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Polycystic liver diseases: advanced insights into the molecular mechanisms

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Abstract

Polycystic liver diseases are genetic disorders characterized by progressive bile duct dilatation and/or cyst development. The large volume of hepatic cysts causes different symptoms and complications such as abdominal distension, local pressure with back pain, hypertension, gastro-oesophageal reflux and dyspnea as well as bleeding, infection and rupture of the cysts. Current therapeutic strategies are based on surgical procedures and pharmacological management, which partially prevent or ameliorate the disease. However, as these treatments only show short-term and/or modest beneficial effects, liver transplantation is the only definitive therapy. Therefore, interest in understanding the molecular mechanisms involved in disease pathogenesis is increasing so that new targets for therapy can be identified. In this Review, the genetic mechanisms underlying polycystic liver diseases and the most relevant molecular pathways of hepatic cystogenesis are discussed. Moreover, the main clinical and preclinical studies are highlighted and future directions in basic as well as clinical research are indicated.

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Review criteria

We searched PubMed (June 2014) using the following terms: “polycystic liver diseases”, “cystogenesis”, “ADPKD”, “ADPLD”, “ARPKD”, “congenital hepatic fibrosis”, “Caroli disease”, “primary cilium”, “ductal plate malformation”, “cystic fluid”, “cystogenesis”, “biliary dilatation”, “cystic cholangiocytes”, “molecular pathways”, “proliferation”, “secretion”, “angiogenesis”, “cell–extracellular matrix”, “microRNAs”, “epigenetics”, “oestrogens”, “cAMP”, “calcium”, “treatment” and “clinical trials”. All selected papers were full-text in English. We searched the reference lists of identified papers for further relevant papers.

Competing interests

The authors declare no competing interests.

Author contributions

All authors contributed equally to all aspects of this manuscript.

Introduction

Polycystic liver diseases (PCLDs) are genetic disorders characterized by bile duct dilatation and/or cyst development derived from the bile duct epithelial cells, cholangiocytes. PCLDs are inherited in a dominant or recessive form and can develop alone or in association with polycystic kidney diseases (PKDs).¹ The most common symptoms and complications of PCLDs are hypertension, back pain, bloating and abdominal discomfort, dyspnea, gastroesophageal reflux, bleeding, infection and cyst rupture. Patients progressively worsen and surgical procedures such as aspiration, sclerotherapy, fenestration and/or segmental hepatic resection are commonly used in the management of patients with PCLDs, but have short-term beneficial effects. High rates of recurrence and complications make liver transplantation the only curative treatment. As the possibilities to prevent and cure PCLDs are limited, this Review appraises the most up-to-date research into the underlying molecular mechanisms of PCLDs and the identification of potential therapeutic targets.

Genetic mechanisms of PCLDs

The formation of multiple cysts scattered throughout the liver alone (autosomal dominant polycystic liver disease [ADPLD]), or in association with similar kidney lesions (autosomal dominant polycystic kidney disease [ADPKD]) and autosomal recessive polycystic kidney disease (ARPKD; Caroli disease and congenital hepatic fibrosis [CHF] in infants), is the result of germ line and/or somatic mutations (listed in Table 1, along with disease frequency). Three genes, *PRKCSH*,^{2,3} *SEC63*⁴ and *LRP5*⁵ are associated with the aetiology of ADPLD, whereas *PKD1*⁶ and *PKD2*⁷ have been identified as causative genes for ADPKD. Mutations in *PKHD1* are responsible for ARPKD as well as Caroli disease and CHF.^{8,9} The genetic diagnosis of ADPKD have revealed that mutations in *PKD1* are more frequent (~85%) than *PKD2* (~15%) and are also associated with a more severe phenotype.¹⁰ On the other hand, 20% of patients with ADPLD show mutations in *PRKCSH*, *SEC63* or *LRP5*, with *PRKCSH* being the most frequent (occurring in ~15% of all ADPLD patients);^{11,12} however, the type of mutation (if any) present in the remaining 80% of patients with ADPLD remains unknown.

PKD1 encodes the mechanoreceptor polycystin-1 and *PKD2* encodes the nonselective calcium channel polycystin-2, which are coupled in the ciliary membrane to form a functional complex. Activation of polycystin-1 facilitates calcium uptake through polycystin-2,¹³ a large pool of which is also present in the endoplasmic reticulum.¹⁴ *PRKCSH* and *SEC63* encode two proteins resident in the endoplasmic reticulum. *PRKCSH* (also known as hepatocystin) is the β subunit of glucosidase 2, a heterodimer complex with *N*-linked glycan-processing activity that is involved in the maturation and/or folding of glyco proteins.^{2,3} *SEC63* is a component of the protein translocation machinery required for transport of glycoproteins into and out of the endoplasmic reticulum (Table 1).⁴ *LRP5* encodes a transmembrane protein that acts as a co-receptor with Frizzled protein members to transduce Wnt signalling.⁵ *PKHD1* encodes fibrocystin (also known as polyductin), a plasma membrane protein localized in the primary cilium that has a role in tubulogenesis and maintaining the architecture of the epithelial duct lumen (Table 1).¹⁵

To date, the Online Mendelian Inheritance in Man (OMIM) database includes 46 mutations that affect genes involved in the development of PCLDs (Table 2), which are functionally and clinically important. Moreover, four new mutations in the *LRP5* gene have been described, but are not yet included in the OMIM database. Additional genetic variations that result in changes to protein functions have been identified but their pathogenic consequences have yet to be elucidated.

