels.

Secondarily, as systemic inflammation is known to inhibit NTCP, this may be a possible bias to observations of NTCP expression in models where inflammation is present[116,117,118]. For our concern over DR, an article studying NTCP inhibition in different models of DR should bear in mind the possible bias of variable systemic inflammation in the models.

Table 5: What are the strategies and outcomes of NTCP inhibition in the liver?

Strategy	Efficiency	Histological data	Other results	Reference
Mouse genetically induced NTCP knock-out	Ntcp mRNA: non-detectable Ntcp protein: non-detectable	No liver damage observed	No effect of Mycludex-B Slower, but preserved, enterohepatic cycle of bile acids Majority of mice (60-75%) were normocholanemic. A subpopulation presented elevated serum BA levels (mainly taurine-conjugated) Reduced body weight	Slijepcevic et al, 2015[132]
Human NTCP deficiency by single homozygous non-synonymous nucleotide mutation	Greatly reduced taurocholate acid uptake. Absence of NTCP in plasma membrane.	ND	Elevated serum BA levels (mainly conjugated) Growth retardation Abdominal ultrasonography: normal liver size and parenchyma without any biliary tract abnormalities. No liver dysfunction.	Vaz et al, 2014[133]
siRNA-mediated NTCP knockdown in mouse with humanized liver	>50% reduction of NTCP mRNA expression level Reduced NTCP immunohistochemical staining after siRNA administration	ND	Suppressed *in vivo* spread of HBV in the presence of uninfected hepatocytes. ND on BA.	Nakabori et al, 2016[134]
HNF-4α knock-down mouse model	95% reduction in Ntcp mRNA	ND	ND	Geier et al, 2008[135]
HBV infection of mouse with humanized liver	Binding of HBV to NTCP limits its function, triggering modifications in genes of BA metabolism	ND	BSEP mRNA: unchanged NTCP mRNA: unchanged CYP7a1 mRNA: up-regulated	Oehler et al, 2014[136]
Myrcludex-B on chimeric mice	ND on NTCP function	ND	ND on serum bile acid levels. CYP7a1 mRNA: up-regulated upon single injection	For efficiency of Myrcludex-B: Ni et al, 2014[137]
Myrcludex-B on WT mice	Abrogation of Na+-dependant TCA uptake	No histological abnormalities after 5 days of Myrcludex-B injections	Transiently delayed conjugated BA clearance. Normal plasma BA levels. Cyp7a1 mRNA: unchanged upon single injection Cyp7a1 mRNA: unchanged after 5 days of daily injections	For usage on chimeric mice: Oehler et al, 2014[138]
Myrcludex-B on Oatp KO mice	Complete inhibition of conjugated BA active transport	No histological abnormalities after 5 days of Myrcludex-B injections	Complete and persistent inhibition of conjugated BA active transport. Elevated plasma BA levels. Cyp7a1 mRNA: up-regulated after single injection Cyp7a1 mRNA: down-regulated after 5 days of daily injections	For usage on WT mice and Oatp KO mice: Slijepcevic et al, 2017[139]

All results presented in this table are statistically significative (p<0,05) unless stated otherwise. "No data" is abbreviated with "ND"

Annex to literature review question 3

(this article was not included in the literature review question 3 because only an abstract is available, therefore information is incomplete)

An abstract presented in October for the 2017th Liver Meeting of the American Association for the Study of Liver Diseases by *Abbing, R.* studying the effect of Myrcludex-B usage on DDC-fed mice and Mdr2 KO mice, noticed increased plasma BA levels in both models, but reduced hepatocellular damage marker ALT (alanine aminotransferase) in DDC[140]. Moreover, when examining bile composition, the authors observed reduced bile salt output with maintained PL levels in the DDC model, unlike in the Mdr2 KO model. These results suggest that although NTCP inhibition causes increased plasma BA levels, it possibly has hepatoprotective effect on the DDC model, but not on the Mdr2 KO model. The hepatoprotective effect of Myrcludex-B administration may originate from the higher PL to BA ratio in bile, that the Mdr2 KO mouse lacking the hepatic PL secretion transporter did not benefit from. In my opinion, this must be verified with BA secretion transporter level measurement in the DDC model before drawing conclusions.

8. Conclusion and perspectives

Liver regeneration after acute injury is achieved by hepatocyte proliferation[52,53]. In chronic liver injury when hepatocyte proliferation is impaired or overwhelmed, ductular reaction (DR) is observed[59,60]. DR is characterized by periportal proliferation of cells with an immature phenotype around the canals of Hering, the narrow link between hepatocyte-lined bile canaliculi and the extralobular cholangiocyte-lined bile ducts[57,58]. DR shows variable phenotype according to the injury type[64]. In cholestatic biliary injury, DR adopts a “duct proliferation” phenotype. We hypothesize that the goal of this duct proliferation is to accommodate excess bile. In CDE-model which causes hepatocellular damage, DR is invasive and spreads towards the centre of the hepatic lobule parenchyma. Because in the CDE model DR-cells only very scarcely differentiate into hepatocytes, DR function in the CDE model is unclear[68]. We hypothesize that DR’s function in the CDE model is to connect with hepatocyte bile canaliculi to ensure bile drainage and prevent superimposed cholestasis.

The aim of this paper was to assess BA synthesis enzymes and transporters expression modifications in the CDE model, as being possible triggers for DR. We analysed mRNA and protein levels of hepatocyte BA synthesis enzymes and transporters in control versus CDE-fed mice. Our results show unchanged BA synthesis, secretion and efflux, but significantly decreased uptake, highlighted by Ntcp down-regulation both at mRNA and protein levels. This does not fit with cholestasis-induced FXR regulation of BA synthesis enzymes and transporters, where BA uptake down-regulation is accompanied by a down-regulation in synthesis, and an up-regulation in secretion. From these results, we hypothesized that in the CDE model, increased blood BA levels from pre-hepatic cholestasis secondary

to Ntcp inhibition, associated with decreases bile BA level was the trigger of DR. To better understand the interactions between chronic liver damage, BA trafficking and DR, three separate literature reviews were carried out.

