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Pathobiology of inherited biliary diseases: a roadmap to understand acquired liver diseases

Luca Fabris[1,2], **Romina Fiorotto**[1], **Carlo Spirli**[1], **Massimiliano Cadamuro**[2], **Valeria Mariotti**[2], **Maria J. Perugorria**[3,4,5], **Jesus M. Banales**[3,4,5], **Mario Strazzabosco**[1,2,*]

[1]Liver Center, Department of Medicine, Yale University, New Haven, CT, USA.

[2]Department of Molecular Medicine, University of Padova, Padova, Italy.

[3]Department of Liver and Gastrointestinal Diseases, Biodonostia Health Research Institute, Donostia University Hospital, University of the Basque Country (UPV/EHU), San Sebastian, Spain.

[4]National Institute for the Study of Liver and Gastrointestinal Diseases (CIBERehd, Instituto de Salud Carlos III), Madrid, Spain.

[5]IKERBASQUE, Basque Foundation for Science, Bilbao, Spain.

Abstract

Bile duct epithelial cells, also known as cholangiocytes, regulate the composition of bile and its flow. Acquired, congenital and genetic dysfunctions in these cells give rise to a set of diverse and complex diseases, often of unknown aetiology, called cholangiopathies. New knowledge has been steadily acquired about genetic and congenital cholangiopathies, and this has led to a better understanding of the mechanisms of acquired cholangiopathies. This Review focuses on findings from studies on Alagille syndrome, polycystic liver diseases, fibropolycystic liver diseases (Caroli disease and congenital hepatic fibrosis) and cystic fibrosis-related liver disease. In particular, knowledge on the role of Notch signalling in biliary repair and tubulogenesis has been advanced by work on Alagille syndrome, and investigations in polycystic liver diseases have highlighted the role of primary cilia in biliary pathophysiology and the concept of biliary angiogenic signalling and its role in cyst growth and biliary repair. In fibropolycystic liver disease, research has shown that loss of fibrocystin generates a signalling cascade that increases β-catenin signalling, activates the NOD-, LRR- and pyrin domain-containing 3 inflammasome, and promotes production of IL-1β and other chemokines that attract macrophages and orchestrate the process of pericystic and portal fibrosis, which are the main mechanisms of progression in cholangiopathies. In cystic fibrosis-related liver disease, lack of cystic fibrosis transmembrane conductance regulator increases the sensitivity of epithelial Toll-like receptor 4 that sustains the secretion of nuclear

* mario.strazzabosco@yale.edu.

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factor-κB-dependent cytokines and peribiliary inflammation in response to gut-derived products, providing a model for primary sclerosing cholangitis. These signalling mechanisms may be targeted therapeutically and they offer a possibility for the development of novel treatments for acquired cholangiopathies.

The bile duct epithelial cells, also known as cholangiocytes, are essential in multiple physiological liver processes, including the fine regulation of bile composition and its flow[1]. The biliary tree is also affected by a group of diseases called cholangiopathies. Most acquired cholangiopathies are complex diseases of unknown aetiology and pathogenesis, such as primary biliary cholangitis (PBC) and primary sclerosing cholangitis (PSC). A proportion of cholangiopathies are caused by inherited or genetically mediated dysfunction of cholangiocytes that progresses chronically, leading to end-stage liver diseases such as congenital hepatic fibrosis and cystic fibrosis-related liver disease[2,3]. Cholangiopathies are rare, but, as a group, they account for up to 20% of liver transplantations in the adult population[4] and up to 80% in the paediatric population[5]. These diseases are complex, and their management usually requires referral to centres of excellence, where clinical expertise is combined with translational research. Treatment options are limited and, besides bile acids, farnesoid X receptor agonists, steroids and liver transplantation, little can be offered to individuals with these diseases at this time. However, new knowledge has been steadily acquired over the past 20 years that will hopefully translate into novel treatments in the near future.

The lack of reliable experimental models has hampered translational research of acquired cholangiopathies. However, the genetic cholangiopathies can be addressed with translational methods owing to the availability of animal and cellular models that phenocopy the disease. These approaches have clarified the functions of the defective genes, improved understanding of important and novel pathobiological concepts and resulted in innovative experimental therapeutic strategies. In addition, the mechanisms uncovered are often relevant for acquired cholangiopathies and, more broadly, for chronic liver diseases. Thus, the genetic cholangiopathies can be viewed as a pathophysiological roadmap for cholangiopathies and chronic liver diseases in general. Among the genetic and congenital cholangiopathies, this Review focuses on examples of monogenic conditions, including Alagille syndrome, polycystic and fibropolycystic liver diseases, and cystic fibrosis-related liver disease. These diseases are associated with changes in major signalling mechanisms that may have the potential to be targeted. We place these new mechanisms in the context of pathobiological mechanisms common to all cholangiopathies and discuss future directions in basic research and potential clinical translation. We do not discuss familial progressive intrahepatic cholestasis type 3 (ABCB4 deficiency) because, even if much has been learned from this model[6,7], the genetic defect is localized in hepatocytes rather than cholangiocytes. Similarly, this Review does not focus on biliary atresia, as in most cases a genetic association is not present.

Biliary epithelium

To better understand genetic and malformative cholangiopathies, a working knowledge of biliary functional anatomy and development is needed. The biliary tree collects the primary bile produced by the hepatocytes and modifies its composition by alkalization and hydration, before delivering it to the intestine where it is indispensable for digestive function and endo-xenobiotic disposal[3]. Besides the extensive transport activities involved in generating and modifying bile that are performed by interlobular and larger bile ducts, the biliary epithelium is intimately involved in the control of liver inflammation and in the regenerative and reparative responses to injury. In response to liver injury, small bile duct cells proliferate and expand and the hepatic progenitor cell (HPC) compartment is activated[8,9]. HPCs, which are bipotential cells that are able to differentiate into either the biliary or the hepatocyte lineage, have two main locations: one is intrahepatic, confined to the periportal niche, in close contact with the canals of Hering[8]; and the other is extrahepatic, in the peribiliary glands present in the submucosal layer of the bile duct wall[10]. In response to chronic liver damage, particularly in the cholangiopathies, ductular epithelial cells acquire a ‘reactive’ phenotype (reactive ductular cells (RDCs)) and grow as irregular strings at the margins of the portal tract[8,9]. By establishing strong paracrine communications with mesenchymal cells, including myofibroblasts, inflammatory cells and endothelial cells, ductular cells orchestrate a reparative response with the side effect of fibrogenesis[11].

The phenotypic differences between intrahepatic and extrahepatic bile ducts mirror their distinct embryological origin and the development of the bile duct epithelium. Whereas the extrahepatic bile ducts derive from the caudal part of the ventral foregut, the intrahepatic system originates from hepatoblasts, which are bipotent endodermal cells present in the fetal liver bud[12,13]. At gestational week 8, hepatoblasts abutting the mesenchyme of the nascent portal space start to differentiate towards a biliary phenotype, leading to a circular layer of single cells called the ductal plate. Initially, ductal plate cells express phenotypic markers of both the hepatocellular lineage (cytokeratin 8 (CK8) and CK18) and the biliary lineage (CK19), but when they start to duplicate and assume a tubular shape from gestational weeks 12–16, they gradually lose hepatocyte markers and gain additional biliary markers, including CK7. Non-duplicating ductal plate cells are usually removed by apoptosis[13,14]. Once ductal plate cells acquire a ductal morphology, these cells migrate to and incorporate into the portal mesenchyme where they enlarge[1,15]. During this phase, an arterial vascularization and a peribiliary plexus develop near the migrating and incorporated bile ducts[16]. This process is finely tuned by the combined interactions of angiogenic growth factors (mainly vascular endothelial growth factor A (VEGFA), and angiopoietin 1 (Ang1) and Ang2) and by several morphogenic signalling pathways, including Wnt–β-catenin, Hedgehog and transforming growth factor-β (TGFβ), and Notch and HIPPO–YAP[17–23]. Notch signalling is a clear example of how activation of these morphogens can become necessary later in life during repair of biliary damage.

Alagille syndrome and Notch signalling

Alagille syndrome is a multisystem disorder involving the liver, vasculature, heart, eyes and skeleton, and is characterized by typical facies. This syndrome is a rare (estimated to affect 1

in 30,000 to 1 in 50,000 live births) autosomal dominant dysmorphogenetic disorder caused by defective Notch signalling owing to mutations in the Notch ligand Jagged1 (JAG1)[24,25] or, less frequently, in a Notch receptor (NOTCH2)[26]. Alagille syndrome is associated with incomplete development of the intrahepatic bile ducts, and this liver disease is characterized by bile duct paucity, with variable degrees of cholestasis, progressive jaundice, itching and failure to thrive[27]. Treatment is only symptomatic; in some instances, jaundice and itching slowly improve, and 20–50% of individuals with this disease require liver transplantation[28].

