

REVIEW ARTICLE

Rodent models of cholestatic liver disease: A practical guide for translational research

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Abstract

Cholestatic liver disease denotes any situation associated with impaired bile flow concomitant with a noxious bile acid accumulation in the liver and/or systemic circulation. Cholestatic liver disease can be subdivided into different types according to its clinical phenotype, such as biliary atresia, drug-induced cholestasis, gallstone liver disease, intrahepatic cholestasis of pregnancy, primary biliary cholangitis and primary sclerosing cholangitis. Considerable effort has been devoted to elucidating underlying mechanisms of cholestatic liver injuries and explore novel therapeutic and diagnostic strategies using animal models. Animal models employed according to their appropriate applicability domain herein play a crucial role. This review provides an overview of currently available *in vivo* animal models, fit-for-purpose in modelling different types of cholestatic liver diseases. Moreover, a practical guide and workflow is provided which can be used for translational research purposes, including all advantages and disadvantages of currently available *in vivo* animal models.

KEYWORDS

drug-induced cholestasis, *in vivo* modelling, intrahepatic cholestasis of pregnancy, primary biliary cholangitis, primary sclerosing cholangitis

1 | INTRODUCTION

Cholestatic liver diseases arise from any situation associated with impaired bile flow, as a result of disturbed hepatobiliary production or bile excretion, accompanied by noxious bile acid accumulation in

hepatocytes or systemic circulation.¹ The word cholestasis is derived from Greek (ie 'chole' and 'stasis') and literally means 'bile halting'.² A schematic overview of the pathogenesis of cholestasis is provided in Figure 1. Different types of cholestasis can be divided depending on specific underlying mechanisms and aetiology, such as biliary atresia,

Abbreviations: AE2, anion exchange protein 2; AMA, antimitochondrial antibody; ANIT, α -naphthylisothiocyanate; ARE(-Del), (deletion in) adenylate-uridylyl-rich element; ATP8B1, ATPase phospholipid transporting 8B1; BDL, bile duct ligation; BRIC, benign recurrent intrahepatic cholestasis; BSEP, bile salt export pump; CD, cluster of differentiation; CFTR, cystic fibrosis transmembrane conductance regulator; DDC, 3,5-diethoxycarbonyl-1,4-dihydrocollidine; DIC, drug-induced cholestasis; DSS, dextran sodium sulphate; (dn)TGF- β (RII) mice, (dominant negative form of) transforming growth factor- β (receptor restricted to T cells); Foxp3, Forkhead box protein 3; IBD, inflammatory bowel diseases; ICP, intrahepatic cholestasis of pregnancy; idd, insulin-dependent diabetes; IL-2(R α), interleukin 2 (receptor α); MDR, multidrug resistance protein; MRP, multidrug resistance-associated protein; NOD(c3c4), non-obese diabetic (with B6/B10 region on chromosomes 3 and 4); PBC, primary biliary cholangitis; PDH-E2, E2 subunit of the pyruvate dehydrogenase; PFIC, progressive familial intrahepatic cholestasis; PSC, primary sclerosing cholangitis; TNBS, 2,4,6-trinitrobenzenesulfonic acid; Treg cells, regulatory T cells.

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benign recurrent intrahepatic cholestasis (BRIC), intrahepatic drug-induced cholestasis (DIC), gallstone disease, iatrogenic cholestatic liver diseases, intrahepatic cholestasis of pregnancy (ICP), primary biliary cholangitis (PBC), primary sclerosing cholangitis (PSC) and progressive familial intrahepatic cholestasis (PFIC).³⁻⁷ The multifaceted liver disease biliary atresia can have devastating consequences including progression into end-stage cirrhosis.⁸ The diagnosis of biliary atresia is based on excluding various causes of neonatal cholestasis, consequently by the time of diagnosis the extrahepatic bile ducts are often already completely obstructed.^{8,9} The incidence of neonatal cholestasis has been estimated to be 1 in 2500 new-born children, from which about 34%-43% have biliary atresia.⁹⁻¹¹ PFIC and BRIC are two different forms of familial intrahepatic cholestasis with autosomal recessive inheritance.⁷ PFIC can be subdivided into three different types, from which type 1 is caused by a mutation in the ATPase phospholipid transporting 8B1 (*ATP8B1*) gene, type 2 by a mutation in the gene encoding the bile salt export pump (*BSEP*) and type 3 by a mutation in the gene encoding multidrug resistance protein 3 (*MDR3*).⁶ PFIC is considered a rare disease typically presenting with cholestasis during infancy or childhood.¹² BRIC was also found associated with mutations in *ATP8B1* and *BSEP*, defined as BRIC type 1 and type 2 respectively. BRIC represents a less severe cholestatic phenotype characterized by intermittent recurrent cholestatic episodes, with extensive pruritus.⁷ Iatrogenic cholestatic liver diseases constitute a group of liver diseases resulting from an

Key points

- There are still significant gaps in the mechanistic understanding of different types of cholestasis, including biliary atresia, drug-induced cholestasis, gallstone liver disease, intrahepatic cholestasis of pregnancy, primary biliary cirrhosis and primary sclerosing cholangitis.
- A better understanding of currently available animal models is urged with their specific properties, since inadequate models may inherently determine a biased research outcome.
- This review provides a practical guide for researchers in the field of translational hepatology by providing a schematic overview of available animal models for cholestasis, including their individual and general advantages and disadvantages relevant for a specific applicability domain.

invasive surgery, including biliary complications evoked by a living donor liver transplantation. Adult recipients from living donor liver transplantation were reported to develop biliary complications in 15%-40% of the cases, including biliary stricture (anastomotic or non-anastomotic), bile leakage, bile duct obstruction, sphincter of

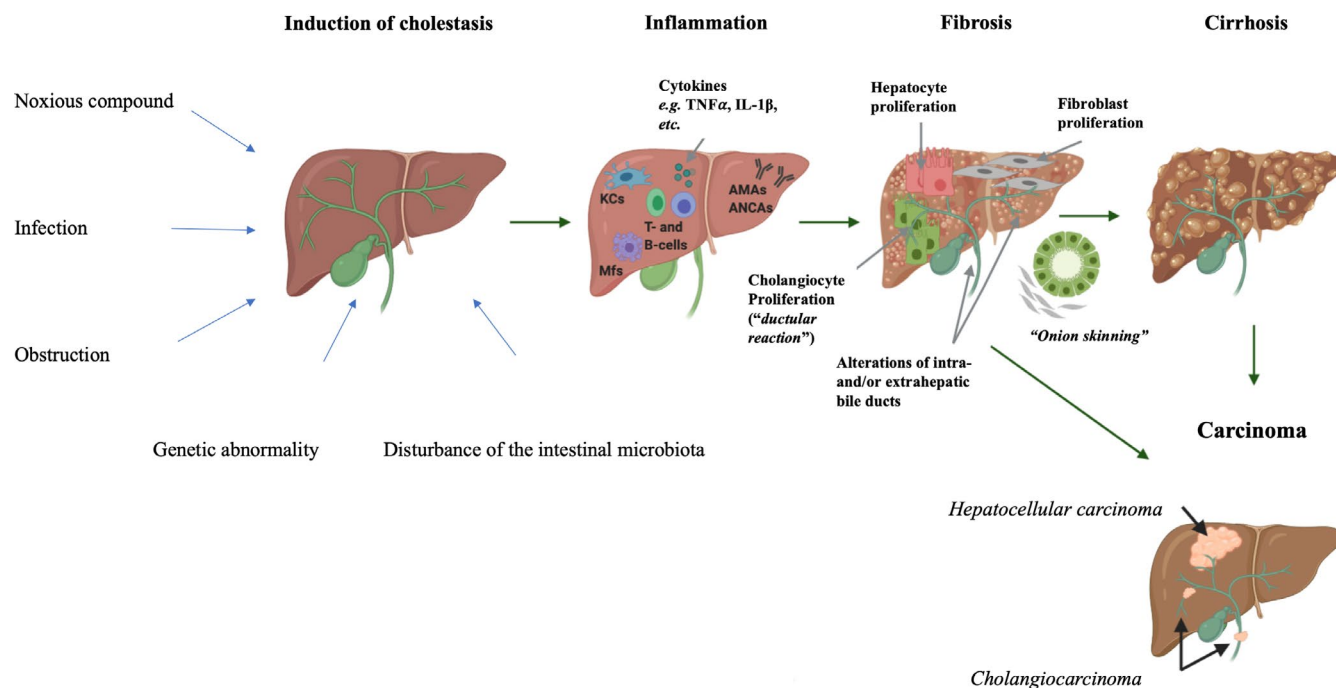


FIGURE 1 Schematic overview of the pathogenesis of cholestatic liver injury. Cholestasis can be induced by a number of factors including, noxious compounds, infection, obstruction of bile flow, disturbance in the intestinal microbiota or genetic abnormalities.^{26,244} These initiating factors evoke an inflammatory injury on hepatocytes and/or cholangiocytes, which may result in activation of hepatocytes, fibroblasts and cholangiocytes.^{48,244} These mature cells, may, in its turn, start to proliferate and cause fibrosis in the liver and/or bile duct.²⁴⁴ In primary sclerosing cholangitis, fibrosis is typically accompanied by the 'onion skinning' around the bile ducts.¹⁸⁶ Moreover, fibrosis might in some cases progress into cirrhosis or even carcinoma (hepatocellular carcinoma or cholangiocarcinoma).²⁴⁵ This figure was created with Biorender Software. IL-1 β , interleukin 1 β ; PSC, primary sclerosing cholangitis; TNF α , tumour necrosis factor α