The first literature review inquired over bile acid synthesis enzymes and transporters expression in other rodent models of ductular reaction. Six rodent models of DR were identified, and separated in two categories: "duct proliferation" and "invasive ductular reaction". The models of the "duct proliferation" category included BDL, Mdr2-/- and DDC-diet: three models of biliary damage-induced DR. Using 3D duct reconstruction in 28 days after BDL, *Vartak et al, 2016* observed that cholangiocytes proliferated to increase duct length in the periportal area, as to increase surface to volume ratio in an effort to alleviate cholestasis[65]. This better clarifies our initial hypothesis stating that DR "duct proliferation" models aims to accommodate excess bile. At a molecular level, I observed that in "duct proliferation" models BA synthesis enzymes and transporter regulation overall comply with the FXR-mediated regulation of BA transporters. The models showing invasive ductular reaction included MCD-diet, 2-AAF/PHx and the CDE diet: three models of hepatocellular damage. Correlating with our observations in the CDE model, the 2-AAF/PHx model showed invasive DR with few LPC-derived hepatocytes. The invasive DR in the MCD model was weaker, and data over LPC differentiation in this model is lacking. Although data was scarce, some correlations were observed at a molecular level such as uptake transporters down-regulation in models featuring invasive DR.

The second literature review inquired over bile acid synthesis enzymes and transporters expression in human diseases involving ductular reaction. Seven human liver diseases portraying DR were compared in this literature review and assigned into two categories: acute liver damage diseases and chronic liver damage diseases. The two acute liver damage diseases were acetaminophen intoxication and biliary obstruction. Acetaminophen toxicity causes hepatocellular damage, and DR-cells had hepatocyte-like features. In this model, data over BA synthesis enzymes and transporter regulation was scarce. In biliary obstruction, DR possessed a "duct proliferation" phenotype. Molecularly, other than unchanged secretion, the observed expression variations overall complied with FXR-mediated regulation of BA synthesis enzymes and transporters. The five chronic models consisted of three hepatocellular-damage models: HCV, NASH, PFIC-2; and two bile-damage models: PBC and PFIC-3. Overall, we observe a lack of data on this subject in the medical literature. This literature review demonstrated that uptake was down-regulated and efflux up-regulated in all models, and that BSEP dysfunction in PFIC-2 human disease has different consequences than mouse Bsep knock-out models. Furthermore, since mRNA-protein results correlation did not appear as absolute I begun reflecting on other ways to assess BA transport, particularly in humans where the invasive act of liver biopsy must be limited. The results of this investigation are included in the "Perspectives" section further below.

Because we hypothesized that the invasive DR in the CDE model was linked with Ntcp inhibition, the third literature review inquired over strategies and outcomes of Ntcp inhibition in the liver. The methods detailed in this literature review are mouse Ntcp KO and its comparison with a human NTCP deficiency, siRNA-mediated NTCP knock-down, HBV infection and Mycludex-B NTCP inhibition, as well as HNF-4α siRNA inhibition. Although most studies exploring NTCP inhibition investigate its effect on HBV infection and not at histological levels, this literature review managed to highlight a very important information for our future research: because unlike human OATPs, mice Oatps seem capable of compensating NTCP inhibition, Oatp-knockout mouse models appear as the most promising way to obtain transposable results to humans when experimentally inhibiting NTCP. In literature review question 2, I observed that BSEP dysfunction had different consequences in mice and humans. With this third literature review, I once again observe that human and animal ortholog KO models have different consequences, this time with NTCP. It is to this date unknown whether other secretion transporters palliate to Bsep knock-out similarly as in Oatps in Ntcp KO mice.

Perspectives

Data collection for my second literature question detailing BA synthesis enzymes and transport in human liver diseases featuring DR was difficult. Because as of this date, the main way to study BA transport in humans is using liver biopsy, and liver biopsies done solely for scientific purpose like BA transport study cannot be justified, non-invasive alternatives to assess BA transporters must be explored. Furthermore, because mRNA-protein variation correlation was not absolute, methods assessing only functional pumps, or which test in a dynamic way BA trafficking would bring new hindsight. Such methods are actually being developed, and their potential applications are immense: prediction of adverse drug reactions in damaged livers, liver function assessment to assess cirrhotic patient prognosis, increased detection rate of focal liver lesions including metastases and early hepatocellular carcinoma... Numerous studies using hepatobiliary contrast agents coupled with imaging methods have proven to live up to expectations.

Nuclear imaging using 99m-technetium-mebrofenin (^{99m}Tc-mebrofenin) is currently proposed as a method to measure future remnant liver function after hepatic resection[141]. ^{99m}Tc-mebrofenin liver-specific agent, substrate of human OATP1b1, OATP1b3[142]. This agent has been tested in MCD-induced rat models of steatosis. The results show that ^{99m}Tc-mebrofenin uptake rate correlated inversely with liver steatosis severity[143]. This meets the conclusions of *Canet et al, 2014* and *Gyamfi et al, 2009*, featured in my literature review over BA synthesis enzymes and transporters in other rodent models of DR, where Oatp mRNA levels were decreased in MCD-fed mice (see Litterature review question 1). A study with 62 human patients infected with HCV, exploring ^{99m}Tc-mebrofenin as an alternative to liver biopsy to study liver fibrosis also showed correlation between decreased uptake rate and the severity of fibrosis[144]. Overall, ^{99m}Tc-mebrofenin appears as a performant non-invasive tool to assess OATP-controlled hepatocellular uptake function.

Radiolabeled BA tracers coupled with positron emission tomography (PET) appear as elegant methods to study hepatic transport. Compared to scintigraphy, PET has much higher spatial and temporal resolution. ^{11}C-CSar (or [N-methyl-^{11}C]cholylsarcosine) is radiolabelled cholylsarcosine, a cholic acid analog artificially conjugated with [^{11}C]amino acid sarcosine. This tracer is not metabolized by hepatocytes, and non-toxic[145]. As most BA, it is up-taken in hepatocytes via NTCP, and secreted into bile via BSEP. This has been proven by inhibition of uptake and secretion of ^{11}C-CSar with pigs pre-treated with cholyltaurine[146]. The first human experiments with this tracer was realized by *Ørntoft et al, 2017*, whose team managed to demonstrate that in patients with cholestasis, uptake of ^{11}C-CSar was reduced and secretion was increased[147]. Although further studies are required to quantify OATP and MRP2 transport of ^{11}C-CSar, this BA tracer may help assess NTCP and BSEP-mediated hepatocellular transport.