Notch signalling

The Notch pathway is an evolutionarily conserved signalling system that regulates cell fate decisions in stem cells and has a major role in the development of the biliary tree[29,30]. Notch signalling enables cells to communicate with their direct neighbours by cell-to-cell ligand–receptor interactions. There are four known Notch receptors (NOTCH1–4) and five ligands (JAG1, JAG2 and Delta-like 1, 3 and 4). In the 'canonical' signalling pathway, ligand–receptor binding leads to proteolytic cleavage of the Notch receptor and subsequent release of the Notch intracellular domain (NICD)[31]. The NICD translocates into the nucleus where it interacts with recombining binding protein suppressor of hairless (RBPJκ), converting RBPJκ from a transcriptional repressor into an activator, thus promoting the transcription of Notch target genes. The main Notch target genes belong to the *HES* or *HEY* family, although several other genes, such as *GATA3*, which is important for T cell development, and those encoding MYC, cyclin D1 and p21 (WAF1), which are implicated in cancer, are also regulated in parallel[29,32]. Notch signalling can generate diverse and even opposite biological effects, depending on the specific context, gene dosage, timing and cell type[33,34]. During liver development, Notch-expressing hepatoblasts localized at the parenchymal interface of the nascent portal tract receive signals from adjacent mesenchymal cells expressing JAG1, and respond by upregulating the expression of *HNF1B* and *SOX9* (FIG. 1a). These factors in turn stimulate the differentiation of hepatoblasts into ductal plate cells and eventually the duplication of the ductal plate and the incorporation of the nascent duct into the portal space. *SOX9*, *HNF1B* and *HES1*, among several candidate genes regulated by Notch, seem to be critical for ductular development[35,36]. However, there is no single overarching factor downstream of Notch that mediates ductal development. Rather, Notch is part of a large transcriptome and signalling network that, among others, includes TGFβ, Wnt–β-catenin, Hedgehog and YAP, and instructs hepatoblasts to enter the biliary lineage and mature into bile ducts[37]. Analysis of histological samples from patients with Alagille syndrome, studies in Notch-related animal models and genomic analysis of human HCC samples have shown that Notch signalling is involved not only in biliary ontogenesis but also after birth in biliary repair and carcinogenesis[34] (FIG. 1b–d). By regulating the homotypic and heterotypic crosstalk between several types of liver cell, Notch signalling regulates biliary repair, tubulogenesis[38,39] and the biliary phenotypic switch of transdifferentiating hepatocytes[40–42]. Furthermore, studies have shown that Notch is also involved in liver metabolism, vascular biology and immunity[31], but these functions are not discussed in this Review.

Role of Notch in cholangiopathies

Studies on liver explants from patients with Alagille syndrome that had progressive liver disease provided the first clue that reactivation of Notch signalling is a key factor in the reparative response to biliary damage[43]. Careful pathological analysis revealed that ductopenia in patients with Alagille syndrome is associated with an imbalance in the epithelial components of the hepatic reparative machinery. In contrast to individuals with biliary atresia, in which RDCs are prevalent, individuals with Alagille syndrome show a near complete absence of RDCs, in parallel with a marked accumulation of cells with an intermediate hepatocyte–biliary (IHBC) phenotype that lack expression of the Notch-dependent transcription factor HNF1β[43]. This finding might indicate that HPCs were forced towards a hepatocellular fate because of defective Notch signalling, or, conversely, that transdifferentiation of hepatocytes to cholangiocytes was blocked at the IHBC level. This study also found that defective Notch signalling reduces liver fibrogenesis. On the one hand, the reduced number of RDCs in individuals with Alagille syndrome is associated with scarce deposition of fibrotic tissue in the portal space, leading to much thinner septa than in biliary atresia. On the other hand, the increased IHBC compartment is accompanied by pericellular fibrosis within the hepatic lobule (a ‘chicken wire’ fibrosis pattern), a fibrotic lesion that is also observed in alcoholic and metabolic liver injury[43]. These findings are consistent with the slow clinical progression to cirrhosis observed in individuals with Alagille syndrome with respect to other conditions such as biliary atresia.

Further studies have clarified that, during liver repair, direct cell–cell interaction between Notch-expressing HPCs and JAG1-expressing portal myofibroblasts favours the conversion of HPCs to RDCs as a default mechanism[38,44]. However, factors such as Numb, an endogenous inhibitor of Notch that targets the NICD to the proteasome and has been shown to promote a progenitor cell fate, can antagonize Notch activation in HPCs, switch off Notch-dependent biliary specification and turn on Wnt–β-catenin activation and HPC differentiation towards a hepatocyte phenotype[38,45]. Notch signalling also regulates ductal branching during biliary repair. The generation of branching tubular structures requires the coordinated and integrated functions of both NOTCH1 and NOTCH2 (REF.[39]). If only NOTCH2 is defective, biliary specification of HPCs in response to biliary injury is preserved; however, the ability to rebuild biliary tubular structures is impaired and the liver cannot restore bile duct mass[39].

The clinical manifestation of Alagille syndrome is quite variable and it is not unusual to witness a reduction in disease severity over time[27]. This phenomenon has been also observed in mice with severe Notch defects, such as deficiency in *Rbpjk* and *Hnf6*, the latter encoding a transcription factor that promotes the expression of HNF1β and of SOX9, two essential factors for biliary development[46]. Work published in 2018 shows that, in these settings, functional bile ducts can be generated by transdifferentiation of hepatocytes into biliary cells and this transition can also occur in the absence of Notch as long as TGFβ signalling is intact[17]. This discovery not only adds an important piece to the puzzle of biliary repair but also offers hope to individuals that have poorly developed bile ducts owing to Notch mutations. In addition, mouse models with specific mutations that affect the ability of JAG1 to bind to NOTCH1 or NOTCH2 have multiple organ abnormalities typical of Alagille

syndrome[47–49]. The clinical manifestations of Alagille syndrome can also be influenced by modifier genes. Among them, thrombospondin 2 (THBS2) is a matricellular protein that can inhibit the JAG1–Notch interaction[50,51]; in addition, studies in mice have shown that *O*-glycosyltransferase 1 (also known as POGLUT1 or RUMI) is a post-translational negative regulator of JAG1 function[52], and that Fringe genes regulate Notch receptors after translation[53].

Notch overactivation

Whereas defective Notch function causes ductopenia during development and hampers biliary repair from an acquired injury, persistent Notch overactivation can result in liver epithelial cell dysplasia and malignant transformation[31,34,54]. Development of dysplastic nodules and subsequent hepatocellular carcinoma (HCC) was observed in mice bearing *Notch*-dependent activation of HPCs[55,56]. In a mouse model tracing the fate of hepatocytes, joint activation of Notch and AKT signalling promoted ductular metaplasia of normal hepatocytes that subsequently behave as precursor cells of rapidly progressing intrahepatic cholangiocarcinoma[40]. It is not currently known whether intrahepatic cholangiocarcinoma in humans derives from hepatocytes through Notch-mediated hepatocyte metaplasia, but increasing evidence supports the association of intrahepatic cholangiocarcinoma with several risk factors for HCC, including cirrhosis, HCV, HBV and nonalcoholic steatohepatitis[57].

Histological studies in PBC and PSC have shown that levels of several Notch ligands and receptors are upregulated in ductular cells near to neovessels[58,59]. Consistent with this observation, the mechanisms unveiled when studying the pathogenesis of Alagille syndrome have demonstrated that Notch signalling plays a major part in biliary repair mechanisms that promote biliary fibrosis, which is the primary factor in disease progression. Studies in animals undergoing experimental liver damage show that modulation of Notch signalling leads to reduced fibrosis deposition[39]. As Notch is a ‘druggable’ signalling pathway[60,61], there is the possibility for pharmacological modulation of Notch signalling, for example, in primary liver tumours.

Polycystic liver disease

Polycystic liver diseases (PLDs) are inherited disorders characterized by the development of multiple (>10) fluid-filled biliary cysts scattered throughout the parenchyma[62,63]. PLDs are typical examples of a group of genetically mediated biliary diseases known as cholangiociliopathies[64,65] that are caused by a lack or dysfunction of proteins located in the cilia. Biliary cells possess a single non-motile cilium that protrudes into the biliary lumen and performs mechanosensing, chemosensing and osmosensing functions[66–70]. Among biliary diseases associated with ciliary dysfunction are ductal plate malformations, such as Bardet–Biedl, Senior–Loken and Joubert syndromes[71]. In the past few years, dysfunction in ciliary proteins or changes in ciliary morphology have been also reported in cases of neonatal sclerosing cholangitis and biliary atresia[72–74]. These conditions are not further discussed in this Review as these observations await confirmation; here, we focus on PLDs.