Oddi dysfunction, etc. Importantly, biliary complications remain the major cause of morbidity and mortality after living donor liver transplantation.^{5,13} Additionally, biliary complications may also occur after invasive abdominal (ie gastrectomy and oesophagectomy), orthopaedic or cardiovascular surgeries and carry significant incidence of morbidity and mortality.¹⁴⁻¹⁶ Gallstone liver disease is reported as major medical problem, especially in Western countries where it has a prevalence of about 15% in adults. Moreover, in Europe, gallstone liver disease is one of the common causes of hospitalization resulting from a gastrointestinal disease.¹⁷⁻²⁰ Approximately 20% of the patients will develop the typical symptoms, such as jaundice and biliary pain, or other types of complications in a time span of 15 years.^{19,21,22} PBC and PSC have a prevalence ranging from 2 to 40 per 100,000 inhabitants and 0 to 16 per 100,000 inhabitants.^{3,23} Ursodeoxycholic acid is the golden standard for safely and effectively treating PBC patients. Nevertheless, still about 15% of the PBC patients show a progressive disease that requires liver transplantation or in some cases results in death, despite ursodeoxycholic acid treatment.^{24,25} For PSC, effective treatment is, thus far, still non-existing, presumably due to considerable gaps in the mechanistic understanding of the disease, which hampers the identification of potential targets.²⁶ More atypical causes of cholestasis, albeit not less important, include DIC and ICP.³ ICP is of high clinical relevance considering their worldwide incidence ranging from 0.2% up to 25% and risk on pre-term birth, respiratory distress syndrome and still birth.²⁷⁻³⁰ DIC is in 73% of the cases evoked by single-prescription medication, mainly cardiovascular, anti-inflammatory, antidiabetic and anti-infectious drugs.³¹ DIC can cause a tremendous financial loss for pharmaceutical companies, mostly due to early drug retraction from clinical trials. This can partly be explained by the fact that current preclinical animal models as well as preclinical in vitro models only predict 50%-60% of all drug-induced liver injuries, including DIC.³¹⁻³⁴ A better understanding of currently available animal models with their specific properties is, therefore, highly demanded, since an inadequate model will inherently determine a biased research outcome.

This review provides a state-of-the-art overview of currently available surgery-induced, genetically modified, chemical-induced, viral-induced and combination rodent models for different types of cholestasis (ie biliary atresia, DIC, gallstone liver disease, ICP, PBC and PSC) together with their characteristics, individual assets, drawbacks and applicability domain, which serves as a tool for researchers in the field of translational hepatology (Tables 1 and 2).

2 | PATHOPHYSIOLOGY OF CHOLESTATIC LIVER DISEASES

2.1 | Gallstone liver disease

Gallstones are hardened deposits of digestive fluid bile, which develop within the gallbladder. They develop when there is an imbalance in the chemical constituents of bile, which results in precipitation of the components.²⁰ Three main pathways have been

identified in the formation of gallstones, cholesterol supersaturation, excess of bilirubin and disturbance in the functionality of the gallbladder. In a healthy situation, bile is able to dissolve the amount of cholesterol excreted through the liver, but in case of excessive cholesterol biosynthesis or excretion cholesterol may precipitate as crystals and evolve to stones (ie cholesterol supersaturation).^{20,21,35} The yellow pigment bilirubin is released upon degradation of red blood cells. In some haemolytic conditions (eg hereditary spherocytosis and sickle cell disease), red blood cell disruption is abnormally high resulting in an overload of bilirubin, which sometimes lead to gallstone formation, referred to as pigment stones.^{20,21,36,37} Finally, gallbladder hypomotility or even impaired contractility can perturb the effective clearance of bile in the gallbladder. The latter may result in a highly concentrated bile, which may, in turn, lead to gallstone formation.^{21,38} Symptoms and complications of these gallstones can occur when the stones obstruct bile ducts and/or cystic ducts. A temporary obstruction of the cystic duct characterized as cholelithiasis is believed to be short-lived and results in biliary pain. A more persistent obstruction occurs when a large stone gets permanently lodged in the neck of the gall bladder (cystic duct) often leading to acute cholecystitis. Other types of complications can also develop depending on the location of gallstone obstruction. As such, obstruction of the common bile duct causes choledocholithiasis and jaundice while obstructing the ampulla in the distal portion of the bile duct may result in gallstone pancreatitis.^{21,39}

2.2 | Biliary atresia

Biliary atresia is a severe inflammatory and fibrosing cholangiopathy of infancy with an obstruction of the extrahepatic bile ducts, which can rapidly progress into end-stage cirrhosis. Intrahepatic bile ducts are not hindered yet, are observed to be hyperplastic and embedded in portal tracts with varying levels of inflammation and fibrosis, and lobules with features of cholestasis and increased multinucleated hepatocytes.^{8,40} Clinically, the disease is typified by pathological jaundice with direct or conjugated hyperbilirubinaemia, hepatosplenomegaly, acholic stool and the onset of symptoms in the first months of life.^{8,41} Precise clinical phenotyping might differ between the perinatal and embryonic form, and cyst-associated or cytomegalovirus-associated variants of the disease. Perinatal biliary atresia also referred to as non-syndromic biliary atresia represents the biggest subgroup with approximately 80% of the biliary atresia patients, characterized by a jaundice-free period after birth.^{8,42} The embryonic form occurs in about 10% of the affected infants and show an earlier onset of jaundice (at birth) together with non-hepatic congenital malformation, such as splenic abnormalities.^{8,43,44} Cystic biliary atresia is defined by a cystic malformation near the obstructed common bile duct, occurring in about 8% of the patients. Cytomegalovirus-associated biliary atresia shows a poor bile drainage and carries the highest risk of death. The incidence of this form is highly variable based on geography as well as the detection methodology used (eg lymphocyte activation

TABLE 1 Reported applicability domain(s), advantages and disadvantages per in vivo rodent model

Cholestatic liver injury	Type of experimental model	Reported rodent species/strains	Applicability domain(s)	Advantages	Disadvantages	References
Biliary atresia	Surgery-induced rodent models	Male and female Sprague-Dawley rat pups, male Wistar rat pups (21-30 days)	Acute and chronic mechanistic studies. Therapeutic studies.	Relatively easy surgical procedure. Progression into fibrosis.	Rat pups have smaller size of biliary structures. Increased mortality.	78,221
	Bile duct ligation	Male C57BL/6 mice	Acute and chronic mechanistic studies. Therapeutic studies.	Minor or no involvement of other tissues. Novel route of administration for testing therapeutic strategies directly in the bile ducts.	Risk of peritonitis. Leakage of bile or the injective solvent into the abdominal cavity. Difficult procedure due to the small anatomic proportions which complicate access to the biliary tree. Model needs to be further characterized.	90-92
	Bile duct injection technique	Male C57BL/6 mice	Acute and chronic mechanistic studies. Therapeutic studies.	Affects both intra- as extrahepatic bile ducts.	No (or delayed) progression into fibrosis. Bad reproducibility. Injection-related injury. Cannibalization of pups. Variable time of infection and virus dosage. Survival rate is 10% at 3 weeks.	222, 223
	Intraperitoneal inoculation of mice with rhesus rotavirus	Balb/c mouse pups (1-2days)	Acute mechanistic studies. Therapeutic studies.			
Drug-induced cholestasis	Genetically modified mice models	Male C57BL/6 and FVB/N mice	Acute mechanistic studies (related to BSEP malfunctioning). Therapeutic studies. Specific studies focused on the role BSEP in bile acid homeostasis and alternative bile acid transport systems.	BSEP malfunctioning is a well-known triggering factor of drug-induced cholestasis.	Time-consuming (model of 60-180 days). Focus on 1 transporter. Not applicable for drugs that simultaneously interfere with multiple hepatobiliary transporters. No development of severe cholestasis.	48,49,97,203
	Chemical-induced rodent models	Male albino and Wistar rats	(Acute) and chronic mechanistic studies. Therapeutic studies.	Short model (5-7 days). Well-known hepatotoxicant.	Acute effects are limited. Interindividual differences in the susceptibility.	139,143,144

(Continues)

TABLE 1 (Continued)

Cholestatic liver injury	Type of experimental model	Reported rodent species/ strains	Applicability domain(s)	Advantages	Disadvantages	References
	Cyclosporin A-induced cholestasis	Male Wistar rats and Sprague-Dawley rats Male C57BL/6 mice	Acute and chronic mechanistic studies. Therapeutic studies.	Short model (11-25 days). Well-known hepatotoxicant.	Development of steatosis. Immunosuppressive effect of cyclosporin A.	145,146,148, 149,151
	Combination rodent models with <i>Bsep</i> ^{-/-} mouse model with cholic acid feeding	Male FVB/N mice	Acute mechanistic studies (related to BSEP malfunctioning). Therapeutic studies. Specific studies focused on the role BSEP in bile acid homeostasis and alternative bile acid transport systems.	BSEP malfunctioning is a well-known triggering factor of drug-induced cholestasis. Development of severe cholestasis. More resemblance with cholestasis than single hit <i>Bsep</i> ^{-/-} mice model.	Time-consuming (model of 66-189 days). Male predominance. High mortality rate.	97,203
Gallstone liver disease	Chemical-induced rodent models	Male albino NMRI-mice Male C57BL/6 mice	Acute and chronic mechanistic studies. Therapeutic studies. Specific studies focused on the genetic susceptibility.	Easy model.	Only cholesterol gallstones. Long model (8 weeks). High variability across different strains and gender.	155,224,225
Intrahepatic cholestasis of pregnancy	Chemical-induced rodent models	Female Sprague-Dawley and Wistar rats	Acute mechanistic studies. Therapeutic studies.	Short model (5 days). Mechanisms of hepatotoxicity are well known.	Strict legislation in purchasing hormones.	165,226,227
Primary biliary cholangiopathy (PBC)	Surgery-induced rodent models	Female C57BL/6 mice	Acute and chronic mechanistic studies. Therapeutic studies.	Minor or no involvement of other tissues. Novel route of administration for testing therapeutic strategies directly in the bile ducts.	Risk of peritonitis. Leakage of bile or the injective solvent into the abdominal cavity. Difficult procedure due to the small anatomic proportions which complicate access to the biliary tree. Model needs to be further characterized.	91

(Continues)