Gadoxetate dimeglumine (Gd-EOB-DTPA or Gdx) is another liver-specific contrast agent, used to enhance liver in magnetic resonance imaging (MRI). It provides liver parenchyma functional information such as hepatocytic uptake and biliary secretion that is unattainable with non-specific contrast agent coupled MRI. Compared to scintigraphy and PET, Gdx-MRI is radiation-free, provides high spatial and temporal resolution and combines anatomical and functional information[148]. Two studies in rats, comparing *in vivo* Gdx uptake, secretion and efflux with Oatp, Mrp2 and Mrp3 mRNA levels respectively, showed correlations of Gdx uptake, secretion and efflux with Oatp, Mrp2 and Mrp3 expression respectively[149,150]. *In vitro* studies on human cells show that Gdx is a substrate of human OATP1b1, OATP1b3, and that Gdx is secreted in bile canaliculi through MRP2[150]. Altogether, Gdx-MRI appear as a very promising biomarker of hepatocyte transporter function, because of all the pre-cited advantages MRI has to offer over both scintigraphy and PET imagery.

In conclusion, we identified three non-invasive methods as perspectives to assess BA transport function in human liver diseases. Using either Gdx or ^{99m}Tc-mebrofenin it is possible to assess OATP, MRP2 and MRP3-mediated transport, and ^{11}C-CSar can be used to assess NTCP and BSEP-mediated transport. These contrast agents may help quantify uptake/efflux/secretion in human diseases featuring DR in a much better way than mRNA or protein level measurement whose interpretation is impeded with many bias such as post-transcriptional or post-translational regulations. With the development and perfection of these non-invasive techniques, it may therefore be possible in the future to assess BA transporter function in various human liver diseases better than with liver biopsy, providing us with precious tools to further define BA transporter modifications and their impact on DR in humans.

9. Personal conclusions

The field of medical research has been a source of curiosity for me since the beginning of my medical studies. I also felt a lack of occasions for hands-on experimentation, critical thinking, and creation in the first few years of my medical studies, so when the opportunity to realise a medical research memoir presented itself during my medical studies I didn't hesitate to take it. What I was looking forward to when beginning my experience in the field of medical research was to discover a medical discipline where knowledge is blended with critical thinking, then tested and interpreted to resolve problems: an approach I felt would very well combine with my theoretical medical courses.

Hands-on skills I have progressively acquired from this three-year experience include know-how in the whole process from mice feeding, surgical procedures, sacrifice, specimen conservation, histological slide cutting and staining, immunological marking and microscope interpretation, polymerase chain reaction, Western blot, spectrophotometer quantification and cell culture. I felt that understanding the whole process from mice feeding to Western blot interpretation helped me identify bias, and develop critical thinking when reading the "Materials and Methods" and "Results and Interpretation" section of scientific articles.

Moreover, accompanying scientists in their daily life at the GAEN laboratory of the UCL made me discover the joys and necessity of teamwork: I had the preconceived vision of medical research as being very solitary. I also discovered the rigorous experimental protocols essential for reproducible results, and I developed my ability to cope with unpredictable and non-controllable conditions affecting experiments, something which at first gave me a hard time. However, the joys of mastering a procedure after multiple unsuccessful attempts is worth it all. This made me better appreciate the long path from animal experimentation to its applications in human medicine, and how vital fundamental research is for medical practice advances. From other clinicians working in the GAEN laboratory, I also discovered the possibility to combine both medical research and medical practice.

My literature review familiarized me with medical search engines, multiple journals, medical article structure and critical analysis, and even a few authors which I learned to trust, or beware. Although I realize that much is still left to be learned, this medical research memoir was overall a very valuable opportunity to familiarize myself with the field of medical research and composing a medical article.

I am very thankful to the Université Catholique de Louvain for this opportunity, to André Geubel for his kind proofreading, and finally to Laure-Alix Clerbaux, Rita Manco, Isabelle Leclercq and to the whole GAEN team for their motivation and for what they invested in me.