In PLD, cystogenesis can arise exclusively in the liver (autosomal dominant PLD (ADPLD)) or in coexistence with renal cysts (autosomal dominant polycystic kidney disease

(ADPKD)), depending on the mutated gene[62,63,75,76] (TABLE 1). The symptoms of PLD are related to the growth of the cysts and the related hepatomegaly (and can include abdominal distension and pain, early satiety, abdominal discomfort and dyspnoea) and/or to cyst damage (for example, bleeding, infection or rupture)[62,63]. The diagnosis of PLD is usually confirmed by liver imaging. Current therapeutic options are only indicated for symptomatic patients and the benefits are short-term and modest. Treatments include surgical procedures (aspiration, sclerotherapy, cyst fenestration or segmental hepatic resection) for symptomatic cysts or chronic treatment with somatostatin analogues for patients with moderate–severe disease and impaired quality of life. Liver transplantation remains the only curative therapy[62,63].

Polycystins and ER-related genes

ADPKD, the most common inherited nephropathy (with a prevalence of 1 in 500–1,000), is caused by mutations in *PKD1* (80–85%)[77] or *PKD2* (10–15%)[78]. The liver is affected in ~85% of patients[62,65,79]. *PKD1* and *PKD2* encode the ciliary proteins polycystin-1 (PC1; a mechanoreceptor) and PC2 (a calcium channel that is also abundant in the endoplasmic reticulum (ER)), respectively. Together, PC1 and PC2 form a functional complex that regulates intracellular Ca^{2+} homeostasis[66]. ADPLD (the PLD variant that does not affect the kidney) is a rare disease (with a prevalence of ~1 in 100,000) triggered by germline mutations in numerous genes, including *PRKCSH*[80,81], *SEC63* (REF.[82]), *SEC61B*[83], *GANAB*[83,84], *ALG8* (REF.[83]) or *LRP5* (REF.[85]), with *PRKCSH* being the most frequently mutated gene (~15% of individuals)[62,76]. Nevertheless, mutations in these genes explain only ~50% of ADPLD cases[62,63,83]. With the exception of *LRP5*, which encodes a plasma membrane co-receptor involved in Wnt signalling, the other ADPLD-related genes encode proteins located at the ER that participate in protein biogenesis (transport, protein folding or glycosylation)[62,63,76,83].

Experimental evidence indicates that defects in PC1 biosynthesis are the rate-limiting determinant of cystogenesis in all forms of PLD[83,86]. The primary cilium is responsible for cholangiocyte cell polarization and the preservation of quiescence. Mutations in *PKD1* or in the aforementioned ER-related genes affect PC1 expression and/or maturation, and result in impaired ciliary structure and, consequently, cholangiocyte proliferation and cystogenesis. Additionally, PC1 regulates Wnt signalling, providing a potential link between *LRP5* mutations and PC1 (REF.[87]).

Liver cystogenesis

Hepatic cystogenesis originates from malformations of the ductal plate during embryogenesis[62,88] and/or from second-hit mutations in the wild-type allele of PLD-related genes in intrahepatic cholangiocytes (loss of heterozygosity)[89–92]. Cystogenesis is characterized by several features[75], including increased cystic cholangiocyte proliferation[93], secretion[94], matrix metalloproteinase activity[95], autophagy[96], ciliary and centrosomal abnormalities[97,98], and alterations in the microRNA expression pattern[99]. An alternative model for initial cyst formation has arisen from work showing that biliary cysts might develop through a proliferation-independent process that involves recruitment and biliary

differentiation of nearby hepatoblasts[100,101]. However, further postnatal growth of cysts requires cell proliferation.

Reduced levels or function of PC1 and/or PC2 directly alters cellular Ca^{2+} signalling and indirectly modifies Ca^{2+}-regulated cAMP levels[102]. Cytoplasmic Ca^{2+} homeostasis is maintained by a balance between extracellular and intracellular Ca^{2+} levels. When ER Ca^{2+} stores are decreased, a mechanism called store-operated Ca^{2+} entry (SOCE) uses extracellular Ca^{2+} to replenish Ca^{2+} stores. In PC2-defective cholangiocytes, SOCE is inhibited and cells respond to acute reductions in Ca^{2+} concentration with increased cAMP production by adenylyl cyclase 5 (AC5), cAMP-mediated protein kinase A (PKA) phosphorylation, and a PKA-dependent increase in extracellular-signal-regulated kinase 1 (ERK1) and ERK2 phosphorylation[103]. Chemical and gene expression inhibition of stromal interacting molecule 1 (*STIM1*) or silencing *AC5* gene expression reduced cAMP production in PC2-defective cholangiocytes[104], suggesting a role for STIM1 and AC5 in ADPKD (FIG. 2). STIM1 is the molecular sensor that couples a reduction in intraluminal ER Ca^{2+} concentrations with the activation of Ca^{2+} entry from the plasma membrane[105,106]. STIM1 translocation from the ER to the plasma membrane activates store-operated Ca^{2+} channels belonging to the ORAI and transient receptor protein channel (TRPC) families[105,106]. Thus, in cholangiocytes, PC2 functions as a necessary component of SOCE and an inhibitor of AC5 function. In the absence of PC2, AC5 is de-repressed and more cAMP is produced[103]. In this context, cAMP drives PKA-dependent cell proliferation and hypersecretion[93,94,103,107]. Somatostatin analogues are approved by the FDA for the treatment of individuals with PLD owing to their downregulation of cAMP levels in cystic cholangiocytes[62,63,108,109]. Moreover, new therapeutic strategies aimed at increasing intracellular Ca^{2+} levels in cystic cholangiocytes are under investigation (for instance, ursodeoxycholic acid (UDCA) and transient receptor potential cation channel subfamily V (TRPV4) agonists)[70,110,111]. The role of PC1 in cholangiocyte ER Ca^{2+} homeostasis has not yet been investigated, but, as interaction with PC2 is necessary for the functions of PC1, it is likely that these changes will also be present in PC1-defective cells.

VEGF and angiogenic signalling.—The pleiotropic growth factor VEGFA and its cognate receptors VEGFR1 and VEGFR2 are overexpressed in cystic cholangiocytes from patients with ADPKD[15]. VEGF has autocrine and paracrine proliferative effects on cystic cholangiocytes and vascular endothelial cells, leading to cyst expansion and pericystic vascularization[15,112]. Notably, cAMP is responsible for VEGF production and VEGFR2-mediated cyst growth in PC2-deficient mouse cholangiocytes via an mechanistic target of rapamycin (mTOR)–ERK1/ERK2–hypoxia-inducible factor 1α (HIF1α) signalling pathway; the pharmacological inhibition of VEGFR2 halts hepatic cystogenesis in these mice[107,113,114]. Similarly, insulin-like growth factor 1, which is also produced in excess by the cystic epithelium, stimulates VEGF secretion via the mTOR pathway[115]. Other angiogenic factors such as Ang1 have a synergic effect with VEGF in enhancing the proliferation of cystic cholangiocytes[15] (FIG. 2) and are increased in PLD.

Post-translational regulation of PC2 has been linked with VEGF secretion and a cholangiocyte response to biliary damage[116]. PC2 is downregulated in the liver from different mouse models of biliary damage (including *Mdr2*$^{-/-}$ knockout, bile duct ligation

and the 3,5-dieth-oxycarbonyl-1, 4-dihydrocollidine (DDC) diet), and by the activity of pro-inflammatory cytokines, nitric oxide donors and ER stressors. Downregulation of PC2 in these contexts triggers ERK–HIF1α-dependent VEGF secretion, and the magnitude of PC2 downregulation promotes ductular reaction or cyst formation[117].

In chronic cholangiopathies, activated cholangiocytes acquire the ability to secrete VEGF and to respond to VEGF, resulting in autocrine stimulation of proliferation or in paracrine stimulation of peribiliary neoangiogenesis[112,114]. This process is important to support the expansion of the bile duct mass during biliary repair. However, VEGF can also stimulate recruitment or migration of fibrogenic stellate cells[118], resulting in pathological repair with excess fibrosis[119]. The mechanisms unveiled while studying PLDs will result in an improved ability to modulate specific steps in biliary angiogenic signalling, acting on VEGFR2, PKA or mTOR.