TABLE 1 (Continued)

Cholestatic liver injury	Type of experimental model	Reported rodent species/strains	Applicability domain(s)	Advantages	Disadvantages	References
Genetically modified mice models	Ae2 _{a,b} ^{-/-} mouse model	Male FVB/N mice	Acute and chronic mechanistic studies. Specific studies focused on the role of AE2 in PBC.	Selective damage of bile ducts.	Time-consuming (model of 180–450 days). Impaired gastric acid secretion, male sterility and osteoporosis. Interindividual differences in the severity of cholangitis. Relatively late onset. Only 30%–80% AMA. No female predominance. No granulomas. Difficulty in breeding.	105,113,114
	ARE Del ^{-/-} mouse model	Female C57BL/6 mice	Acute and chronic mechanistic studies. Therapeutic studies. Specific studies focused on the role of interferon γ in PBC. Specific studies focused on the female predominance in PBC.	Female predominance.	Time-consuming (model of 70 days). Lupus-like autoimmune features.	114,118–120
	<i>dInTGF-βRII</i> mouse model	Female and male C57BL/6 mice	Acute mechanistic studies. Specific studies focused on the role of (regulatory) T cells and TGF- β pathways in PBC.	100% AMA.	Time-consuming (model of 154–196 days). Development of intestinal inflammation. No granulomas. No progression into chronic cholestasis. No female predominance.	105,112–114
	<i>IL-2Rα^{-/-}</i> mouse model	Female and male C57BL/6 mice	Acute mechanistic studies. Specific studies focused on the role of (regulatory) T cells in PBC.	100% AMA.	Time-consuming (model of 56–154 days). Development of inflammatory bowel diseases, haemolytic anaemia and lymphoproliferative autoimmune disorder. High mortality rate. Short life span. No granulomas and eosinophilic infiltration. No progression into chronic cholestasis. No female predominance.	105,111,113

(Continues)

TABLE 1 (Continued)

Cholestatic liver injury	Type of experimental model	Reported rodent species/strains	Applicability domain(s)	Advantages	Disadvantages	References
	MRL/lpr mouse model	Female and male C57BL/6 mice	Acute mechanistic studies. Specific studies focused on the role of T cells in PBS.	First spontaneous model developed for PBC.	Time-consuming (model of 140 days). No progression to cirrhosis. No significant increase in bilirubin and hepatobiliary enzymes. No female predominance. Only 50% of mice develop a PBC-like disease.	98,99
	NOD c3c4 mouse model	Female and male NOD c3c4 mice	Acute and chronic mechanistic studies. Specific studies focused on the role of B and T cells in PBC. Specific studies focused on the peculiar switch from diabetes to a PBC-like disease.	Only existing model that could unravel the genetic switch from diabetes to a PBC-like disease.	Time-consuming (model of 67 days). Biliary dilatation and cystic lesions. Only 50%-60% AMA. Granulomas are rare. No progression to chronic cholestasis. No female predominance.	105,114,116,117
	Scurfy mice model	Male C57BL/6 mice	Acute mechanistic studies. Specific studies focused on the role of regulatory T cells in PBC.	Short model (21-28days). 100% AMA.	Lupus-like autoimmune features. High mortality rate. No female predominance.	102,104,105
Chemical-induced rodent models	2-octynoic acid and 2-nonynoic acid-induced cholestasis	Female C57BL/6 mice	Chronic mechanistic studies. on the role of regulatory T cells in PBC.	Short model (28 days). 100% AMA. Easy model.	Peritonitis. No female predominance. Less pronounced portal inflammation.	105,114,170
Primary sclerosing cholangitis (PSC)	Bile duct ligation	Male C57BL/6 and 129/Sv mice Male Sprague-Dawley rats	Chronic mechanistic studies.	Short model (14-56 days). Relatively easy surgical procedure.	High variability across different strains, species and gender. Technical pitfalls. No development of inflammatory bowel diseases.	74,75,83,86

(Continues)

TABLE 1 (Continued)

Cholestatic liver injury	Type of experimental model	Reported rodent species/strains	Applicability domain(s)	Advantages	Disadvantages	References
	Bile duct injection technique	Female C57BL/6 mice	Acute and chronic mechanistic studies. Therapeutic studies.	Minor or no involvement of other tissues. Novel route of administration for testing therapeutic strategies directly in the bile ducts.	Risk of peritonitis. Leakage of bile or the injective solvent into the abdominal cavity. Difficult procedure due to the small anatomic proportions which complicate access to the biliary tree. Model needs to be further characterized.	91
Genetically modified mice models	<i>Cft^{r/-}</i> mouse model	Male C57BL/6 mice	Chronic mechanistic studies. Therapeutic studies. Specific studies focused on the long-term pro-tumorigenic aspects of PSC.	Only existing model that could unravel the link between cystic fibrosis liver disease and PSC.	Time-consuming (model of 30-728 days). High variability across strains, gender and age. No development of inflammatory bowel diseases. Intestinal obstruction. No progression into hepatic fibrosis. Contrasting results from different studies.	81,82,127,129, 200,210
	<i>fch/fch</i> mouse model	Male BALB/c mice	Acute and chronic mechanistic studies. Specific studies focused on the long-term pro-tumorigenic aspects of PSC and the coincidence of cholestasis in erythropoietic protoporphyria.	Only existing model that could unravel the link of cholestasis in erythropoietic protoporphyria.	Time-consuming (model of 84-496 days). Model needs to be further characterized.	81,94,135,136
	<i>Mdr2^{-/-}</i> mouse model	Male BALB/c and Female FVB/N mice.	Chronic mechanistic studies. Test novel therapeutic strategies. Biomarker discovery.	Short model (28 days). Male predominance (only in the BALB/c strain).	No development of inflammatory bowel diseases.	123,124,201
Chemical-induced rodent models	α -naphthylisothiocyanate-induced cholestasis	Male Sprague-Dawley rats. Female and male C57BL/6 mice	Acute and chronic mechanistic studies. Therapeutic studies. Diagnostic studies (biomarkers).	Single dose suffices. Well-known hepatotoxicant. Very short model (2 days). Easy model.	No large bile duct injury. No development of inflammatory bowel diseases.	79,171,172,174

(Continues)

TABLE 1 (Continued)

Cholestatic liver injury	Type of experimental model	Reported rodent species/ strains	Applicability domain(s)	Advantages	Disadvantages	References
	3,5-diethoxycarbonyl-1,4-dihydrocollidine-induced cholestasis	Male 129 DV mice, C57BL/6, FVB/N mice and Swiss albino mice.	Chronic mechanistic studies. Therapeutic studies.	Short model (7-56 days). Available in 4 mice strains. Easy model.	No development of inflammatory bowel diseases.	82,175-177
	Lithocholic acid-induced cholestasis	Male 129 DV mice, C57BL/6, FVB/N mice and Swiss albino mice. Female and male Sprague-Dawley rats.	Acute mechanistic studies. Therapeutic studies. Specific studies focused on the role of bile acids in PSC.	Very short model (4 days). Available in 4 mice strains and rats. Easy model.	High mortality rate. Short lifespan. No development of inflammatory bowel diseases.	81,184,185
	2,4,6-trinitrobenzenesulfonate acid-induced cholestasis (intraportal administration)	Female Lewis rats and male Sprague-Dawley rats.	Acute and chronic mechanistic studies.	Short model (7-56 days).	Mild phenotype. No development of inflammatory bowel diseases. AMA not directed towards PDH-E2. Biliary tree remains intact upon administration in the portal vein.	205,207
Combinational rodent models	<i>Cftr</i> ^{-/-} mice with dextran sodium sulphate feeding	Male C57BL/6 mice	Chronic mechanistic studies. Specific studies focused on the role of the coincidence of an inflammatory bowel disease in PSC.	Better representation of the coincidence of an inflammatory bowel disease in PSC. Better resemblance of PSC cholestasis than the single hit <i>Cftr</i> ^{-/-} mice model.	Time-consuming (model of 54 days). No progression to hepatic fibrosis.	81,127,129
	<i>Cftr</i> ^{-/-} or <i>Cftr</i> [±] mice with dextran sodium sulphate feeding and 3,5-diethoxycarbonyl-1,4-dihydrocollidine feeding	Male C57BL/6 mice	Chronic mechanistic studies. Therapeutic studies. Specific studies focused on the role of the gut-liver axis in PSC. Specific studies focused on the role of CFTR dysfunction in developing cholestatic liver diseases.	Better representation of the coincidence of an inflammatory bowel disease in PSC. Progression into hepatic fibrosis. Better resemblance of PSC cholestasis than the double hit <i>Cftr</i> ^{-/-} mice model with dextran sodium sulphate feeding.	Time-consuming (model of 70 days).	129,200

(Continues)

TABLE 1 (Continued)

Cholestatic liver injury	Type of experimental model	Reported rodent species/strains	Applicability domain(s)	Advantages	Disadvantages	References
	<i>Mdr2</i> ^{-/-} mice with dextran sodium sulphate feeding	Male C57BL/6 mice	Acute and chronic mechanistic studies. Specific studies focused on the role of the gut-liver axis in PSC.	Better representation of the coincidence of an inflammatory bowel disease in PSC	Time-consuming (model of 49–56 days).	201
	Bile duct ligated rats with 2,4,6-trinitrobenzenesulfonic acid administration	Female and male Lewis rats	Acute and chronic mechanistic studies.	Better resemblance of PSC cholestasis than the single hit 2,4,6-trinitrobenzenesulfonic acid administration model and bile duct ligation model.	Time-consuming (model of 42–378 days). High mortality rate. No development of inflammatory bowel diseases.	202,208,209

to cytomegalovirus proteins with or without other biochemical markers).^{8,45} Several factors have been described in the pathogenesis, including abnormal foetal or prenatal circulation, genetic factors, embryogenesis defects, an abnormal inflammatory response, autoimmunity, viral infection and environmental toxins.⁸