10. Bibliography

1. Blouin A., Bolender R., Weiber E. Distribution of organelles and membranes between hepatocytes and nonhepatocytes in the rat liver parenchyma. A stereological study. The Journal of Cell Biology. 1977;72(2):441-455.
2. Lőw P., Molnár K., and Kriska G. Atlas of Animal Anatomy and Histology. Switzerland: Cham Springer International Publishing, 2016. p.379.
3. Bataller R., Brenner DA. Liver fibrosis. J Clin Invest 2005; 115(2):209–218.
4. Ruetz, S., Gros, P. Phosphatidylcholine translocase: a physiological role for the mdr2 gene. Cell 1994;77:1071–1081.
5. Elferink RP., Tytgat GN., Groen AK. Hepatic canalicular membrane 1: the role of mdr2 P-glycoprotein in hepatobiliary lipid transport. FASEB J 1997;11:19–28.
6. Fickert P., Fuchsbichler A., Wagner M. and al. Regurgitation of bile acids from leaky bile ducts causes sclerosing cholangitis in Mdr2 (Abcb4) knockout mice. Gastroenterology. 2004;127(1), pp.261-274.
7. Inamine T., Schnabl B. Immunoglobulin A and liver diseases. Journal of Gastroenterology. 2017.
8. Saxena R., Theise N. Canals of Hering: Recent Insights and Current Knowledge. Seminars in Liver Disease. 2004;24(1), pp.43-48.
9. Bauer E., Jakob S., Mosenthin R. Principles of Physiology of Lipid Digestion. Asian-Australasian Journal of Animal Sciences. 2005;18(2), pp.282-295.
10. Dorrell C., Erker L., Schug J. et al. Prospective isolation of a bipotential clonogenic liver progenitor cell in adult mice. Genes & Development. 2011:25(11), pp.1193-1203.
11. Yovchev M., Grozdanov P., Zhou H. et al. Identification of adult hepatic progenitor cells capable of repopulating injured rat liver. Hepatology. 2007;47(2):636-647.
12. Kordes C., Häussinger D. Hepatic stem cell niches. Journal of Clinical Investigation. 2013;123(5):1874-1880.
13. Russell DW., Setchell KDR. Bile acid biosynthesis. Biochemistry.1992;31(20):4737-4749
14. Kaur A., Patankar J., de Haan W., et al. Loss ofCyp8b1Improves Glucose Homeostasis by Increasing GLP-1. Diabetes. 2014;64(4):1168-1179.
15. Björkhem I., Araya Z., Rudling M. et al. Differences in the Regulation of the Classical and the Alternative Pathway for Bile Acid Synthesis in Human Liver. Journal of Biological Chemistry. 2002;277(30):26804-26807.
16. Agellon L., Torchia E. Intracellular transport of bile acids. Biochimica et Biophysica Acta (BBA) - Molecular and Cell Biology of Lipids. 2000;1486(1), pp.198-209.
17. EASL Clinical Practice Guidelines: management of cholestatic liver diseases. J Hepatol. Netherlands; 2009 Aug;51(2):237–67.
18. Gerloff T., Stieger B. The Sister of P-glycoprotein Represents the Canalicular Bile Salt Export Pump of Mammalian LiverJ. Biol. Chem. 1998;273:10046.
19. Bahar RJ., Stolz A. Bile acid transport. Gastroenterol. Clin. N. Am. 1999;28:27–58.
20. Staels B., Fonseca VA. Bile Acids and Metabolic Regulation: Mechanisms and clinical responses to bile acid sequestration. Diabetes Care. 2009;32(Suppl 2):S237-S245.
21. Rudman D., Kendall F. Bile Acid Content of Human Serum. II. The Binding of Cholanic Acids by Human Plasma Proteins1. Journal of Clinical Investigation. 1957;36(4), pp.538-542.
22. Hagenbuch B., Meier PJ. Organic anion transporting polypeptides of the OATP/ SLC21 family: Phylogenetic classification as OATP/SLCO superfamily, new nomenclature and molecular/functional properties. Pflügers Arch.2004, 447, 653–665.
23. Schuetz EG. Disrupted bile acid homeostasis reveals an unexpected interaction among nuclear hormone receptors, transporters, and cytochrome P450. J Biol Chem. 2001;276:39411–39418

24. Thomas C., Pellicciari R., Pruzanski M. et al. Targeting bile-acid signalling for metabolic diseases. Nat Rev Drug Discov. 2008; 7: 678-693
25. Roberts LR., Kurosawa M. Cathepsin B contributes to bile salt-induced apoptosis of rat hepatocytes. Gastroenterology. 1997;113: 1714–1726
26. Billingtonn D., Evans CE., Godfrey PP. et al. Effects of bile salts on the plasma membranes of isolated rat hepatocytes. Biochem J. 1980; 188: 321-327
27. Allen K., Jaeschke H., Copple BL. Bile acids induce inflammatory genes in hepatocytes: a novel mechanism of inflammation during obstructive cholestasis. Am J Pathol. 2011; 178: 175–86.
28. Shibatani N., Yamamoto S. Neutrophil chemotaxis in bile duct-obstructed rats, and effect of internal biliary drainage. Hepatogastroenterology. 2002;49:918–923.
29. Santoro P., Raimondi F. Unconjugated bile acids modulate adult and neonatal neutrophil chemotaxis induced in vitro by N-formyl-met-leuphe-peptide. Pediatr Res. 2002;51:392–396.
30. Kinnman N., Hultcrantz R. PDGF-mediated chemoattraction of hepatic stellate cells by bile duct segments in cholestatic liver injury. Lab Invest 2000;80:697–707.
31. Safadi R., Friedman SL. Hepatic fibrosis--role of hepatic stellate cell activation. MedGenMed. United States; 2002 Jul;4(3):27.
32. Forman BM., Goode E. Identification of a nuclear receptor that is activated by farnesol metabolites, Cell. 1995;81, pp.687-693.
33. Wang Y., Chen W., Moore D. et al . FXR: a metabolic regulator and cell protector. Cell Research. 2008;18(11):1087-1095.
34. Arana M., Tocchetti G., Rigalli J. et al. Physiological and pathophysiological factors affecting the expression and activity of the drug transporter MRP2 in intestine. Impact on its function as membrane barrier. Pharmacological Research. 2016;109:32-44.
35. Huang L., Zhao A. Farnesoid X receptor activates transcription of the phospholipid pump MDR3. J Biol Chem. United States. 2003;Dec;278(51):51085–90.
36. Chen F., Ma L., Dawson P. et al. Liver Receptor Homologue-1 Mediates Species- and Cell Line-specific Bile Acid-dependent Negative Feedback Regulation of the Apical Sodium-dependent Bile Acid Transporter. Journal of Biological Chemistry. 2002;278(22):19909-19916.
37. Lee H., Zhang Y., Lee F. et al. FXR regulates organic solute transporters α and β in the adrenal gland, kidney, and intestine. Journal of Lipid Research. 2005;47(1):201-214.
38. Inagaki T., Choi M., Moschetta A. et al. Fibroblast growth factor 15 functions as an enterohepatic signal to regulate bile acid homeostasis. Cell Metabolism. 2005;2(4):217-225.
39. Holt J., Luo G., Billin A. Definition of a novel growth factor-dependent signal cascade for the suppression of bile acid biosynthesis. Genes & Development. 2003;17(13):1581-1591.
40. Watanabe M., Houten S., Mataki C. et al. Bile acids induce energy expenditure by promoting intracellular thyroid hormone activation. Nature. 2006;439(7075):484-489.
41. Katsuma S., Hirasawa A., Tsujimoto G. Bile acids promote glucagon-like peptide-1 secretion through TGR5 in a murine enteroendocrine cell line STC-1. Biochemical and Biophysical Research Communications. 2005;329(1):386-390.
42. Keitel V., Häussinger D. TGR5 in the Biliary Tree. Digestive Diseases. 2011;29(1):45-47.
43. Wang Y., Chen W., Yu D., et al. The G-Protein-coupled bile acid receptor, Gpbar1 (TGR5), negatively regulates hepatic inflammatory response through antagonizing nuclear factor kappa light-chain enhancer of activated B cells (NF-κB) in mice. Hepatology. 2011;54(4):1421-1432.
44. Pols T., Noriega L., Nomura M., et al. The Bile Acid Membrane Receptor TGR5: A Valuable Metabolic Target. Digestive Diseases. 2011;29(1):37-44.
45. Wagner M., Fickert P., Zollner G. et al. Role of farnesoid X receptor in determining hepatic ABC transporter expression and liver injury in bile duct-ligated mice. Gastroenterology. 2003;125(3):825-838.