CHF and Caroli disease

Fibropolycystic liver disease is a collective term for a group of different diseases characterized by bile duct dysmorphogenesis and fibrosis[120,121], by ductal plate malformation and that result from mutations in the *PKHD1* gene, which encodes the protein fibrocystin (FPC; also known as polyductin). FPC is localized in the primary cilia, basal bodies and centromeres of several epithelial ductal structures, such as pancreatic and renal ducts, salivary glands and biliary cells[122,123]. The distinctive feature of fibropolycystic liver diseases is the presence of cyst-like dilatations of the biliary tree surrounded by a macrophage-dominated immune infiltrate and dense fibrosis[124]. Mutation of *PKHD1* can generate three different diseases: autosomal recessive polycystic kidney disease (ARPKD), congenital hepatic fibrosis (CHF) and Caroli disease[125]. Despite sharing some common pathological traits, such as biliary cyst development, these diseases differ in several aspects (for example, age of onset). CHF is a rare disease (diagnosed in 1 in 20,000 live births) characterized by cystic malformation of the biliary tree and a prominent peribiliary fibrotic response[113]. ARPKD is characterized by a very early onset (at late pregnancy or at birth), massive kidney involvement, severe outcomes and eventually death due to kidney and liver enlargement and lung hypoplasia[126]. Whereas ARPKD and CHF usually affect newborn babies and children, individuals with Caroli disease are usually young adults (around the fourth decade)[127]. Patients with Caroli disease have a prominent liver involvement characterized by fibrosis deposition and recurrent cholangitis, which can lead to development of cholangiocarcinoma[127]. The precise function of FPC is still largely unknown but it is thought to be involved in various cellular functions, including regulation of proliferation, secretion, differentiation, tubulogenesis, planar cell polarity and cell–matrix interactions[93,95,123,128,129]. Membrane-bound FPC undergoes Notch-like proteolytic processing, with translocation of the C terminus fragment to the nucleus where it can affect transcriptional regulation[130]; however, the signals stimulating this cleavage are not clear. Pharmacological treatments to slow disease progression are not available and therapy is aimed at controlling the consequences of portal hypertension, which relies on hepatic resection or liver transplantation in the most advanced cases[131,132].

Fibropolycystic diseases are characterized by ductal dysgenesis with generation of biliary microhamartomas (irregularly shaped bile duct dilatation originating from ductal plate malformations and embedded in fibrous stroma) and segmental dilations that display a fetal-like phenotype, ductal plate remnants that remain in connection with the biliary system, and progressive accumulation of peribiliary fibrosis leading to portal hypertension and hepatic failure[133]. Dysgenic bile ducts, which originate from duct plate malformations with increased recruitment of nearby hepatoblasts[100], progressively enlarge after birth in association with dense and slow-growing worsening fibrosis and the accumulation, over the course of the disease, of a rich portal inflammatory infiltrate recruited within the fibrotic area. However, overt necro-inflammatory damage to the biliary epithelium is absent[134]. This pathogenetic sequence is different from that observed in PBC, in which the biliary epithelium is damaged by inflammation due to immune activation, and cholangiocyte necrosis or apoptosis is the cause of portal scarring. Rather, the context is reminiscent of PSC, in which stricture and dilatation of the biliary tree become associated with extensive peribiliary fibrosis.

β-Catenin signalling

Cystic structures are characterized by a high mitotic index[98] and by perturbations in several signalling mechanisms. In particular, in orthologous rodent models of ARPKD, cellular levels of cAMP are increased[135], whereas intracellular Ca^{2+} levels are downregulated[93]. Data published in the past few years show that, in mice with mutations in *Pkhd1* (*Pkhd1*$^{del4/del4}$, in which expression of *Pkhd1* is at ~30% of the wild-type level), cAMP, in addition to its known effects on biliary proliferation and secretion, also modulates the hepatic fibro-inflammatory response by stimulating β-catenin nuclear import[136,137]. β-Catenin has a double role in cellular physiology, acting as a structural protein in adherence junctions and as a transcriptional regulator as part of the Wnt–β-catenin pathway[138]. In the absence of Wnt signals, this protein is retained in the cytoplasm in an inactive form as part of the β-catenin destruction complex. Upon Wnt stimulation, the destruction complex is inhibited and β-catenin is able to translocate to the nucleus, where it binds to transcription factors such as TCF and LEF and regulates target gene expression[139]. Notably, in the *Pkhd1*$^{del4/del4}$ mouse, higher concentrations of cAMP and increased PKA activity induces the phosphorylation of β-catenin at Ser675, which stabilizes the protein, prevents its catabolism and enables its nuclear transfer where it acts as a transcription factor[137] (FIG. 3). This mechanism is responsible for the increased secretion into the portal microenvironment of several chemokines (including CXC-chemokine ligand 1 (CXCL1), CXCL10 and CXCL12) that are involved in the development of the fibroinflammatory response typical of CHF[124,136]. These chemokines are responsible for the pericystic recruitment of inflammatory cells, mostly M1 macrophages and then, at later stages of the disease, M2 macrophages[124,136]. Macrophages secrete TGFβ and TNF, which stimulate cystic cholangiocytes to express αVβ6 integrin, a protein of fundamental importance for fibrogenesis owing to its activation of the latent form of TGFβ[124]. Furthermore, CXCL10 secretion by FPC-defective cholangiocytes is mediated not only by β-catenin nuclearization but also by the secretion of IL-1β through the activation of the NLRP3 inflammasome complex[136] (FIG. 3). Active IL-1β secretion in epithelial cells leads to a self-perpetuating feedforward loop that sustains an inflammatory response. This vicious cycle of inflammation

is the basis of autoinflammatory conditions, either caused by abnormal production of IL-1β owing to *NLRP3* mutation or deficient antagonism of the IL-1β receptor. IL-1β is the major driver of these diseases and has the potential to cause both systemic and organ-specific immunopathology[140,141].

Relevance to acquired cholangiopathies

The pathophysiological mechanisms involved in CHF and Caroli disease are relevant for acquired cholangiopathies as they demonstrate the roles of β-catenin in biliary inflammation and cholangiocyte dysfunction in biliary fibrosis. Furthermore, the identification of these signalling pathways has clarified the sequence of events leading from cholangiocyte dysfunction to biliary fibrosis, through increased intracellular cAMP levels, β-catenin-mediated secretion of pro-inflammatory cytokines and chemokines, early recruitment of macrophages, activation of latent TGFβ and accumulation of myofibroblasts. These pathobiological mechanisms are potential therapeutic targets for the treatment of cholangiopathies characterized by the accumulation of peribiliary fibrosis accompanied by inflammation and possibly by cholangiocyte hyperplasia. Several studies have examined the use of pasireotide and octreotide, two somatostatin analogues that are able to bind to the somatostatin receptors and block cAMP signalling; both compounds were able to slow hepatic cyst growth and, to a lesser extent, the deposition of peribiliary fibrosis in PCK rats, a rodent model orthologous to human ARPKD carrying mutations in the *Pkhd1* gene[108,135]. To inhibit cell proliferation directly, $Cdc25A^{+/-}$ mice (heterozygous for a defective version of the gene encoding M-phase inducer phosphatase 1, which is responsible for the regulation of all cell cycle phases) were cross-bred with $Pkhd1^{del2/del2}$ mice. The offspring of these animals had reduced cystogenesis and pericystic fibrosis owing to the effect on CDC25A that partially reverted the pathogenic effect of FPC depletion[142]. Treatment of PCK rats with agonists of peroxisome proliferator-activated receptor-γ (PPARγ), such as pioglitazione or telmisartan, inhibited the activation of the ERK1–ERK2 and mTOR–S6 kinase signalling pathways and reduced cyst area, cholangiocyte proliferation and the extent of pericystic fibrosis[143,144]. Another promising approach to treat ARPKD and CHF is to target macrophages. Administration of clodronate, a bisphosphonate able to inhibit monocyte–macrophage transdifferentiation, reduced cyst growth and the accumulation of pericystic inflammatory infiltrate and fibrosis in $Pkhd1^{del4/del4}$ mice[124]. Using the same mouse model, inhibition of CXC-chemokine receptor 3 (CXCR3; the receptor for CXCL10, a chemokine involved in macrophage recruitment) showed similar effects to clodronate, further indicating that the presence of a macrophage infiltrate is pivotal for the pathogenesis of fibropolycystic diseases[136]. Similar approaches aiming to inhibit the recruitment of inflammatory cells were applied also to chronic liver diseases, such as PBC. Starting from the observation that patients with PBC have increased plasma levels of CXCL10 (REF.[145]), a clinical trial using NI-0801, a monoclonal anti-CXCL10 antibody, was proposed. Unfortunately, this clinical trial failed to reach the main endpoint of the trial: a reduction in the levels of liver function tests[146]. Using a rodent model of PSC ($Mdr2^{-/-}$ mice), two groups demonstrated that the use of cenicriviroc, an inhibitor of CCR2 and CCR5, is able to reduce the peribiliary accumulation of T cells and macrophages with a concomitant reduction in peribiliary fibrosis and normalization of liver function tests[147,148]; on this basis, a clinical trial involving human patients with PSC is ongoing.