2.3 | Drug-induced cholestasis

Different types of DIC can be distinguished depending on (i) the location of the insult, intrahepatic DIC *versus* extrahepatic DIC, (ii) the reversibility of the insult, acute DIC *versus* chronic DIC and (iii) the clinical phenotype of the insult, cholestasis without hepatitis, with hepatitis or with bile duct injury.⁴⁶ Clinical symptoms may include jaundice or, when associated with parenchymal liver injury, non-specific symptoms, such as anorexia, nausea, fatigue and malaise.⁴⁶ Moreover, chronic DIC may also be accompanied by xanthomas, pruritus and melanoderma.^{46,47} Increasing efforts have been made to identify a set of mechanisms involved in all these different types of DIC. In this regard, inhibition of the BSEP was earlier denoted as a key molecular initiating event. Inhibiting the transporter involved in bile acid export may result in an accumulation of bile acids in the hepatocytes. In turn, bile acid accumulation was observed to trigger two types of cellular responses, namely a deteriorative response and an adaptive response. The deteriorative response is characterized by inflammation, oxidative stress, opening of the mitochondrial membrane permeability pore and cell death. The adaptive response is the curious phenomenon of cholestasis, as this type of cellular response strives to counteract cholestasis by regulating nuclear receptors involved in bile acid homeostasis.^{48,49}

2.4 | Intrahepatic cholestasis of pregnancy

ICP is a cholestatic liver disease that refers to the sporadic occurrence of recurrent jaundice, pruritus, increased bile acid levels and/or elevated alanine/aspartate aminotransferase during the last trimester of pregnancy, sometimes resulting in foetal risks, such as preterm birth, respiratory distress syndrome or stillbirth.^{27–29} There are still a lot of uncertainties in the aetiology of ICP. Genetic, environmental and hormonal factors are all believed to contribute to the pathogenesis of ICP. Accordingly, mutations in the MDR3 have been estimated to account for up to 15% of all ICP cases.^{29,50,51} Additionally, oestrogen is proven to play an important role in ICP. Oestrogen reaches its maximum concentration in the last trimester of pregnancy, in which ICP occurs most frequently. Twin and triplet pregnancies with increased oestrogen levels appear to be more susceptible for ICP compared to single gestations. Finally, women with a family or personal history of ICP were seen to develop ICP when treated with high doses of oestrogen oral contraceptive.^{52,53} Environmental factors that have been identified include an increased incidence of ICP associated with increased plasma levels of selenium, which varies over different seasons.⁵⁴ ICP has a mainly symptomatic treatment,

TABLE 2 Serum and histological characteristics including the relevant disease phenotypes per in vivo model

Type of experimental model	Experimental in vivo model	Serum characteristics	Histological characteristics	Relevant human disease phenotype	References
Chemical-induced models	2-octynoic acid- and 2-nonynoic acid-induced cholestasis	AMA: IgM [↑] and IgG [↑]	Mild portal inflammation. Granuloma formation. Mild necrosis in hepatic parenchyma.	Primary biliary cholangitis.	168,170
	2,4,6-trinitrobenzenesulfonate acid-induced cholestasis (intraperitoneal administration)	ALP [↑] AST [↑] Bilirubin [↑] SBA [↑]	Focal non-parenchymal necrosis. Fibrous scarring of liver. Mild ductular reaction.	Primary sclerosing cholangitis.	207
	3,5-diethoxyacetyl-1,4-dihydrocollidine-induced cholestasis	ALP [↑] AST [↑] ALT [↑] Bilirubin [↑] SBA [↑]	Necrosis in liver lobules. Intrahepatic porphyrin plug. Portal inflammation. Ductular reaction. Damaged biliary epithelia. Periductular fibrosis (onion-skin type). Increased wall thickness ductus hepatocholedocus.	Primary sclerosing cholangitis.	177,228
	α-naphthylisothiocyanate-induced cholestasis	ALP [↑] AST [↑] ALT [↑] Bilirubin [↑] SBA [↑] γGT [↑]	Inflammation. Multifocal periportal necrosis. Fatty metamorphosis. Sinusoid congestion. Vacuole degeneration.	Primary sclerosing cholangitis.	174,229,230
	Chlorpromazine-induced cholestasis	Albumin [↓] ALT [↑] AST [↑] Bilirubin [↑] SBA [↑]	Expansion hepatic sinus. Hepatocellular necrosis. Ductular reaction.	Drug-induced cholestasis.	143
	Cyclosporin A-induced cholestasis	ALP [↓] Bilirubin ^{↑/±} SBA [↑]	Vacuolization of liver cells Submembranous vesicle formation.	Drug-induced cholestasis.	145,149,151
	Ethinylestradiol and estradiol-17β-D-glucuronide-induced cholestasis	ALP [↑] AST [↑] ALT [↑] Bilirubin [↑] SBA [↑]	Inflammation. Widening of intercellular spaces. Oedema. Nuclear pyknosis of cells. Rearranged hepatocytes.	Drug-induced cholestasis. Intrahepatic cholestasis of pregnancy.	165,231
	Lithocholic acid-induced cholestasis	ALP [↑] AST [↑] ALT [↑] Bilirubin [↑] SBA [↑]	Inflammation. Hepatocellular necrosis. Bile infarcts. Crystals obstructing interlobular bile ducts. Larger bile ducts with periductal oedema. Pericholangitis. Periductal fibrosis. Ulceration of bile duct epithelium.	Primary sclerosing cholangitis. Role of BA accumulation in cholestasis.	185,232,233

(Continues)

TABLE 2 (Continued)

Type of experimental model	Experimental in vivo model	Serum characteristics	Histological characteristics	Relevant human disease phenotype	References
	Lithogenic diet	ND	Liver Vacuolar degeneration. Neutrophil infiltration into acini and portal area of liver. Gallbladder Inflammation. Thickened muscular walls. Altered mucosal papillary architecture. Reactive epithelial changes.	Gallstone liver disease.	225,234
Genetically modified mice models	<i>Ae2_{a,b}</i> ^{-/-} mouse model	ALP↑ ALT↑ AMA: IgM↑ and IgG↑	Mild to intense portal inflammation. (Slight portal fibrosis)	Primary biliary cholangitis.	113
	ARE <i>Del</i> ^{1/-} mouse model	AST↑ ALT↑ SBA↑ AMA: IgM↑ and IgG↑	Portal and lobular inflammation. Small bile duct damage. Granuloma formation. Mild fibrosis.	Primary biliary cholangitis.	113,119
	<i>Bsep</i> ^{-/-} mouse model	No significant changes of albumin, ALP, AST, ALT, bilirubin and γ GT	Only ultrastructural changes: Dilatation of canalicular lumens. Loss of microvilli. Increased peroxisomes, lysosomes and lipid droplets in hepatocytes.	Benign recurrent intrahepatic cholestasis type 2 Drug-induced cholestasis. Type 2 progressive familial intrahepatic cholestasis.	203,235
	<i>Cftr</i> ^{-/-} mouse model	No significant increase in ALP	Ductular cell proliferation. Portal inflammation. (Periportal and bridging fibrosis.) Hepatic steatosis. (Cirrhosis). Cave: contrasting studies described <i>Cftr</i> ^{-/-} mice to only exhibit an intestinal phenotype with mild or non-existing pathologies in other organs. ^{81,130-132}	Cystic fibrosis liver disease. Primary sclerosing cholangitis.	129
	<i>dnTGF-βRII</i> mouse model	AMA: IgM no increase Other cholestatic parameters ND	Portal inflammation. Bile ductular destruction. Inflammation in the parenchyma of the liver.	Primary biliary cholangitis.	106,112
	<i>fch/fch</i> mouse model	Bilirubin↑ SBA↑	Ductular reaction. Periportal or septal fibrosis. Protophyrin deposits in small bile ductules.	Erythropoietic protoporphyria (link with primary sclerosing cholangitis).	135,236

(Continues)

TABLE 2 (Continued)

Type of experimental model	Experimental in vivo model	Serum characteristics	Histological characteristics	Relevant human disease phenotype	References
Surgical-induced model	<i>IL-2Rα^{-/-}</i> mouse model	AMA: IgA \uparrow and IgG \uparrow Other cholestatic parameters ND	Liver Portal inflammation. Mild interface hepatitis. Bile ductular destruction. Colon Colon inflammation.	Primary biliary cholangitis.	111,237
	<i>Mdr2^{-/-}</i> mouse model	AST \uparrow ALT \uparrow ALP \uparrow Bilirubin \uparrow SBA \uparrow	Focal necrosis and eosinophilic bodies. Portal inflammation. Ductular reaction. Slight fibrosis. Dilatation of large bile ducts.	Intrahepatic cholestasis of pregnancy. Primary sclerosing cholangitis. Progressive familial intrahepatic cholestasis type 3.	76,121,122,238
	MRL/lpr mouse model	AMA: IgA \uparrow and IgG \uparrow No significant increase in the other cholestatic parameters.	Portal inflammation. Granuloma formation. Bile ductular destruction. Focal necrosis and/or acidophilic bodies.	Primary biliary cholangitis	98,99
	NOD c3c4 mouse model	AST \uparrow ALT \uparrow AMA: IgG \uparrow (females) and IgM \uparrow	Peribiliary lymphocytic infiltration. Bile ductular destruction. Intrahepatic biliary cyst formation. (Fibrosis and granuloma formation). Dilated bile ducts.	Primary biliary cholangitis	117,239
	Scurfy mice model	AMA: IgA \uparrow , IgG \uparrow and IgM \uparrow	(Peri)portal and perisinusoidal inflammation. Bile ductular destruction. Interface hepatitis. Focal necrosis.	Primary biliary cholangitis. Systemic lupus erythematosus.	102,104
Bile duct ligation		Albumin \downarrow ALP \uparrow/\downarrow AST \uparrow ALT $\uparrow/=$ Bilirubin \uparrow SBA \uparrow γ GT \uparrow	Liver fibrosis (F1 \rightarrow F4). Portal hypertension. Biliary infarcts. Dilatation of bile canaliculi. Portal tract enlargement. Portal inflammation. Ductular reaction.	Biliary atresia. (Extrahepatic cholestasis) Gallstone liver disease. Hepatic encephalopathy. Iatrogenic cholestatic liver diseases Obstructive cholestasis. Primary sclerosing cholangitis. Secondary biliary cirrhosis.	74,75,78,83, 86,240,241
	Bile duct injection technique	ALT \uparrow at day 1 (normalization after 7 days) No significant changes in AST, ALP and bilirubin.	Necrosis Fibrosis Portal inflammation Cave: these histological features occur after 1 week postoperation and resolve after 2-6 weeks.	Biliary atresia. Biliary cirrhosis. Cholangiocarcinoma. Primary biliary cholangitis. Primary sclerosing cholangitis.	90-92