46. Fickert P., Wagner M. Biliary bile acids in hepatobiliary injury – What is the link?. Journal of Hepatology. 2017;67(3):619-631.
47. Perez M., Briz O. Bile-acid-induced cell injury and protection. World Journal of Gastroenterology. 2009;15(14):1677.
48. Allen K., Jaeschke H., Copple B. Bile Acids Induce Inflammatory Genes in Hepatocytes. The American Journal of Pathology. 2011;178(1), pp.175-186.
49. Guicciardi M., Gores G. Bile acid-mediated hepatocyte apoptosis and cholestatic liver disease. Digestive and Liver Disease. 2002;34(6), pp.387-392.
50. Barker N., van Oudenaarden A., Clevers H. Identifying the stem cell of the intestinal crypt: strategies and pitfalls. Cell Stem Cell. 2012; 11, 452–460.
51. Blanpain C., Fuchs E. Epidermal homeostasis: a balancing act of stem cells in the skin. Nat. Rev. Mol. Cell Biol. 2009; 10, 207–217.
52. Fausto N., Campbell J. The role of hepatocytes and oval cells in liver regeneration and repopulation. Mechanisms of Development. 2003; 120(1), pp.117-130.
53. Miyaoka Y., Ebato K., Kato H. et al. Hypertrophy and Unconventional Cell Division of Hepatocytes Underlie Liver Regeneration. Current Biology. 2012;22(13), pp.1166-1175.
54. Bataller R., Brenner DA. Liver fibrosis. J Clin Invest 2005;115(2):209–218.
55. Friedman SL. Liver fibrosis-from bench to bedside. J Hepatol. 2003;38(Suppl 1):S38–S53
56. Byass P. The global burden of liver disease: a challenge for methods and for public health. BMC Medicine. 2014;12(1).
57. Roskams T. Progenitor cell involvement in cirrhotic human liver diseases: from controversy to consensus. Journal of Hepatology. 2003;39(3):431-434.
58. Paku S., Schnur M., Nagy P. et al. Origin and structural evolution of the early proliferating oval cells in rat liver. Am. J. Pathol. 2001;158, 1313–1323.
59. Shafritz DA., Dabeva MD. Liver stem cells and model systems for liver repopulation. J Hepatol 2002;36:552–564.
60. Duncan AW., Dorrell C., Grompe M. et al. Stem cells and liver regeneration. Gastroenterology 2009;137:466–481.
61. Wang X., Foster M., Al-Dhalimy M., et al. The origin and liver repopulating capacity of murine oval cells. Proc Natl Acad Sci USA. 2003, 100 (Suppl 1): 11881-11888.
62. Arends B., Vankelecom H., Vander Borght S., et al. The dog liver contains a "side population" of cells with hepatic progenitor-like characteristics. Stem Cells Dev. 2009, 18 (2): 343-350.
63. Schotanus BA., van den Ingh TS., Penning LC., et al. Cross-species immunohistochemical investigation of the activation of the liver progenitor cell niche in different types of liver disease. Liver Int. 2009, 29 (8): 1241-1252.
64. Gouw A., Clouston A., Theise N. Ductular reactions in human liver: Diversity at the interface. Hepatology. 2011;54(5):1853-1863.
65. Vartak N., Damle-Vartak A., Richter B. et al. Cholestasis-induced adaptive remodeling of interlobular bile ducts. Hepatology. 2016;63(3):951-964.
66. Malato Y., Naqvi S., Schürmann S. et al. Fate tracing of mature hepatocytes in mouse liver homeostasis and regeneration. Journal of Clinical Investigation 2011;121, 4850–4860.
67. Schaub JR., Malato Y., Gormond C. et al. Evidence against a stem cell origin of new hepatocytes in a common mouse model of chronic liver injury. Cell Reports 2014;8, 933–939.
68. Español–Suñer R., Carpentier R., Van Hul N. et al. Liver Progenitor Cells Yield Functional Hepatocytes in Response to Chronic Liver Injury in Mice. Gastroenterology 2012;143(6), pp.1564-1575.e7.
69. Martínez A., Maroni L. Mouse Models of Liver Fibrosis Mimic Human Liver Fibrosis of Different Etiologies. Current Pathobiology Reports. 2014;2(4), pp.143-153.