Cystic fibrosis-related cholangiopathy

Cystic fibrosis is a common monogenic disease (with a prevalence of 1 in 2,500 and 1 in 3,500 in Europe and the United States, respectively) that predominantly affects white individuals[149,150]. The condition is an autosomal recessive disease caused by mutations affecting the function of cystic fibrosis transmembrane conductance regulator (CFTR)[151], a chloride channel expressed by secretory epithelia including the biliary epithelium in the liver[152,153] (FIG. 4a). Cystic fibrosis is a multiple organ disease, with the lungs being the most severely affected followed by the pancreas, intestines and the liver[150]. Owing to the improvement of supportive treatments for the lung disease, the life expectancy in patients with cystic fibrosis has greatly increased and the number of adult individuals with cystic fibrosis-related cholangiopathy (also known as cystic fibrosis liver disease (CFLD)) is rising[154,155]. CFLD affects 5–10% of patients with cystic fibrosis and has a heterogeneous spectrum of manifestations that ranges from abnormal biochemical liver tests (elevated levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), γ-glutamyl transferase (γGT) or alkaline phosphatase (ALP)) to biliary architecture changes, cholestasis and, finally, to clinically severe sclerosing cholangitis, focal biliary cirrhosis and multi-lobular biliary cirrhosis complicated by portal hypertension[156,157]. Furthermore, the development of cirrhosis and portal hypertension aggravates the respiratory function of these individuals, and CFLD is the third most common cause of mortality after cardiorespiratory and transplant complications[157]. The variability of the liver phenotype and the fact that these individuals are often asymptomatic makes the early diagnosis of CFLD challenging[158].

UDCA is the current treatment of choice for patients with CFLD (10–20 mg per kg body weight per day) and this drug has been shown to reduce serum levels of ALT, AST, γGT and ALP[159,160]. However, doses of UDCA higher than commonly used (25–30 mg per kg body weight per day) have raised safety concerns in patients with PSC; furthermore, the beneficial effects of UDCA on the progression of CFLD has not been proven by long-term randomized clinical studies[161]. Small-molecule treatments aimed at correcting the basal defect of CFTR have been approved in the past 7 years for the treatment of specific cystic fibrosis genotypes (ivacaftor, lumacaftor–ivacaftor and ivacaftor–tezacaftor) and several more therapies are in development. These molecules can be categorized as compounds that are able to rescue the specific mutation dysfunction (correctors, such as lumacaftor and tezacaftor) or as compounds that are able to restore CFTR channel activity (potentiators, such as ivacaftor). These treatments can be used in combination, depending on the functional and/or genetic defect[151,162,163]. However, most of the clinical outcome data about their efficacy are restricted to the pulmonary function.

The pathogenesis of CFLD is still unclear, and further studies are needed to design specific interventions; however, progress has been made in the past few years. Furthermore, novel stem cell technologies, such as induced pluripotent stem cells and 3D culture of epithelial organoids, have increased the availability of patient-derived cells to model the disease, and these advances will hopefully accelerate the translation of novel findings into treatment[164–166]. CFTR plays an essential role in the regulation of bile secretion by the biliary tree[1] (FIG. 4a). These fundamental mechanisms are impaired in cystic fibrosis, and bile flow and the secretion of chloride and bicarbonate are substantially reduced. Biliary

secretion is defective in all individuals with cystic fibrosis and, although different mutations might have different effects on CFTR function, there is no clear correlation between the mutation genotype and the progression of liver disease, suggesting that other comorbidities or modifier genes might contribute[155,167].

Role of CFTR in epithelial innate immunity

CFTR has a role in multiple cell functions[168,169]; for example, CFTR can act as a hub protein that forms macromolecular complexes with several other proteins at the apical membrane of epithelia. CFTR has been reported to interact directly or indirectly with proteins that regulate its channel activity (such as AKAP and PKA), integrate signalling pathways (such as adenosine 2b receptors or the β_2-adrenergic receptor) and coordinate other transport activities (for example, amiloride-sensitive sodium channel (ENaC), ROMK, AE2 and AQP1). Usually, PDZ-domain-containing proteins (such as NHERF1–4, CAL and CAP70) mediate these interactions by connecting CFTR to other proteins in the complex and to the sub-membrane actin cytoskeleton that stabilizes the complex[168,169].

One such example is the discovery of the interaction between CFTR and Src family tyrosine kinases (SFKs) in cholangiocytes[170] (FIG. 4a). This physical interaction is important for the regulation of innate immune responses and to maintain endotoxin tolerance in cholangiocytes. CFTR, in association with NHERF1, binds CBP and CSK that retain Src kinase in an inactive state[170]. When this complex is not assembled, unbound Src self-activates and phosphorylates Toll-like receptor 4 (TLR4), the pattern recognition receptor for lipopolysaccharides (LPS)[170,171]. Phosphorylation of TLR4 is important for the activation of its signalling pathway and the recruitment of downstream signal-competent adaptor proteins (for example, MyD88) and kinases (such as IRAK4 and IRAK1) in response to LPS binding[172,173]. However, in the normal biliary epithelium, TLR4 phosphorylation is markedly and constitutively downregulated, yielding endotoxin tolerance[140]. This tolerance is important to avoid inappropriate inflammatory responses, given that the biliary epithelium expresses a full set of TLRs (TLR2, TLR3, TLR4 and TLR5) and is continuously exposed to microbial components coming from the gut[174,175].

This regulatory mechanism has important implications for the pathogenesis of CFLD. In vitro data show that cholangiocytes isolated from *Cftr*$^{-/-}$ mice are hyperresponsive to gut-derived endotoxins, and, when exposed to LPS, these cells elicit an abnormal inflammatory response with the production of several pro-inflammatory cytokines[176]. In cultured mouse *Cftr*$^{-/-}$ cholangiocytes, Src tyrosine kinase activity and TLR4 phosphorylation are substantially increased (FIG. 4b). Pharmacological inhibition of SFKs (with PP2 for example) or targeting inflammation using PPARγ agonists (pioglitazone and rosiglitazone) decreased the inflammatory phenotype in *Cftr*$^{-/-}$ cholangiocytes both in vivo and in vitro[170,177]. Similar findings were confirmed in human cholangiocytes derived from an individual carrying the most common mutation in cystic fibrosis (ΔF508) using induced pluripotent stem cell technology[164]. Remarkably, the association of the SFK inhibitor with CFTR modulators (lumacaftor and ivacaftor) that correct the ΔF508 misfolding mutation further ameliorated the secretory defect in human cystic fibrosis cholangiocytes. Taken together, these results support a role for CFTR in controlling innate immunity responses in

the biliary epithelium, challenge the current view of CFLD as a classic ‘channelopathy’ and suggest potential synergistic targets for therapy (TABLE 2).

Microbiome and gut–biliary axis in CFLD

The gut and liver share important anatomical and functional connections and their crosstalk has been implicated in the pathogenesis of several liver diseases[178,179]. Data published in the past few years indicate that CFLD exemplifies this axis. The *CFTR* genetic defect is present in both the biliary epithelium and the intestine, and increased intestinal permeability and dysbiosis have been described in mouse models and in patients with cystic fibrosis[180–182]. Early studies in mouse models of cystic fibrosis showed that induction of colitis with dextran sodium sulfate, which increases intestinal permeability and the portal release of bacterial products, causes biliary damage and inflammation in $Cftr^{-/-}$ mice but not in wild-type littermates. Interestingly, the biliary damage is attenuated by treatment with broad-spectrum antibiotics, supporting a contribution of the intestinal microbiota in the establishment of the disease[176]. More studies are needed to clarify the pathophysiological role of the gut microbiota in the development of CFLD. The sequence of events discovered in CFLD is relevant for acquired cholangiopathies, such as PSC. In fact, they demonstrate that changes in microbiota and intestinal permeability can cause a cholangiopathy in the presence of an altered regulation of epithelial innate immunity. The involvement of the gut microbiota in the pathogenesis of PSC is strongly supported by the association of PSC with IBD and ulcerative colitis (~60–80% of individuals with PSC)[183], by genome-wide association study data that have identified genetic variants in PSC that are associated with ulcerative colitis (such as those encoding GPR35)[184] or influence biliary bacterial composition (such as those encoding FUT2)[185], and by the presence of bacterial products in the liver explants of patients with PSC[186]. Conversely, in vitro data have shown that biliary cells isolated from patients with PSC have aberrant TLR–nuclear factor-κB (NF-κB) immune responses to intestinal endotoxins with increased production of pro-inflammatory cytokines, such as IL-8 and TNF[187].

Conclusions

Studies in inherited and congenital cholangiopathies have generated important new information that has not yet been discussed in a unified view. Importantly, investigations into these conditions have yielded insights into cholangiocyte pathobiology that are relevant also to acquired cholangiopathies. The central mechanism of cholangiocyte pathobiology[2,3,188] is persistent inflammation with ongoing biliary damage and repair, resulting in progressive fibrosis, changes in bile production and evolution to biliary cirrhosis. Recent studies demonstrated that these processes require the reactivation of morphogenetic mechanisms typical of biliary development, such as Notch signalling (for Alagille syndrome), and the Hedgehog pathway and Wnt–β-catenin signalling (for CHF). Defective Notch signalling causes congenital ductopenia, but also impairs liver repair after damage when compensatory biliary hyperplasia in response to cholestasis does not occur, leading to extravasation of bile in the interstitial tissue (bile lakes). Conversely, the blunted ductular reaction dampens the fibrotic response. Unrestrained Notch activity might be involved in liver carcinogenesis and, specifically, in the formation of intrahepatic cholangiocarcinomas through biliary

transdifferentiation of hepatocytes. We have also learned that there is considerable redundancy in the mechanisms regulating the formation of ductular cholangiocytes, as other pathways may, in the long run, vicariate for Notch effects by stimulating hepatocyte transdifferentiation into biliary cells.