(Continues)

TABLE 2 (Continued)

Type of experimental model	Experimental in vivo model	Serum characteristics	Histological characteristics	Relevant human disease phenotype	References
Viral-induced rodent models	Intraperitoneal inoculation of mice with rhesus rotavirus	Bilirubin ↑	Obstruction of extrahepatic bile ducts by inflammatory cells. Portal inflammation. Ductular reaction. Focal stenosis of the common bile duct. (Distal cystic dilatation)	Biliary atresia	196,223,242
Combinational rodent models	<i>Bsep</i> ^{-/-} mouse model and cholic acid feeding	ALP ↑ AST ↑ Bilirubin ↑ SBA ↑ 5'-nucleotidase ↑	Mild ductular reaction. Dilated bile ducts with infiltration of inflammatory cells. Necrosis in liver parenchyma.	Drug-induced cholestasis. Type 2 progressive familial intrahepatic cholestasis.	97
	<i>Cftr</i> ^{-/-} mice with dextran sodium sulphate feeding	ALT ↑	Liver Portal inflammation. Biliary epithelial damage. Ductular reaction. Colon Mononuclear cell infiltrates. Loss of crypts. Mucosal ulcerations in the colonic resection specimens.	Primary sclerosing cholangitis.	127,210
	<i>Cftr</i> ^{-/-} or <i>Cftr</i> [±] mice with dextran sodium sulphate feeding and 3,5-diethoxycarbonyl-1,4-dihydrocollidine feeding	ALP ↑ ALT ↑ AST ↑	Periportal and sinusoidal inflammation. Ductular reaction. Periductular fibrosis. 'Onion skin' fibrosis. Bridging fibrosis. Porphyrin bile plugs.	Primary sclerosing cholangitis.	200
	<i>Mdr2</i> ^{-/-} mice with dextran sodium sulphate feeding	ALT ↑	Liver Ductular reaction. Bridging fibrosis. Colon Colonic inflammation. Colonic shortening.	Primary sclerosing cholangitis.	201,243

Abbreviations: ALP, alkaline phosphatase; ALT, alanine aminotransferase; AMA, antimitochondrial antibody; AST, aspartate aminotransferase; Ig, immunoglobulin; ND, not determined; (S)BA, (total serum) bile acids; γ GT, gamma glutamyltransferase.

since the pathophysiology is still unresolved. Ursodeoxycholic acid is being supported as first-line therapy in ICP with beneficial effects on the mother, foetus and new-borns.^{55,56}

2.5 | Primary biliary cholangitis

PBC is a slowly progressive cholestatic liver disease with an important autoimmune aspect. This autoimmune disease is characterized by immune-mediated destruction of the intrahepatic bile ducts and portal inflammation. In a more advanced stage of the disease, loss of bile ducts may lead to diminished bile secretion, accumulating noxious substances within the liver, fibrosis, cirrhosis and ultimately liver failure. Accordingly, four different histological stages can be distinguished during PBC: (i) portal inflammation with or without 'florid' bile duct lesions (ie intense inflammatory infiltration and necrosis around bile ducts), (ii) enhanced periportal lesions accompanied by interface hepatitis, (iii) abnormalities in the hepatic architecture and several fibrous septa and finally (iv) cirrhosis. Typical for PBC is the seropositivity for antimitochondrial antibodies (AMAs) towards the E2 subunits of 2-oxo-acid dehydrogenase complexes, such as pyruvate dehydrogenase (PDH-E2).⁵⁷ AMAs are present in 90%-95% of the patients and can often be detected years before clinical symptoms, including fatigue, pruritus, jaundice, xanthomas, osteoporosis and dyslipidaemia.⁵⁷⁻⁵⁹ The pathogenesis of PBC still remains to be further elucidated; however, an important genetic aspect has already been identified. The latter is related to an increased susceptibility in first-degree relatives⁶⁰ but a full characterization of the exact genetic influences is still missing.^{61,62} PBC primarily affects woman, with a ratio 10 to 1 versus men, presumably related to a higher incidence of X-chromosome monosomy in lymphoid cells.^{57,63} Environmental factors play an additional important role in PBC. Several causatives have been proposed to initiate a strong autoimmune response due to molecular mimicry and cross reactivity with human pyruvate dehydrogenase complex autoepitopes, including bacteria, viruses and environmental chemicals.^{57,64}

2.6 | Primary sclerosing cholangitis

PSC is a long-term, chronic liver disease featured by inflammation and fibrosis of intra- and/or extrahepatic bile ducts, leading to end-stage liver disease and reduced life expectancy. PSC primarily affects young, middle-aged men. Most patients complain of right upper quadrant abdominal pain, pruritus, fatigue and jaundice. In addition, hepatomegaly and splenomegaly are regularly observed in PSC patients. 10%-20% of PSC patients spontaneously develop cholangiocarcinoma, making this a very dangerous liver disease.⁶⁵ Interestingly, PSC is also closely associated with inflammatory bowel diseases (IBD), with up to 70% of the PSC patients having underlying IBD, particularly ulcerative colitis. PSC-IBD represents a phenotypically different disease with a higher risk of hepatobiliary and colorectal malignancy compared to non-IBD-PSC. Unfortunately, the

link between PSC and IBD is incompletely understood.⁶⁶⁻⁶⁹ Both hereditary and environmental factors were shown to contribute to PSC development. It has been hypothesized that genetically predisposed pathways may initiate a persistent injury on cholangiocytes lining the bile ducts after being exposed to an environmental source.^{26,65} With respect to the genetic susceptibility, a large cohort genome-wide association study showed a strong correlation with specific human leukocyte antigens (HLA) class I, II and III regions.^{26,70} Recently, novel insights were also gathered with regard to the role of peribiliary glands and the biliary tree stem cell compartment in the pathogenesis of PSC and related carcinogenesis.⁷¹⁻⁷³ There are three subtypes established for PSC being (i) classic PSC, affecting small and large bile ducts; (ii) small-duct PSC, affecting only small bile ducts and (iii) PSC associated with autoimmune hepatitis, affecting small and large bile ducts.⁶⁵

3 | SURGERY-INDUCED RODENT MODELS

3.1 | Obstructive cholestasis

The most frequently used surgery-induced animal model of cholestasis relies on bile duct ligation (BDL), where a ligature is placed or surgical ligation is performed on the common bile duct. This surgical procedure creates an extrahepatic obstruction of the biliary system, which results in cholestasis and inflammation.^{74,75} The BDL model induces acute extrahepatic obstructive biliary lesions, reflecting the clinical setting of gallstone liver disease and biliary atresia.⁷⁶⁻⁷⁸ However, while BDL is *stricto sensu* an extrahepatic obstruction, the model has also been extensively used as platform for investigating the subsequent pathophysiological processes in hepatic morphology and function resulting from obstructive cholestasis. The BDL model has therefore also been widely used to study PSC.^{74,75,79-81} The model was initially developed for rats, since they lack the gallbladder^{82,83} and later successfully adapted to mice.^{84,85} BDL in mice causes jaundice, release of transaminases, elevated bilirubin serum levels accompanied by development of bile duct proliferation with leukocyte infiltration, which eventually results in liver fibrosis.⁸⁶ In contrast to rats and humans, mice develop a biliary type of hepatocytic necrosis (bile infarcts) instead of apoptosis.^{86,87} Additionally, unlike rats, the gallbladder of mice can drastically dilate after BDL and subsequently perforate resulting in bilioperitoneum, which may burgeon into death. Two strategies are followed to avoid these events, namely removal of the gall bladder *via* a cholecystectomy and placement of a surgical clip on the cystic duct.⁸⁸

In the last decade, modifications were also described of total BDL, including partial BDL and selective BDL.^{85,87,89} Selective BDL is defined as ligating the left hepatic bile duct before it enters the common bile duct, while in partial BDL, a single ligation around the common bile duct is performed with leaving a defined lumen that allows a limited degree of bile flow.⁸⁷ The advantage of selective BDL is that the cholestatic injury is solely located in the left hepatic lobe, wherein bile infarcts develops as well as infiltrating neutrophils.^{85,87}

Partial BDL exhibits a less extensive tissue injury compared to selective BDL, believed to better mimic obstructive cholestasis in humans compared to total BDL. It should however be noted that cholestasis in this model seemed to spontaneously resolve after already 5 days, as a result partial BDL is particularly eligible for modelling acute cholestasis.⁸⁵

3.2 | Cholangiopathies

Recently, the bile duct injection technique has been reported in which bile ducts are directly accessed without causing harm to the hepatobiliary tissue.⁹⁰⁻⁹² Studies have been reported injecting different types of solutions in the bile ducts varying from a harmless phosphate buffered saline solution to toxic dimethyl sulfoxide solutions, plasmid solutions and exogenous compounds including oxazolone.⁹⁰⁻⁹² The model has great potential to mimic several cholangiopathies, such as biliary atresia, PBC and PSC, depending on the causative agent that is being injected. The biggest advantage of this surgical model is the minimal injury to neighbouring tissue of the biliary tree. However, an important obstacle is the small anatomic proportion of mice (gall bladder has a diameter of 1-2 mm), which complicate access to the biliary tree. Other drawbacks are the risk of peritonitis and possible leakage of bile or the injected solvent into the abdominal cavity. Noteworthy, a clear sex difference has been observed in this model. Female mice are described with a higher postoperative morbidity and mortality compared to male mice.⁹¹