70. Ito R., Takahashi T., Katano I. et al. Current advances in humanized mouse models. Cell Mol Immunol. Chinese Society of Immunology and The University of Science and Technology; 2012 May;9(3):208–14.
71. Higashiyama H., Sumitomo H., Ozawa K. et al. Anatomy of the Murine Hepatobiliary System: A Whole-Organ-Level Analysis Using a Transparency Method. Anat. Rec. 2016 ;299: 161–172.
72. Baratta J., Ngo A., Lopez B. et al. Cellular organization of normal mouse liver: a histological, quantitative immunocytochemical, and fine structural analysis. Histochemistry and Cell Biology. 2009;131(6):713-726.
73. Botham K., Boyd G. The Metabolism of Chenodeoxycholic Acid to beta-Muricholic Acid in Rat Liver. European Journal of Biochemistry. 1983;134(1):191-196.
74. Zhang Y., Hong J., Rockwell C. et al. Effect of bile duct ligation on bile acid composition in mouse serum and liver. Liver International. 2011;32(1):58-69.
75. Lu WY., Bird T., Boulter L. et al. Hepatic progenitor cells of biliary origin with liver repopulation capacity. Nature cell biology. 2015;17, 971–983.
76. Clerbaux LA., Van Hul N., Gouw A. et al. Relevance of the CDE and DDC mouse models to study ductular reaction in chronic human liver diseases in animal models for human diseases**,** Editor Bartholomew Ibeh. April 2017. ISBN 978-953-51-5440-2
77. Lombardi B., Pani P., Schlunk F.F. Choline-deficiency fatty liver : impaired release of hepatic triglycerides. Journal of lipid research. 1968;9, pp.437–446.
78. Zeisel S. Choline: an essential nutrient for humans. Nutrition. 2000;16(7-8):669-671.
79. Shinozuka H. Early histological and functional alterations of ethionine liver carcinogenesis in rats fed a choline-deficient diet. Cancer research. 1978;38, pp.1092–1098.
80. Alix JH. Molecular Aspects of the In Vivo and In Vitro Effects of Ethionine, an Analog of Methionine. Microbiological reviews. 1982;46(3), pp.281–295.
81. Sell S. Comparison of liver progenitor cells in human atypical ductular reactions with those seen in experimental models of liver injury. Hepatology. 1998;27(2), pp.317–331.
82. Shin S., Upadhyay N., Greenbaum L. et al. Ablation of Foxl1-Cre–Labeled Hepatic Progenitor Cells and Their Descendants Impairs Recovery of Mice from Liver Injury. Gastroenterology. 2015;148(1):192-202.e3.
83. Liu J., Lu H., Lu Y. et al. Potency of Individual Bile Acids to Regulate Bile Acid Synthesis and Transport Genes in Primary Human Hepatocyte Cultures. Toxicological Sciences. 2014;141(2):538-546.
84. Hagenbuch B., Meier P. The superfamily of organic anion transporting polypeptides. Biochimica et Biophysica Acta (BBA) - Biomembranes. 2003;1609(1):1-18.
85. Nakabori T., Hikita H., Murai K. et al. Sodium taurocholate cotransporting polypeptide inhibition efficiently blocks hepatitis B virus spread in mice with a humanized liver. Scientific Reports. 2016;6(1).
86. Wagner M., Fickert P., Zollner G. et al. Role of farnesoid X receptor in determining hepatic ABC transporter expression and liver injury in bile duct-ligated mice. Gastroenterology. Elsevier; 2017 Oct 22;125(3):825–38.
87. Zollner G., Fickert P., Silbert D. et al. Induction of short heterodimer partner 1 precedes downregulation of Ntcp in bile duct-ligated mice. American Journal of Physiology-Gastrointestinal and Liver Physiology. 2002;282(1):G184-G191.
88. Zhang Y., Hong JY., Rockwell CE. et al. Effect of bile duct ligation on bile acid composition in mouse serum and liver. Liver Int. 2012 Jan 1;32(1):58–69.
89. Eipel C., Menschikow E., Sigal M., et al. Hepatoprotection in bile duct ligated mice mediated by darbepoetin-α is not caused by changes in hepatobiliary transporter expression. International Journal of Clinical and Experimental Pathology. 2013;6(1):80-90.
90. Vartak N., Damle-Vartak A., Richter B., et al. Cholestasis-induced adaptive remodeling of interlobular bile ducts. Hepatology. 2016;63(3):951-964.

91. Cai S., Mennone A., Soroka C., et al. Altered expression and function of canalicular transporters during early development of cholestatic liver injury in Abcb4-deficient mice. American Journal of Physiology-Gastrointestinal and Liver Physiology. 2014;306(8):G670-G676.
92. Fickert P., Fuchsbichler A., Wagner M. et al. Regurgitation of bile acids from leaky bile ducts causes sclerosing cholangitis in Mdr2 (Abcb4) knockout mice. Gastroenterology; 2004;127(1), pp.261-274.
93. Fickert P., Fuchsbichler A., Wagner M. et al. The role of the hepatocyte cytokeratin network in bile formation and resistance to bile acid challenge and cholestasis in mice. Hepatology. 2009;50(3):893-899.
94. Fickert P., Stöger U., Fuchsbichler A. et al. A New Xenobiotic-Induced Mouse Model of Sclerosing Cholangitis and Biliary Fibrosis. Am J Pathol. Elsevier; 2007 Oct 21;171(2):525–36.
95. Canet, M., Hardwick, R., Lake, A. et al. Modeling Human Nonalcoholic Steatohepatitis-Associated Changes in Drug Transporter Expression Using Experimental Rodent Models. Drug Metabolism and Disposition. 2014;42(4):586-595.
96. Gyamfi, M., Tanaka, Y., He, L. et al. Hepatic effects of a methionine–choline-deficient diet in hepatocyte RXRα-null mice. Toxicology and Applied Pharmacology. 2009;234(2):166-178.
97. Ros, J., Roskams, T., Geuken, M. et al. ATP binding cassette transporter gene expression in rat liver progenitor cells. Gut. 2003;52(7):1060-1067.
98. Dusabineza, A., Van Hul, N., Abarca-Quinones, J. et al. Participation of liver progenitor cells in liver regeneration: lack of evidence in the AAF/PH rat model. Laboratory Investigation. 2011;92(1):72-81.
99. Schmelzer E., Zhang L., Bruce A. et al. Human hepatic stem cells from fetal and postnatal donors. The Journal of Experimental Medicine. 2007;204(8):1973-1987.
Schmelzer E., Zhang L., Bruce A. et al. Human hepatic stem cells from fetal and postnatal donors. The Journal of Experimental Medicine. 2007;204(8):1973-1987.
100. Gouw A., Clouston A., Theise N. Ductular reactions in human liver: Diversity at the interface. Hepatology. 2011;54(5):1853-1863.
101. Barnes S., Aleksunes L., Augustine L. et al. Induction of Hepatobiliary Efflux Transporters in Acetaminophen-Induced Acute Liver Failure Cases. Drug Metabolism and Disposition. 2007;35(10):1963-1969.
102. Chai J., He Y., Cai S. et al. Elevated hepatic multidrug resistance-associated protein 3/ATP-binding cassette subfamily C 3 expression in human obstructive cholestasis is mediated through tumor necrosis factor alpha and c-Jun NH2-terminal kinase/stress-activated protein kinase-signaling pathway. Hepatology. 2012;55(5):1485-1494.
103. Ros J., Libbrecht L., Geuken M. et al. High expression of MDR1, MRP1, and MRP3 in the hepatic progenitor cell compartment and hepatocytes in severe human liver disease. The Journal of Pathology. 2003;200(5):553-560.
104. Wang L., Collins C., Kelly E. et al. Transporter Expression in Liver Tissue from Subjects with Alcoholic or Hepatitis C Cirrhosis Quantified by Targeted Quantitative Proteomics. Drug Metabolism and Disposition. 2016;44(11):1752-1758.
105. Hardwick R., Fisher C., Canet M. et al. Variations in ATP-Binding Cassette Transporter Regulation during the Progression of Human Nonalcoholic Fatty Liver Disease. Drug Metabolism and Disposition. 2011;39(12):2395-2402.
106. Davit-Spraul A., Gonzales E., Baussan C. et al. Progressive familial intrahepatic cholestasis. Orphanet J Rare Dis. 2009;Jan 8. 4:1.
107. Keitel V., Burdelski M., Warskulat U. Expression and localization of hepatobiliary transport proteins in progressive familial intrahepatic cholestasis. Hepatology. 2005;41(5):1160-1172.
108. Nakanuma Y., Harada K. Florid duct lesion in primary biliary cirrhosis shows highly proliferative activities. Journal of Hepatology. 1993;19(2):216-221.
109. Desmet V. Ductal plates in hepatic ductular reactions. Hypothesis and implications. I. Types of ductular reaction reconsidered. Virchows Archiv. 2011;458(3):251-259.