Studies in PLDs have revealed a fundamental role of the cilium, an organelle that was previously neglected. The cilium hosts many proteins that are able to sense physical and chemical properties of the biliary milieu, and its dysfunction is associated with increased cholangiocyte proliferation and profound changes in cholangiocyte intracellular signalling and in the ability of cholangiocytes to sense biliary composition and flow. Using PC2-defective mice, we have unveiled novel mechanisms in which loss of Ca^{2+} homeostasis in the ER might increase cAMP levels via AC5, when PC2 expression is absent or heavily reduced. This novel mechanism, ultimately leading to increased cholangiocyte proliferation, is also activated when PC2 is downregulated by inflammatory factors, as in acquired cholangiopathies.

By studying PLD, we have understood the role played by VEGF and VEGFR2 in regulating autocrine biliary proliferation and paracrine peribiliary vascularization. Angiogenic factors also play a fundamental role in biliary repair by increasing cholangiocyte proliferation and provide a clue for neoangiogenesis. Future studies will clarify the role and whether this mechanism can be targeted to modify cholangiocyte repair.

The role of the biliary epithelium in innate immunity has been the focus of intensive investigation in recent years, driven by interest in autoimmune and inflammatory cholangiopathies. In spite of these research efforts, the mechanism of these cholangiopathies remains elusive. It is important to consider that cholangiocytes, in addition to being the target of immune cell aggression and presenting antigens to immune cells, might also be the originators of an inflammatory and immune reaction. This concept is highlighted by biliary diseases caused by a genetically determined defect in FPC (CHF and Caroli disease) or in CFTR. It is worth mentioning that loss of homeostasis in epithelial cells, as a consequence of a mutation or exposure to damaging agents, generates a sequence of signals directed towards the re-establishment of new homeostatic set points. In this scenario, epithelial cells secrete numerous molecules and factors that are able to instruct immune cells or to generate an inflammatory reaction (the epi-immunome). The studies discussed in this Review have also led to the identification of several possible actionable targets and experimental treatments, as highlighted in TABLE 2. We expect that future research into the pathobiology of these fascinating diseases will be further rewarding and will also reveal important insights on the function of the healthy biliary epithelium.

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Key points

- Reactivation of morphogen signalling (such as Notch, Wnt–β-catenin and Hedgehog) takes place during biliary repair and orchestrates the balance between biliary remodelling and fibrogenesis and transdifferentiation and carcinogenesis.
- Polycystins control fundamental Ca^{2+}–cAMP-dependent cell signalling processes in cholangiocytes and, when defective, these proteins enhance cell proliferation and activate angiogenic signalling that leads to cystogenesis.
- Polycystin-2 can be modulated in response to biliary inflammation and mediates vascular endothelial growth factor secretion and cholangiocyte proliferation in acquired cholangiopathies.
- Genetic defects in fibrocystin are associated with altered β-catenin signalling, which generates an auto-inflammatory response with secretion of chemokines that are able to attract macrophages, resulting in biliary fibrogenesis.
- Cystic fibrosis transmembrane conductance regulator (CFTR) regulates cholangiocyte innate immunity and maintains Toll-like receptor tolerance; loss of CFTR predisposes the biliary epithelium to inflammation and damage in response to gut-derived microbial components.
- Pathological mechanisms identified in genetic cholangiopathies can be applied to acquired cholangiopathies and might represent potential targets for the next generation of treatments.

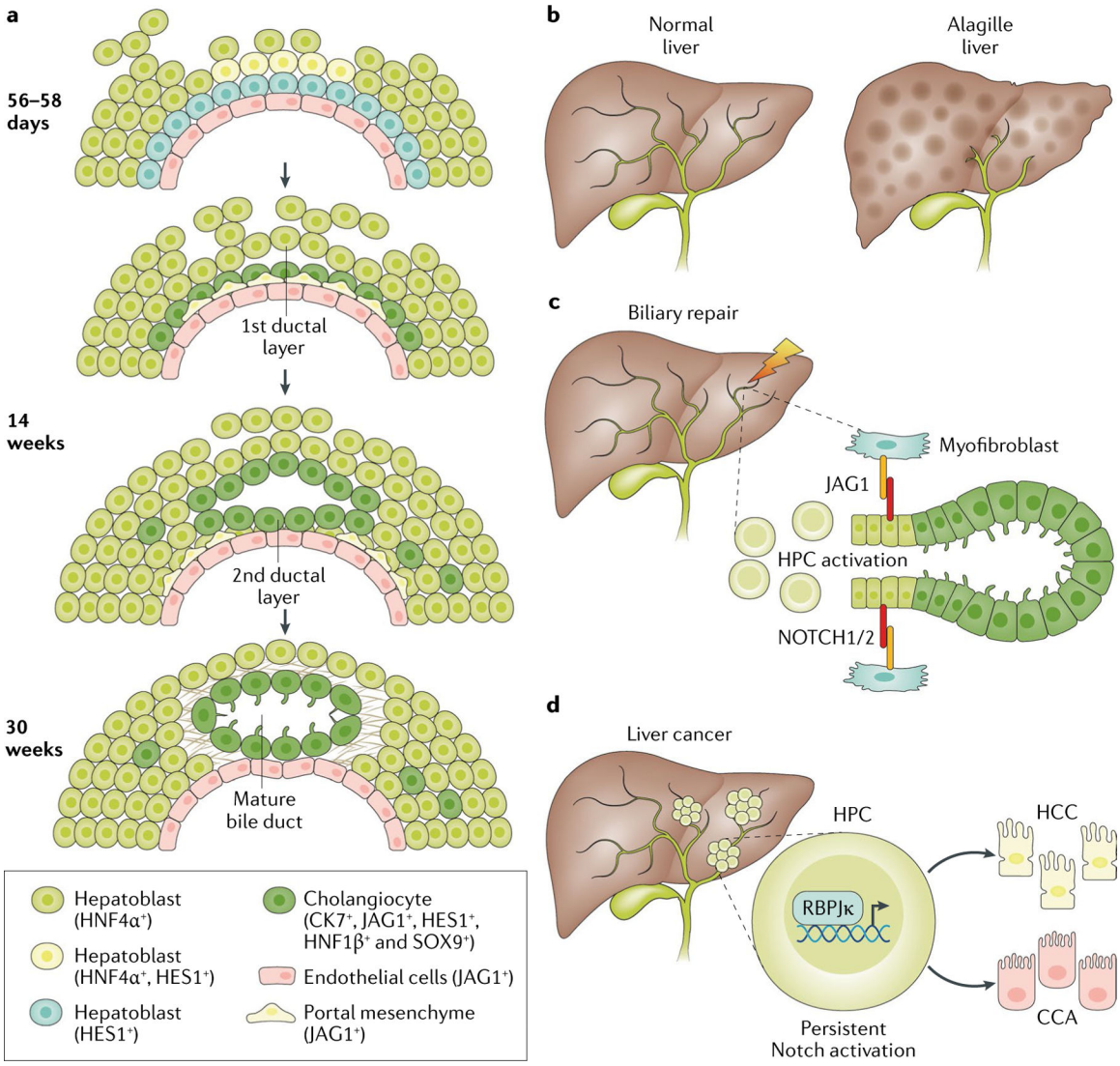

Fig. 1 |. Notch signalling in biliary development and disease.

a | Between 56 and 58 days to 14 weeks gestational age, Notch signalling in the developing liver becomes activated in hepatoblasts by interaction with Jagged-1 (JAG1)-expressing mesenchymal cells. Activation of Notch is involved in the biliary specification of the hepatoblasts in contact with the portal vein mesenchyme. This process generates ductal biliary structures that become mature at ~30 weeks of gestation. **b** | When Notch signalling is disrupted, as in Alagille syndrome, small branches of the biliary tree do not develop, causing ductopenia, jaundice and pruritus. In some cases, the condition improves with time, whereas in 20–50% of cases, it can progress to end-stage liver disease. **c** | Notch signalling is also involved in biliary morphogenesis during the repair process in the context of chronic biliary damage. In this setting, Notch has a dual function that includes the activation of hepatic progenitor cells (HPCs) and ductular cells followed by the formation of the tubular structures and crosstalk with mesenchymal cells. **d** | Persistent overactivation of Notch signalling in HPCs, leading to downstream RBPJκ-dependent transcriptional activity,

favours malignant transformation in hepatocellular carcinoma (HCC) or cholangiocarcinoma (CCA).