4 | GENETICALLY MODIFIED MICE MODELS

Researchers have entered a new era of modelling human diseases concomitant with the emergence of genetically manipulating the laboratory mouse (*Mus musculus*). The mouse is particularly relevant due to its high degree of conservation with humans regarding its anatomy, physiology and genetics.⁹³ Genetically modified models are sometimes referred to as 'spontaneous' models or described to 'spontaneously develop' the respective liver pathology. The word 'spontaneous' indicates the absence of surgical or toxic/infectious insults to induce cholestasis.^{52,94,95}

4.1 | Drug-induced cholestasis

Malfunctioning of the BSEP, via drug inhibition, internalization, mutations or altered expression, drastically affects the bile acid homeostasis and inhibition of this transporter has been denoted as the key triggering factor of DIC.^{48,49} Consequently, *Bsep* (*Abcb11*)^{-/-} mice may serve as an essential tool elaborating the consecutive effects of *Bsep* perturbation while identifying possible alternative bile acid transport systems. A shortcoming of this model is the development of mild non-progressive cholestasis, presumably due to the more

hydrophilic properties of endogenous bile acids in mice compared to humans.⁹⁶ The latter can be tackled by administering additional cholic acids to *Bsep*^{-/-} mice.⁹⁷ Finally, *Bsep*^{-/-} mice can additionally serve as a model for studying different genetic forms of cholestasis characterized by *Bsep* mutations, such as progressive familial cholestasis 2 and benign recurrent intrahepatic cholestasis 2. However, this goes beyond the scope of this review paper and thus will not further be discussed.^{82,96}

4.2 | Primary biliary cholangitis

The first model created to mimic PBC consists of MRL/*lpr* mice bearing the lymphoproliferative (*lpr*) gene.^{98,99} This gene results in the spontaneous development of severe autoimmune diseases, including lymphadenopathy, hypergammaglobulinaemia, glomerulonephritis, arthritis and Sjögren's syndrome.⁹⁸ The latter is frequently associated with PBC.¹⁰⁰ In accordance, MRL/*lpr* mice present PBC-like histological features and infiltration of inflammatory cells, such as cluster differentiation 4 (CD4)⁺ T cells and AMAs. The latter, however, only occurs in about 50% of the mice. In addition, MRL/*lpr* mice exhibit several other clinical features, incompatible with human PBC, including divergent bilirubin serum levels and hepatobiliary enzymes.^{98,99} Ergo, development of additional models to study PBC urged, among them are mouse strains based on deficiencies in regulatory T (Treg) cells, including scurfy mice.¹⁰¹ Scurfy mice contain a missense mutation in the transcription factor Forkhead box protein 3 (*Foxp3*) gene, essential in development, maintenance and function of Treg cells, resulting in complete abolition of CD4⁺ *Foxp3*⁺ Treg cells.¹⁰²⁻¹⁰⁴ This experimental model was constructed on the assumption that mice genetically deficient in regulatory mechanisms that provide immune homeostasis, including naturally occurring Treg cells, would gain increased susceptibility for developing autoimmune diseases, such as PBC.¹⁰² Indeed, scurfy mice manifest a similar serological, immunological and histopathological profile, albeit on the background of a multi-system autoimmunity.^{101,104,105} A similar disease phenotype was achieved in transgenic mice that express a dominant negative form of transforming growth factor- β receptor restricted to T cells (*dnTGF- β RII* mice), and in knockout mice with homozygous interleukin 2 receptor α (*IL-2R α*) deficiency, both containing a prominent dysfunction in Treg cell function.¹⁰¹ Dysfunctional TGF- β signalling results in reduced tolerance towards autoantigenic proteins present in the liver and leads to a PBC-like liver disease, including the characteristic AMAs directed to mitochondrial autoantigens, such as PDH-E2.¹⁰⁶⁻¹⁰⁸ The discovery of PBC-like disease in a child with homozygous *IL-2R α* deficiency¹⁰⁹ gave rise to the construction of the *IL-2R α* ^{-/-} mice.¹¹⁰ Accordingly, both human PBC and *IL-2R α* ^{-/-} mice exhibit portal lymphoid infiltrates and increased numbers of cytokines.¹¹¹ *dnTGF- β RII* and *IL-2R α* ^{-/-} mice are especially interesting to study acute mechanisms and address the role of Treg cells in PBC.^{111,112} However, both models exhibit a coinciding IBD, absence of granulomas and lack of progression into chronic cholestasis.^{105,111,113,114}

An additional model was later developed to reduce the number of Treg cells, by introducing a mutation that disables the anion exchange protein 2 (AE2) gene.¹⁰¹ AE2 encodes the $\text{Cl}^-/\text{HCO}_3^-$ anion exchanger 2, which regulates the intracellular pH and trans-epithelial acid-base transport, including secretin-stimulated biliary bicarbonate excretion and proton gastric secretion. Thus, disturbed AE2 activity may have an influence on the bile acid equilibrium, but also gastric acid equilibrium. Regulation of pH appears an important aspect in modulating lymphocyte function. Indeed, mature *Ae2_{a,b}*^{-/-} mice show immunological and hepatobiliary characteristics similar to PBC, but equally also PBC-unlike alterations, including impaired gastric acid secretion and only 30%-80% AMA.¹¹³ A different strategy was used based on altered T-cell functionality, called non-obese diabetic (NOD)c3c4 mice. NOD mice develop an immune-mediated destruction of pancreatic β cells, which underlies human type I diabetes and were created in the hope of identifying specific loci linked to the occurrence of type I diabetes.¹¹⁵ This led to the discovery of complete abolishment of diabetes development when congenic segments from chromosomes 3 and 4 (NOD.c3 and NOD.c4) were used to replace the identified specific loci for insulin-dependent diabetes (*idd*) in NOD mice. Rather than diabetes, these NOD.c3c4 mice develop an autoimmune biliary disease similar to PBC with a comparable immunologic and serologic profile (ie AMAs towards PDH-E2).^{95,116} Nevertheless, NODc3c4 mice also exhibit some PBC-unspecific features, including biliary dilation and cystic lesions and absence of chronic destructive cholangitis.^{114,117}

No murine model was able to fully recapitulate the female preponderance present in human PBC, until the a new 'designer' mouse consisting of an adenylate-uridylyl-rich element (ARE) deletion in the interferon γ gene that results in chronic expression of interferon γ .¹¹⁸ ARE-*Del*^{-/-} mice represent a phenotype very comparable to human PBC, particularly on histopathological, immunological and serological levels. Above all, this mouse model is the pioneer in exhibiting a female predominance in the matter of developing PBC, making this characteristic its biggest asset. Moreover, this specific advantage may finally open the research field in terms of elucidating the crucial mechanisms behind increased susceptibility of women in developing PBC.^{114,119,120}

4.3 | Primary sclerosing cholangitis

One of the first genetically modified models to study hepatobiliary diseases related to PSC was described in 1994, the *Mdr2*^{-/-} mice.¹²¹ The *Mdr2* gene, orthologue of the human *MDR3* (*ABCB4*) gene, encodes a transporter that secretes phospholipids into bile across the canalicular membrane.¹²² As such, homozygous disruption of *Mdr2* leads to absence of phospholipids (ie phosphatidylcholine) in bile and, thus interferes with the formation of biliary micelles resulting in liver injury.¹²² This injury particularly translates into cholelithiasis and sclerosing cholangitis. The model was initially

used to investigate the role of PFIC3 but also showed several histological lesions resembling human features of PSC, including the rapid progression into hepatic fibrosis.^{52,122-124} The main drawback of the *Mdr2*^{-/-} mouse model for PSC is the absence of co-existing IBD and the spontaneous development of hepatocellular carcinoma in in vivo models.^{94,123}

A second model was created based on the observation that PSC and cystic fibrosis liver disease share several features being cholestasis, chronic inflammation and portal tract injury.¹²⁵⁻¹²⁷ Moreover, abnormalities in the liver cystic fibrosis transmembrane conductance regulator (*CFTR*) gene product were earlier denoted to play a key role in the development of cholestasis in cystic fibrosis patients and, *vice versa*, mutations in the *CFTR* gene were also reported in PSC patients.^{127,128} Hence, it was hypothesized that *Cftr*^{-/-} mice, harbouring an exon 10 deletion in the *Cftr* gene, might also develop a similar cholestatic liver injury. *Cftr*^{-/-} mice have been reported to, indeed, develop a progressive liver disease with focal cholangitis, inspissated bile and bile duct proliferation.¹²⁹ However, contrasting studies described *Cftr*^{-/-} mice to only exhibit an intestinal phenotype with mild or non-existing pathologies in other organs.^{81,130-132} Moreover, this intestinal phenotype in *Cftr*^{-/-} mice resembles more a distal intestinal obstruction syndrome and meconium ileus rather than an IBD phenotype typical for PSC.^{69,129,133} *Cftr*^{-/-} mice, as such, are therefore rarely preferred as experimental model for PSC.

Erythropoietic protoporphyria is an inherited disorder typified by accumulating protoporphyrins as a result of reduced ferrochelatase activity. Severe malfunctioning in this ferrochelatase activity predisposes to the development of severe liver diseases with a progressive character, which frequently require liver transplantation.¹³⁴ Mice with a homogeneous mutation in genes encoding the ferrochelatase enzyme (*fch/fch*) display an increasing number of protoporphyrin deposits in the lumen of small and large bile ducts, resulting in an incomplete obstruction. The toxic effect of the accumulating protoporphyrin additionally causes damage and hepatocyte death.^{81,135} *fch/fch* mice present an extreme cholestatic phenotype with increased serum liver enzymes, increased bile salt levels and conjugated hyperbilirubinaemia, yet bile formation remains unchanged.¹³⁶ Not much is known about this model. A more in-depth study is needed to characterize the longitudinal changes of *fch/fch* mice as well as underlying pathogenetic mechanisms.