110. Zollner G., Fickert P., Silbert D. et al. Adaptive changes in hepatobiliary transporter expression in primary biliary cirrhosis. Journal of Hepatology. 2003;38(6):717-727.
111. Jacquemin E. Progressive familial intrahepatic cholestasis. Journal of Gastroenterology and Hepatology. 2002;14(6):594-599.
112. Wang R., Salem M., Yousef IM. et al. Targeted inactivation of sister of P-glycoprotein gene (spgp) in mice results in nonprogressive but persistent intrahepatic cholestasis. Proceedings of the National Academy of Sciences. 2001;98(4):2011-2016.
113. Song X., Vasilenko A., Chen Y., et al. Transcriptional dynamics of bile salt export pump during pregnancy: mechanisms and implications in intrahepatic cholestasis of pregnancy. Hepatology. 2014;60(6):1993-2007
114. Halilbasic E., Claudel T., Trauner M. Bile acid transporters and regulatory nuclear receptors in the liver and beyond. Journal of Hepatology. 2013;58(1):155-16
115. Tsutsumi T., Suzuki T., Shimoike T. et al. Interaction of hepatitis C virus core protein with retinoid X receptor alpha modulates its transcriptional activity. Hepatology. 2002;35(4):937-946.
116. Trauner M., Arrese M., Lee H. et al. Endotoxin downregulates rat hepatic Ntcp gene expression via decreased activity of critical transcription factors. Journal of Clinical Investigation. 1998;101(10):2092-2100.
117. Denson L., Auld K., Schiek D. et al. Interleukin-1β Suppresses Retinoid Transactivation of Two Hepatic Transporter Genes Involved in Bile Formation. Journal of Biological Chemistry. 2000;275(12):8835-8843.
118. Bouezzedine F., Fardel O., Gripon P. Interleukin 6 inhibits HBV entry through NTCP down regulation. Virology. 2015;481:34-42.
119. Engelke M., Mills K., Seitz S. et al. (2006). Characterization of a hepatitis B and hepatitis delta virus receptor binding site. Hepatology, 43(4), pp.750-760.
120. Oehler N., Volz T., Bhadra OD. et al. Binding of hepatitis B virus to its cellular receptor alters the expression profile of genes of bile acid metabolism. Hepatology. 2014;60: 1483–1493.
121. Kim K., Shin H., Kim K. et al. Hepatitis B Virus X Protein Induces Hepatic Steatosis Via Transcriptional Activation of SREBP1 and PPARγ. Gastroenterology. 2007;132(5), pp.1955-1967.
122. Na T., Shin Y., Roh K. et al. Liver X receptor mediates hepatitis B virus X protein-induced lipogenesis in hepatitis B virus-associated hepatocellular carcinoma. Hepatology. 2008;49(4), pp.1122-1131.
123. Lempp F., Urban S. Inhibitors of Hepatitis B Virus Attachment and Entry. Intervirology. 2014;57(3-4), pp.151-157.
124. Dong Z., Ekins S., Polli J. Structure–Activity Relationship for FDA Approved Drugs As Inhibitors of the Human Sodium Taurocholate Cotransporting Polypeptide (NTCP). Molecular Pharmaceutics. 2013;10(3), pp.1008-1019.
125. Azer S., Stacey N. Differential effects of cyclosporin a on the transport of bile acids by human hepatocytes. Biochemical Pharmacology. 1993;46(5), pp.813-819.
126. Blank A., Markert C., Hohmann N. et al. First-in-human application of the novel hepatitis B and hepatitis D virus entry inhibitor myrcludex B. Journal of Hepatology. 2016;65(3):483-489.
127. Gripon P., Cannie I., Urban S. Efficient Inhibition of Hepatitis B Virus Infection by Acylated Peptides Derived from the Large Viral Surface Protein. Journal of Virology. 2005;79(3):1613-1622.
128. Ni Y., Lempp F., Mehrle S. et al. Hepatitis B and D Viruses Exploit Sodium Taurocholate Co-transporting Polypeptide for Species-Specific Entry into Hepatocytes. Gastroenterology. 2014;146(4):1070-1083.e6.
129. Schieck A., Schulze A., Gahler C. et al. Hepatitis B virus hepatotropism is mediated by specific receptor recognition in the liver and not restricted to susceptible hosts. Hepatology. 2013; 58: 43–53