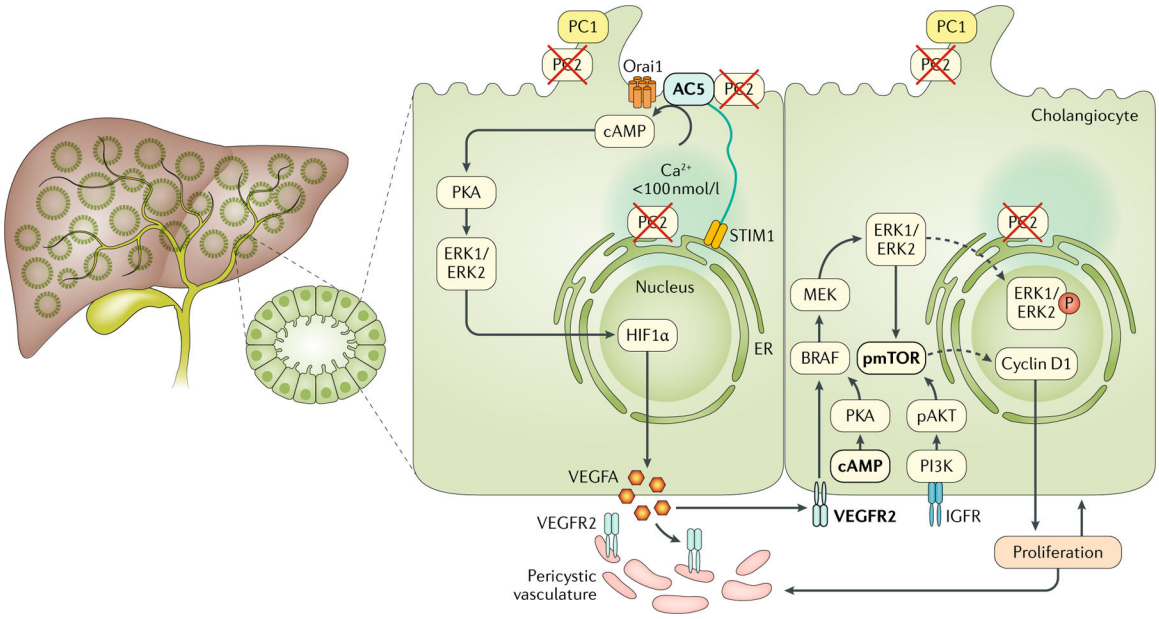

Fig. 2 |. Signalling mechanisms involved in cyst growth in ADPKD.

Autosomal dominant polycystic kidney disease (ADPKD) is associated with mutations in one of two genes, *PKD1* or *PKD2*, which encode polycystin-1 (PC1) and PC2, respectively. ADPKD is characterized by the presence of multiple cysts in the liver parenchyma that progressively dilate and grow. PC2-defective cholangiocytes are characterized by inappropriate production of cAMP and increased protein kinase A (PKA)-dependent activation of extracellular-signal-regulated kinase 1 (ERK1)/ERK2 and subsequently mechanistic target of rapamycin (mTOR), and hypoxia-inducible factor 1α (HIF1α)-increased production of cAMP is caused by changes in intracellular Ca^{2+} homeostasis. In PC2-defective cholangiocytes, store-operated Ca^{2+} entry is inhibited and cells respond to an acute reduction in endoplasmic reticulum (ER) Ca^{2+} levels with stromal interacting molecule 1 (STIM1)-dependent and adenylyl cyclase 5 (AC5)-dependent stimulation of cAMP production, which drives PKA-dependent activation of ERK1/ERK2. Defective PC2 also inhibits the interaction between STIM1 and Orai channels and maximizes the functional coupling of STIM1 to AC5, resulting in increased production of cAMP. PC2-defective cells respond to conditions that decrease ER Ca^{2+} levels and trigger oligomerization and membrane translocation of STIM1, with an overproduction of cAMP. In turn, cAMP activates the PKA–Ras–Raf–ERK pathway and stimulates vascular endothelial growth factor (VEGF) production through an mTOR–HIF1α-mediated mechanism. VEGF produced by cystic cholangiocytes increases perivascular microvascular density and cholangiocyte proliferation through binding with VEGF receptor 2 (VEGFR2). Stimulation of mTOR through AKT, for example, by insulin-like growth factor receptor (IGFR) ligand binding, can also activate ERK1/ERK2. Signalling molecules that are druggable are highlighted in bold and detailed in TABLE 2. PI3K, phosphoinositide 3-kinase; pmTOR, phosphorylated mTOR; pAKT, phosphorylated AKT.

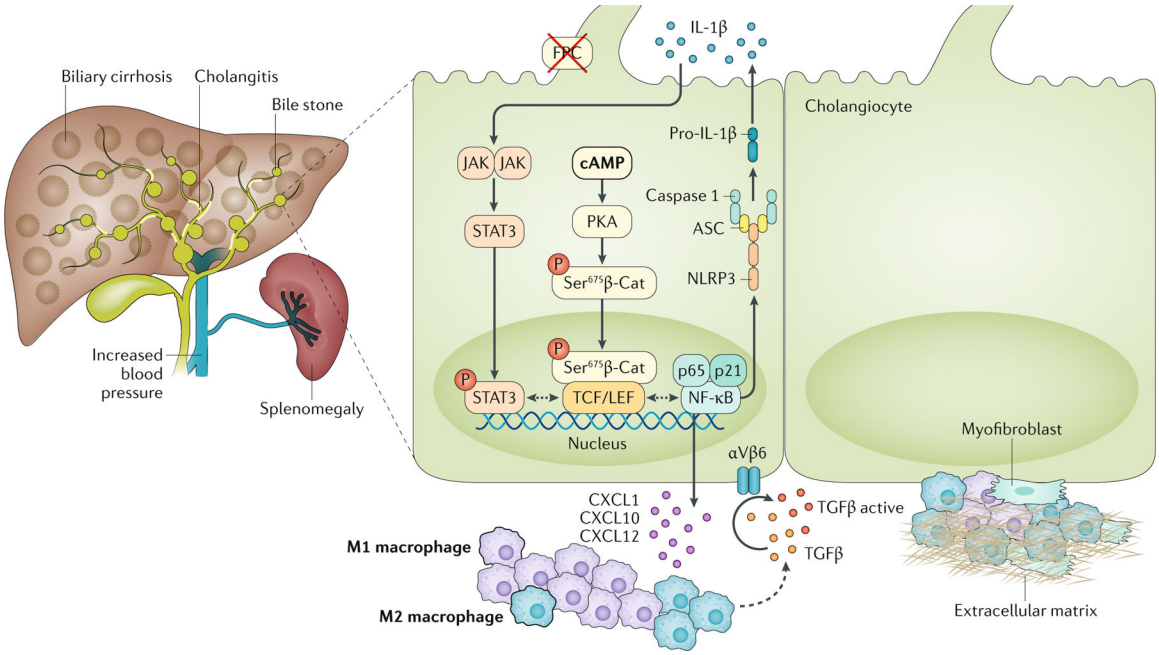

Fig. 3 |. Novel mechanisms of biliary fibrosis in ARPKD.

Liver disease in autosomal recessive polycystic kidney disease (ARPKD), which is caused by mutations in the *PKHD1* gene (encoding fibrocystin (FPC)), is characterized by cystic dysgenesis of the intrahepatic bile ducts that retain an immature phenotype reminiscent of the embryonic biliary structures (ductal plate malformations). The disease is associated with progressive portal fibrosis, leading to portal hypertension and related complications. In FPC-defective cholangiocytes, increased levels of cAMP activate protein kinase A (PKA)-dependent phosphorylation of β-catenin (β-Cat) at Ser675 that leads to the nuclear translocation of pSer^{675}β-catenin and transcriptional activation. This mechanism mediates the secretion of CXC-chemokine ligand 1 (CXCL1), CXCL10 and CXCL12 that recruit inflammatory cells, mostly M1 and then M2 macrophages, around the cystic epithelium. Macrophages secrete transforming growth factor-β (TGFβ) and TNF and stimulate the expression of αVβ6 integrins on cystic cholangiocytes that in turn activate latent TGFβ. CXCL10 secretion is further increased by the production of IL-1β through the activation of the Janus kinase (JAK)–signal transducer and activator of transcription 3 (STAT3) pathway. IL-1β secretion is mediated by an activated inflammasome. Signalling molecules that are druggable are highlighted in bold and detailed in TABLE 2. ASC, apoptosis-associated speck-like protein containing a CARD; NF-κB, nuclear factor-κB; NLRP3, NOD-, LRR- and pyrin domain-containing 3.