5 | CHEMICAL-INDUCED RODENT MODELS

Chemical-induced cholestasis is a well-recognized problem attributed to compounds, such as drugs, pesticides, food additives, cosmetic ingredients or industrial compounds.^{137,138} Different types of cholestatic liver injuries have been identified depending on the causative agent, varying from (hepato-)canalicular, hepatocellular to ductular cholestasis.¹³⁹

5.1 | Drug-induced cholestasis

Chlorpromazine is an antipsychotic drug, which belongs to the largest class of first-generation phenothiazines.¹⁴⁰ Although chlorpromazine is one of the three listed medicines in the World Health Organization's Essential Drug List for treating psychotic disorders, the drug contains many adverse effects, among which cholestasis.¹⁴¹ Chlorpromazine is believed to induce cholestasis *via* direct BSEP inhibition in accessory to repressing BSEP and MDR3 gene expression.¹⁴² Chlorpromazine has been commonly used to induce DIC in rodents while investigating its underlying mechanisms and the protective effect of chemicals against cholestasis.^{143,144}

Cyclosporin A is used to prevent graft rejection after organ transplantation. The two most serious side effects observed during cyclosporin A treatment are nephrotoxicity and hepatotoxicity, from which the latter is delineated as cholestasis with low to moderate hyperbilirubinaemia.¹⁴⁵ Cholestasis is presumably triggered by a number of factors, including direct inhibition and internalization of BSEP, disruption of the cytoskeleton and altered permeability of the canalicular membrane.¹⁴⁵⁻¹⁴⁸ Similar to chlorpromazine, cyclosporin-A-treated rodents have been extensively employed as experimental model for DIC.^{145,148-151}

5.2 | Gallstone liver disease

In 1964, Tepperman et al discovered that feeding mice a cholesterol-cholic acid containing diet results in the formation of cholesterol gall stones in mice.¹⁵² This lithogenic diet contains high fat, high cholesterol and 0.5% cholic acid. The combination of cholic acid with cholesterol is essential, cholesterol alone cannot induce gallstones.¹⁵² Fujihira et al revealed the significant genetic contribution to gallstone formation in inbred mice.¹⁵³ Resultantly, numerous studies were performed on different mouse strains striving to identify the candidate genes behind the increased susceptibility towards gallstone development, among which *Lith1* and *Lith2* genes are believed to be major players.¹⁵⁴⁻¹⁵⁷ Additional research are focussed on the impact of nuclear receptors, intestinal microbiota, gender and comorbidities, such as non-alcoholic fatty liver disease, diabetes and obesity in the occurrence of gallstones.¹⁵⁷

5.3 | Intrahepatic cholestasis of pregnancy

The first case of unexplained pruritus and jaundice in the last trimester of pregnancy was notified in 1883.¹⁵⁸ Still, the disease remained unnoticed until mid-1950s.¹⁵⁹ Researchers started to highlight its importance and spread some of the drastic consequences the disease withholds, including premature births, fetal distress or even stillbirths.¹⁶⁰ As a result, research related to this subject was increased and resulted in the discovery of oestrogens, like estradiol-17 β -D-glucuronide, as inducers of reversible

cholestasis in animals.^{27,161} Estradiol-17 β -D-glucuronide inhibits BSEP and MDR1 transporters, initiates an internalization of transporters BSEP and multidrug resistance-associated protein 2 (MRP2) and increases the permeability of tight junctions.^{146,161,162} Administration of the synthetic ethinylestradiol or estradiol-17 β -D-glucuronide to rodents can be used to study the underlying mechanisms of ICP as well as DIC, since oestrogens are often used in oral contraceptives and hormone substitution therapies (ie menopausal hormone therapy).¹⁶³⁻¹⁶⁵

5.4 | Primary biliary cholangitis

2-octynoic acid and 2-nonynoic acid are chemicals regularly used as ingredients in food additives and perfumed cosmetics, such as toilet waters, soaps, detergents, lipstick, perfumes and facial creams, thanks to their violet scent.^{137,166} 2-octynoic and 2-nonynoic acid are mimics of lipoic acid and replace the lipoyl group of the immunodominant E2 domain of PDH (ie PDH-E2), thereby generating a high specific and reactive antimitochondrial response.¹⁶⁶ This was also demonstrated in patients suffering from PBC, who presented an increased reactivity towards PDH-E2 coupled with 2-octynoic acid.¹⁶⁷ Accordingly, a xenobiotic-induced model was designed by means of administering 2-octynoic acid coupled with bovine serum albumin to mice.¹⁶⁸⁻¹⁷⁰ These mice manifest auto-immune cholangitis, concurrent with the typical AMAs of PBC.¹⁷⁰

5.5 | Primary sclerosing cholangitis

α -naphthylisothiocyanate (ANIT) is a chemical used for preparation of cationic aromatic urethane.¹³⁷ The compound is also well known among researchers for adequately inducing cholestasis in rats and mice,^{79,171} by specifically targeting bile duct epithelial cells followed by hepatocellular necrosis.^{137,172} Chronic and recurrent exposure to ANIT *via* the diet resembles an experimental setting of chronic cholangitis, bile duct hyperplasia and peribiliary fibrosis.¹⁷²⁻¹⁷⁴ Moreover, mechanisms underlying peribiliary fibrosis in chronic ANIT models show similarities with other chronic cholestasis models, such as long-term BDL and *Mdr2*^{-/-} mice.¹⁷²

3,5-diethoxycarbonyl-1,4-dihydrocollidine (DDC) is a second compound found to induce cholestasis. DDC is a porphyrinogenic agent and strong initiator of δ -aminolevulinic acid synthetase.^{137,175} Administration of DDC induces an increasing secretion of hepatotoxic protoporphyrins, concomitant with formation of protoporphyrin plugs. The latter induces an obstruction in the small bile ducts, which initiates cholestasis.¹⁷⁶ Moreover, DDC-induced cholestasis is typified by sclerosing cholangitis and pronounced biliary fibrosis accompanied by ductular proliferation.^{135,176,177} DDC feeding to rodents can therefore be applied to investigate (xenobiotic-induced) chronic cholangiopathies.^{82,177} Furthermore, lithocholic acid, a noxious endogenous bile acid, also induces cholestasis when present in abnormally high concentrations.^{178,179} Accordingly, the effect

of this endogenous bile acid has been widely studied in rodents to understand the potential role of the bile acid in the pathogenesis of cholestasis.¹⁸⁰⁻¹⁸⁴ It has been postulated that cholestasis in lithocholic-acid-administered mice results from partial obstruction of the bile ducts, due to crystal formation (presumably lithocholic acid precipitates), along with bile infarcts, destructive cholangitis and periductal fibrosis within days.¹⁸⁵ The lithocholic acid rodent model is claimed a valid short-term model in terms of investigating early changes in sclerosing cholangitis, thanks to the rapid induced changes after lithocholic acid administration.¹⁸⁶

6 | VIRAL-INDUCED MODELS

6.1 | Biliary atresia

Although the aetiology of biliary atresia remains unclear, viral agents were proposed as one of the root causes of neonatal obstructive cholangiopathies, including biliary atresia.¹⁸⁷ Moreover, several studies discovered the presence of pathologic viruses inside the liver of biliary atresia patients namely, rotavirus,^{188,189} reovirus,^{190,191} cytomegalovirus^{192,193} and human papillomavirus.¹⁹⁴ Together with these findings, a first murine model was developed based on the inoculation of the rhesus rotavirus to new-born mice, which rendered a biliary atresia-like obstruction of extrahepatic bile ducts.¹⁹⁵ Later on, this model has been frequently used to study disease mechanisms of biliary atresia.¹⁹⁶⁻¹⁹⁸ Unfortunately, this model contains several practical limitations, including timing and dosing of the virus, which harms the reproducibility in addition to injection-related injury to abdominal organs, inter-strain differences and a low survival rate.¹⁹⁹ Bile duct proliferation and lymphocyte infiltration inside the portal triads are apparent in the model, resembling features of human biliary atresia, albeit without liver fibrosis.¹⁹⁹

7 | COMBINATION MODELS

To achieve a better reflection of the in vivo situation, well-acknowledged rodent models can be combined and yield an improved rodent model of cholestasis. These combination models regularly consist of genetically modified rodent models or a surgery-induced model merged with a chemical-induced rodent model.^{97,127,200-202}

7.1 | Drug-induced cholestasis

BSEP disturbance is well known as one of the key players in DIC, yet *Bsep*^{-/-} mice only show mild non-progressive cholestasis and lack important histopathological features of cholestasis. Adding cholic acids to the diet of *Bsep*^{-/-} mice can be used to overcome this drawback by (i) altering the hydrophobicity of the bile acid pool, (ii) saturating bile acid hydroxylation and (iii) resulting in a more pronounced intrahepatic accumulation of hydrophobic bile acids. Accordingly,

male *Bsep*^{-/-} mice supplemented with cholic acids manifested severe progressive cholestasis associated with periportal fibrosis, inflammation and cell death.^{97,203}