130. Odom D., Zizlsperger N., Gordon B. et al. Control of Pancreas and Liver Gene Expression by HNF Transcription Factors. Science. 2004;303(5662):1378-1381.
131. Hayhurst G., Lee Y., Lambert G. et al. Hepatocyte Nuclear Factor 4 (Nuclear Receptor 2A1) Is Essential for Maintenance of Hepatic Gene Expression and Lipid Homeostasis. Molecular and Cellular Biology. 2001;21(4):1393-1403.
132. Slijepcevic, D., Kaufman, C., Wichers, C. et al. Impaired uptake of conjugated bile acids and hepatitis b virus pres1-binding in na+-taurocholate cotransporting polypeptide knockout mice. Hepatology. 2015;62(1):207-219.
133. Vaz, F., Paulusma, C., Huidekoper, H., et al. Sodium taurocholate cotransporting polypeptide (SLC10A1) deficiency: Conjugated hypercholanemia without a clear clinical phenotype. Hepatology. 2014;61(1):260-267.
134. Nakabori, T., Hikita, H., Murai, K. et al. Sodium taurocholate cotransporting polypeptide inhibition efficiently blocks hepatitis B virus spread in mice with a humanized liver. Scientific Reports. 2016;6(1).
135. Geier, A., Martin, I., Dietrich, C. et al. Hepatocyte nuclear factor-4α is a central transactivator of the mouse Ntcp gene. American Journal of Physiology-Gastrointestinal and Liver Physiology. 2008;295(2):G226-G233.
136. Oehler, N., Volz, T., Bhadra, O. et al. Binding of hepatitis B virus to its cellular receptor alters the expression profile of genes of bile acid metabolism. Hepatology. 2014;60(5):1483-1493.
137. Ni, Y., Lempp, F., Mehrle, S. et al. Hepatitis B and D Viruses Exploit Sodium Taurocholate Co-transporting Polypeptide for Species-Specific Entry into Hepatocytes. Gastroenterology. 2014;146(4):1070-1083.e6.
138. Oehler, N., Volz, T., Bhadra, O. et al. Binding of hepatitis B virus to its cellular receptor alters the expression profile of genes of bile acid metabolism. Hepatology. 2014;60(5):1483-1493.
139. Slijepcevic, D., Roscam Abbing, R., Katafuchi, T. et al. Hepatic uptake of conjugated bile acids is mediated by both sodium taurocholate cotransporting polypeptide and organic anion transporting polypeptides and modulated by intestinal sensing of plasma bile acid levels in mice. Hepatology. 2017;66(5):1631-1643.
140. Inc. M. NTCP inhibition has hepatoprotective effects in cholestasis by lowering bile salt load on hepatocytes and increasing the biliary phospholipid vs bile salt ratio, by Mr. Reinout Roscam Abbing [Internet]. Liverlearning.aasld.org. 2018 [cited 4 January 2018]. Available from:https://liverlearning.aasld.org/aasld/2017/thelivermeeting/195302/reinout.roscam.abbing.ntcp.inhibition.has.hepatoprotective.effects.in.html
141. de Graaf W., van Lienden K., van Gulik T. et al. 99mTc-Mebrofenin Hepatobiliary Scintigraphy with SPECT for the Assessment of Hepatic Function and Liver Functional Volume Before Partial Hepatectomy. Journal of Nuclear Medicine. 2010;51(2):229-236.
142. de Graaf W., Häusler S., Heger M. et al. Transporters involved in the hepatic uptake of 99mTc-mebrofenin and indocyanine green. Journal of Hepatology. 2011;54(4):738-745.
143. Veteläinen R., Bennink R., de Bruin K. et al. Hepatobiliary function assessed by 99mTc-mebrofenin cholescintigraphy in the evaluation of severity of steatosis in a rat model. European Journal of Nuclear Medicine and Molecular Imaging. 2006;33(10):1107-1114.
144. Kula M., Karacavus S., Baskol M. et al. Hepatobiliary function assessed by 99mTc-mebrofenin cholescintigraphy in the evaluation of fibrosis in chronic hepatitis: histopathological correlation. Nuclear Medicine Communications. 2010;31(4):280-285.
145. Schmassmann A., Fehr H., Locher J. et al. Cholylsarcosine, a new bile acid analogue: Metabolism and effect on biliary secretion in humans. Gastroenterology. 1993;104(4):1171-1181.
146. Frisch K., Jakobsen S., Sorensen M. et al. [N-Methyl-11C]Cholylsarcosine, a Novel Bile Acid Tracer for PET/CT of Hepatic Excretory Function: Radiosynthesis and Proof-of-Concept Studies in Pigs. Journal of Nuclear Medicine. 2012;53(5):772-778.

147. Ørntoft N., Munk O., Frisch K. et al. Hepatobiliary transport kinetics of the conjugated bile acid tracer 11 C-CSar quantified in healthy humans and patients by positron emission tomography. Journal of Hepatology. 2017;67(2):321-327.
148. Van Beers B., Pastor C., Hussain H. Primovist, Eovist: What to expect?. Journal of Hepatology. 2012;57(2):421-429.
149. Lagadec M., Doblas S., Giraudeau C.. Advanced Fibrosis: Correlation between Pharmacokinetic Parameters at Dynamic Gadoxetate-enhanced MR Imaging and Hepatocyte Organic Anion Transporter Expression in Rat Liver. Radiology. 2015;274(2):379-386.
150. van Montfoort JE., Stieger B., Meijer DK. et al. Hepatic uptake of the magnetic resonance imaging contrast agent gadoxetate by the organic anion transporting polypeptide Oatp1. J Pharmacol Exp Ther. 1999;290(1):153–157.