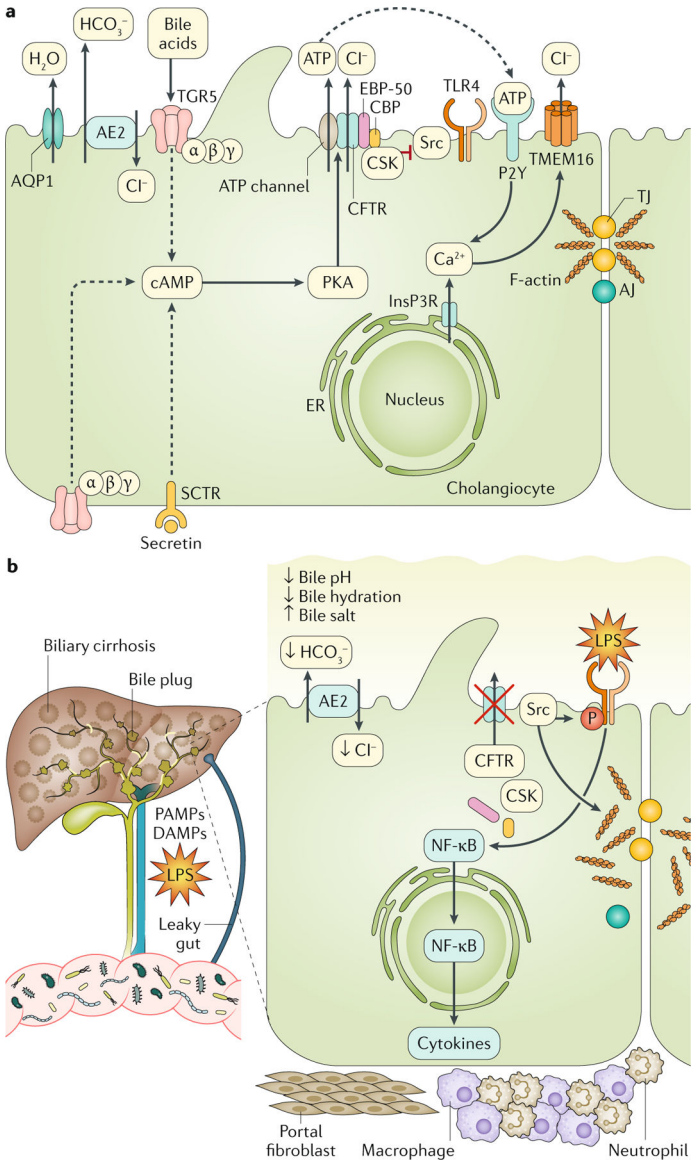

Fig. 4 |. CFTR function in cholangiocytes.

a | Cystic fibrosis transmembrane conductance regulator (CFTR) is located on the apical membrane of cholangiocytes where it has a major role in modifying bile properties (fluidity and pH). Bicarbonate (HCO_3^-) secretion into bile is necessary to sustain bile flow, to enable clearance of xenobiotics and to accomplish digestive needs (digestion and absorption of fats) within the intestine. Secretin is the main hormone that controls the secretory functions of the biliary epithelium. Secretin interacts with the G protein-coupled secretin receptor (SCTR) expressed on the basolateral membrane of cholangiocytes and triggers the production of cAMP, which activates protein kinase A (PKA). In turn, PKA phosphorylates the R domain of CFTR and opens the chloride conductivity channel. Apical chloride secretion is mediated by bicarbonate exchange through the anion exchanger 2 (AE2), generating an electrolyte–osmotic gradient that favours the passive movement of water through aquaporins (AQPs). These mechanisms are responsible for the normal hydration and alkalization of bile. CFTR-

dependent biliary secretion is also triggered in response to TGR5 receptor–PKA signalling when TGR5 is stimulated by bile acids. TGR5, a membrane-bound bile acid receptor coupled to a stimulatory G protein, is expressed in the liver by several non-parenchymal cells (including the sinusoidal epithelium, Kuppfer cells and hepatic stellate cells) and by cholangiocytes. On cholangiocytes, TGR5 is localized both on the apical membrane and on the primary cilium and is strongly activated by taurolithocholic acid and taurocholate. In vitro data in mouse cholangiocytes have shown that CFTR can mediate the apical release of ATP that binds to P2Y purinergic receptors on the apical membrane of biliary cells and activates the Ca^{2+}–calmodulin chloride channel (TMEM16). CFTR also regulates the function of proteins involved in biliary innate immunity and endotoxin tolerance (such as Src family tyrosine kinase and its regulatory proteins CBP and CSK). **b** | Mutations in the gene encoding CFTR cause a chronic cholangiopathy that can eventually progress to biliary cirrhosis. When CFTR is absent, biliary secretion is impaired and the protein complex formed with Src and its regulatory proteins is disrupted, resulting in the self-activation of Src. Src activation is responsible for the phosphorylation of Toll-like receptor 4 (TLR4), which enhances its response to lipopolysaccharide (LPS) and increases nuclear factor-κB (NF-κB) activation and cytokine secretion. This inflammatory milieu also affects the F-actin cytoskeleton and tight junction (TJ) integrity, altering the epithelial barrier function. CFTR loss also promotes changes in the gut that favour the colonization of a more pro-inflammatory microbiota and the translocation of their products to the liver. The altered biliary epithelial innate immunity and changes in the gut microbiota synergistically lead to the progression of cystic fibrosis-related liver disease. Signalling molecules that are druggable are detailed in TABLE 2. AJ, adherens junction; DAMP, damage-associated molecular pattern; ER, endoplasmic reticulum; InsP3R, inositol-1,4,5-trisphosphate receptor; PAMP, pathogen-associated molecular pattern.

Table 1 |

Genes and proteins affected in polycystic liver diseases

Mutated genes	Protein	Localization	Function
ADPLD (frequency of ~1:100,000)			
PRKCSH	Hepatocystin or glucosidase II subunit-β	ER	*N*-linked glycan-processing enzyme in the ER
SEC63	Translocation protein Sec63 homologue (SEC63)	ER	Translocation of proteins into the ER lumen
LRP5	Low-density lipoprotein receptor-related protein 5	Plasma membrane	Canonical Wnt signalling
GANAB	Glucosidase II subunit-α	ER	*N*-linked glycan-processing enzyme in the ER
SEC61B	Sec61 translocon β-subunit (SEC61β)	ER	Translocation of proteins into the ER lumen
ALG8	α–1,3-glucosyltransferase	ER	Protein glycosylation
ADPKD (frequency of ~1:500–1:1,000)			
PKD1	Polycystin-1	Primary cilium, plasma membrane and cell junctions	Mechanoreceptor involved in calcium signalling
PKD2	Polycystin-2	Primary cilium and ER	Non-selective calcium channel
ARPKD, CHF and Caroli disease (frequency of ~1:20,000)			
PKHD1	Fibrocystin	Primary cilium	Tubulogenesis and/or maintenance of bile duct architecture

ADPKD, autosomal dominant polycystic kidney disease; ADPLD, autosomal dominant polycystic liver disease; ARPKD, autosomal recessive polycystic kidney disease; CHF, congenital hepatic fibrosis; ER, endoplasmic reticulum.

Table 2 |

Actionable therapeutic targets in genetic cholangiopathies

Disease	Target	Agent	Refs
PLD	Somatostatin receptors (inhibitor)	Octreotide	108,135
		Lanreotide	62,63
		Pasireotide	108
	AC5 (inhibitor)	SQ22,536	104
	VEGFR2 (inhibitor)	SU5416	113,114
	BRAF	Sorafenib	107
	Intracellular calcium and toxic bile acid removal	UDCA	110
	pmTOR	Rapamycin	115
CHF/CD	Somatostatin receptors (inhibitor)	Pasireotide	108
		Octreotide	108,135
	Intracellular calcium and toxic bile acid removal	UDCA	111
	Matrix metalloproteases	Marimastat	95
	PPARγ (agonist)	Pioglitazione	144
		Telmisartan	143,144
	Macrophages (depletion)	Clodronate	124
	CXCR3 expressed by macrophages	AMG-487	136
CFLD	PPARγ (agonist)	Pioglitazione	177
		Rosiglitazone	177
	CFTR function (correctors and potentiators)	Ivacaftor, lumacaftor and tezacaftor	162,163
	Src tyrosine kinase family (inhibitor)	PP2	164,170

In polycystic liver disease (PLD), octreotide, lanreotide and pasireotide are currently under evaluation in phase II–III clinical trials (octreotide in , lanreotide in and pasireotide in). In cystic fibrosis, ivacaftor and the combinations of ivacaftor plus lumacaftor and ivacaftor plus tezacaftor are currently in use by patients with specific mutations. However, no data are available about their effect on cystic fibrosis-related liver disease (CFLD). AC5, adenylyl cyclase 5; CD, Caroli disease; CFTR, cystic fibrosis transmembrane conductance regulator; CHF, congenital hepatic fibrosis; CXCR3, CXC-chemokine receptor 3; pmTOR, phosphorylated mechanistic target of rapamycin; PPARγ, peroxisome proliferator activated receptor-γ; UDCA, ursodeoxycholic acid; VEGFR, vascular endothelial growth factor receptor.