7.2 | Primary sclerosing cholangitis

Next to liver injury, about 70%-86% of the PSC patients present an IBD, most frequently ulcerative colitis.²⁶ To investigate concomitant IBD presentation, additional toxics are used, including water-soluble dextran sodium sulphate (DSS).^{127,200,201} DSS is negatively charged, water-soluble and contains a highly variable molecular weight between 5 and 1400 kilodalton. This sulphated polysaccharide can be administered via drinking water, after which it can damage the monolayer of epithelial cells lining the large intestine, hereby disrupting the upper barrier. As a result, intestinal contents, including bacteria and their products, can disseminate into underlying tissue and cause an inflammatory response resulting in an IBD phenotype.²⁰⁴ 2,4,6-trinitrobenzenesulphonate acid (TNBS) is a chemical that can act as a hapten upon coupling with lysine moieties in proteins and provoke a cell-mediated immune response.²⁰⁵ Initially, the compound was used for inducing inflammatory colitis in rats by means of intracolonic instillation of TNBS.²⁰⁶ The successful use of TNBS for evoking an inflammatory disease of the colon raised the question what the inflammatory effect of TNBS would be on the liver, when locally administered. Consequently, new models were created that inject TNBS in the portal vein and in the ductus choledochus (ie intracholedochal). TNBS administration in the portal vein yields an active liver injury in rats with PSC-characteristic antineutrophil cytoplasmic antibodies, albeit without the involvement of the biliary tree²⁰⁷ while intracholedochal injection does involve the biliary tree and induces a rather mild chronic cholangitis.²⁰⁵ Orth and colleagues made a first attempt in combining models by administering TNBS to BDL rats.²⁰² The latter resulted in a PSC-like appearance featured with irregularities in the intra- and extrahepatic bile ducts, inflammation and the development of antineutrophil cytoplasmic antibodies. Although this model clearly shows an improvement compared to the single hit model, coinciding IBDs are still missing.^{202,208,209}

To integrate the IBD component, previously defined in vivo models can also be conjugated with DSS feeding. To date, three such combinational models have been described in *Cftr*^{-/-} mice or *Mdr2*^{-/-} mice.^{127,176,200,201,204,210,211} Administering DSS to *Mdr2*^{-/-} mice results in an exacerbated sclerosing cholangitis along with increased ductular reaction and bridging fibrosis.²⁰¹ Similarly, DSS can be administered to *Cftr*^{-/-} mice, producing a significant increase in bile duct injury compared to the phenotype observed in *Cftr*^{-/-} mice. Yet, fibrosis remains absent and a clear association with CFTR dysfunction cannot be identified, since homozygote, heterozygote and wild-type mice manifest similar phenotypes.¹²⁷ A follow-up study was performed and indicates the need of three separate factors to elicit bile duct injury accompanied by progressive periductal fibrosis in *Cftr*^{-/-} mice. Firstly, CFTR functionality should be harmed (ie *Cftr*^{-/-} mice). Secondly, mice should be exposed to profibrogenic activators (ie

DDC feeding). Thirdly, intestinal permeability needs to be disturbed (ie DSS feeding). Altogether, this one-of-a-kind model may serve as an ideal tool to elaborate the full pathogenic mechanism by which CFTR dysfunction predisposes fibrotic liver diseases.²⁰⁰

8 | DRAWBACKS OF CURRENT IN VIVO RODENT MODELS

Liver diseases are a major cause of illness and mortality accounting for up to 2 million deaths per year.^{212,213} Many researchers have focused on unravelling the aetiology and pathogenesis of liver diseases, including cholestatic liver disease. In vivo models have led to numerous breakthroughs in this field. However, they are also associated with inter- and intraspecies differences as well as model-specific bottlenecks, which urges an additional consideration to facilitate proper selection and application of the described models.

8.1 | Intraspecies differences

Three important aspects deserve specific attention, namely strain, gender and age. To exemplify, conflicting results were reported using *Cftr*^{-/-} mice and DDC-fed mice (partially) according to their genetic background. *Cftr*^{-/-} mice present a progressive hepatobiliary disease in C57BL/6 strains while other strains only exhibit a strong intestinal phenotype, but lack any abnormality in the liver.⁸¹ In addition, DDC feeding appears to specifically result in the highest degree of large duct disease when using Swiss albino mice.¹⁷⁷ Similarly,

inadequate gender selection can interfere considerably in establishing a sensitive model of cholestasis. Indeed, male mice were considered more suitable for BDL than female counterparts because of lower mortality rates; however, the latter does not relate to *Sv129* mice.^{86,214} Gender differences may also enhance the modelling of PBC or PSC, considering their female or male preponderance, as is the case for the ARE Del^{-/-} mice model and *Mdr2*^{-/-} mice model respectively.^{58,118,124} Likewise, the age of an animal should not be neglected, as the inflammatory response may alter in function of the rodents' age.²¹⁵

8.2 | Interspecies differences

The interspecies differences that should be encountered when modelling cholestasis include (i) a more hydrophilic bile acid composition in rodents compared to humans, (ii) differences in the substrate specificity of bile acid transporters, (iii) alterations in metabolic detoxification and (iv) hepatocellular excretion of bile acids and drugs.^{216,217} Moreover, *Cftr*^{-/-} knockouts and DDC feeding are not feasible in rats; hence, variation in the clinical phenotype between mice and rats may also occur.^{129,177,218,219} Experimental in vivo models should preferably be benchmarked towards human samples. This strategy could also identify additional crucial interspecies differences, not yet established, and hence complement some missing gaps in the mechanistic understanding of the cholestatic pathology. It would be highly relevant to use human-derived in vitro models and in silico modelling, based on structural and/or physical-chemical parameters, in addition to in vivo models. These models lack the

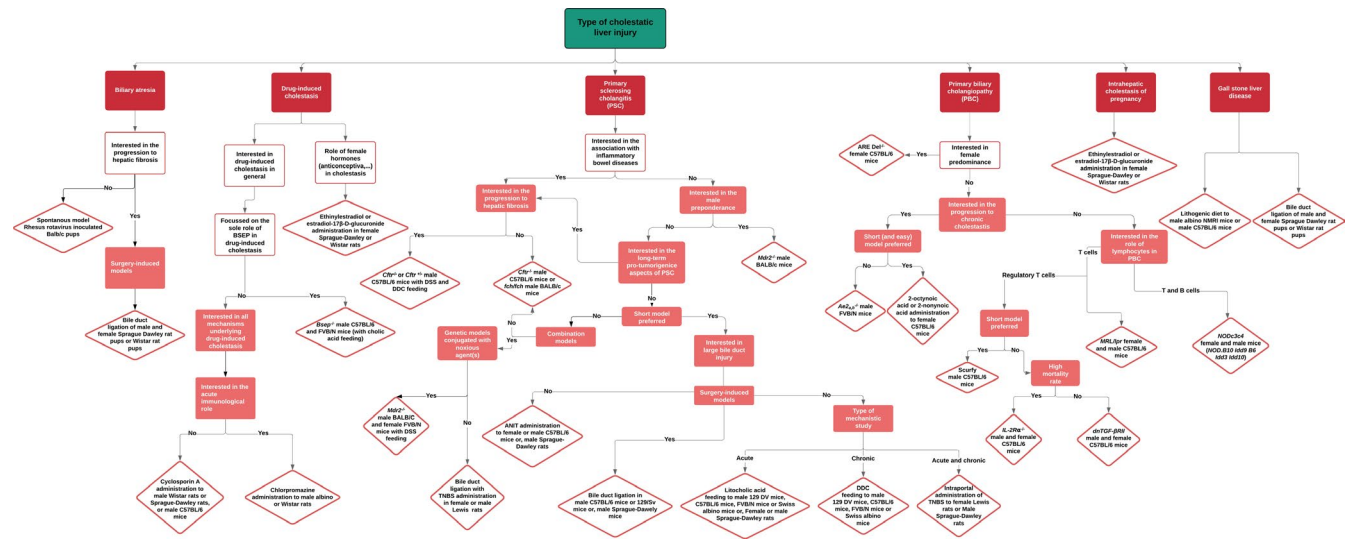


FIGURE 2 Practical workflow for selecting of the most applicable in vivo rodent model considering the applicability domain, advantages and disadvantages. This figure was created with Lucidchart software. The selection of experimental models was based on the information available in literature, as such the most appropriate well-described models were integrated. AE2, anion exchange protein 2; AMA, antimitochondrial antibody; ARE-Del, (deletion in) adenylate-uridylylate-rich element; BSEP, bile salt export pump; CFTR, cystic fibrosis transmembrane conductance regulator; DDC, 3,5-diethoxycarbonyl-1,4-dihydrocollidine; DSS, dextran sodium sulphate; dnTGF-βRII mice, dominant negative form of transforming growth factor-β receptor restricted to T cells; IL-2Rα, interleukin 2 receptor α; NOD3c4, non-obese diabetic with B6/B10 region on chromosomes 3 and 4; PBC, primary biliary cholangitis; PDH-E2, E2 subunit of the pyruvate dehydrogenase; PSC, primary sclerosing cholangitis; TNBS, 2,4,6-trinitrobenzenesulfonic acid

burden of a complex environment, allow high-throughput screening and may further improve the accuracy in predicting cholestasis while keeping interspecies differences in mind.^{49,220}

8.3 | Specific bottleneck within a model

In addition to inter- and intraspecies differences, it is of the utmost importance to be adequately informed about specific aspects of a certain model relevant for the respective applicability domain(s) (Table 1). Additionally, a practical workflow is provided that guides researchers to make an informed decision in the most appropriate in vivo rodent model currently available while considering the specific bottlenecks (Figure 2).

9 | CONCLUSION

Considerable fundamental clues have been unveiled with the generation of experimental animal models related to the pathophysiological mechanisms of biliary and cholestatic diseases. Although extensive information has been gathered in the last couple of decades with regard to the mechanistic understanding of cholestatic liver diseases, still considerable gaps are left behind due to inadequate animal models exemplified by the difficulty of inducing the female preponderance of PBC and the coincidence of PSC and IBD. Although breakthroughs are made in the design of new models, which try to tackle these setbacks, still no experimental animal is able to perfectly mimic the real cholestasis phenotype seen in humans. For this reason, we should acknowledge the specific limitation of each model and design novel models by means of combining multiple in vivo rodent models, hereby circumventing limitations while increasing the assets. A number of these combination models have already been described in the last decade but are still limited. Further exploration of these combination models will undoubtedly pave the way for improved resemblance of human cholestasis in vivo and provide the possibility of more accurate mechanistic and therapeutic studies.

CONFLICT OF INTEREST

The authors have no conflicts of interest to declare.

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