Although ADPLD and ADPKD are inherited in a dominant fashion, how heterozygous mutations lead to disease is unclear. Previous reports have indicated that mutations in a single allele do not have severe consequences for cholangiocyte function and that PCLD will only develop after loss of heterozygosity.¹⁶ Indeed, *PRKCSH* loss of heterozygosity has been found in a high proportion of the cysts in patients carrying a germ line mutation in this gene,¹⁷ whereas only a small proportion of *SEC63*-mutated cysts acquire *SEC63* loss of heterozygosity.¹⁶ Second-hit mechanisms, such as loss of heterozygosity, have also been reported in cysts of patients with ADPKD who have *PKD1* germ line mutations¹⁸ and in people with *PKD2* germline mutations.¹⁹ Additionally, transheterozygous mutations in other genes associated with PCLDs have been suggested.²⁰ The importance of somatic second-hit mutations in the pathogenesis of PCLDs has been reviewed elsewhere.²¹

Hepatic cystogenesis

Accumulating evidence suggests that pathophysiological alterations in ductal plate remodelling, the primary cilium and in many intracellular signalling pathways and cellular functions (proliferation, angiogenesis, secretion, cell–matrix interaction) account for hepatic cystogenesis. Given the multifactorial nature of these defects, their relative involvement in cyst growth is discussed below.

Embryology and ductal plate malformation

Hepatic cystogenesis in PCLDs is associated with ductal plate malformation, which is a defect of ductal plate remodelling.^{1,22–26} Development of the human liver starts on the eighteenth day of gestation when the hepatic diverticulum is formed.²⁷ At the sixth week of gestation, hepatoblasts immediately adjacent to the portal tract mesenchyme flatten and establish the ductal plate, a layer of biliary-type cuboidal cells expressing the biliary markers CK19 and SOX9, as well as high levels of cadherin-1.^{23,28} Ductal plate malformation is defined as embryological arrest of ductal plate development²⁹ and falls into three categories: the inability of biliary precursor cells to differentiate; defects in maturation of primitive bile ducts; and abnormal bile duct enlargement.^{24,30}

ARPKD and CHF are both characterized by the presence of ductal plate remnants along the portal tract margins. In patients with ARPKD, liver histology depicts incompletely developed ductal plates,³¹ whereas in CHF bile ducts are embedded in fibrous stroma and form cystic lesions (called von Meyenburg complexes).²⁹ Both ADPKD and ADPLD are associated with ductal plate malformation; however, is not clear to which category of malformation they belong.²⁴

Hepatoblast commitment to biliary lineage and ductal plate remodelling is controlled by a network of the following: signalling molecules including Notch,^{32–34} transforming growth factor β ,^{35,36} Wnt^{37–39} and fibroblast growth factor;^{40–42} transcription factors;^{43–50} and microRNAs⁵¹ (Box 1). Many components of ductal plate remodelling have also been implicated in the regulation of ciliary function and are aberrantly expressed in cystic cholangiocytes, thus linking ductal plate malformation and hepatic cystogenesis (Box 1).

Cholangiocyte abnormalities

Primary cilium and centrosomes—Cholangiocytes, the epithelial cells that line the bile ducts, are key liver cells involved in the regulation of the flow and composition of bile. Cholangiocytes contain a single primary cilium that extends from the apical membrane into the bile duct lumen, which is formed by an axoneme and a centriole-derived basal body.⁵² The primary cilium, an antenna-like bulge, functions as a sensory organelle detecting changes in bile flow, composition and osmolarity and has an important role in cholangiocyte physiology and pathophysiology (Figure 1a).^{52,53} Hepatic cystogenesis is thought to be associated with disturbances in ciliary sensation that result from structural and functional changes owing to aberrant expression of PCLD-related and ciliary-associated proteins. Indeed, shortened, unusually long or entirely absent cilia are present in the cystic cholangiocytes of animal models with PCLD^{47,54–56} and patients with ADPKD (Figure 1b).⁵⁷ Abnormalities in the primary cilium are accompanied by atypical centrosome positioning, supernumerary centrosomes and multipolar spindles.^{48,49,58} Notably, cholangiocytes with hyperamplified centrosomes represent a small but notably abnormal portion of cells lining liver cysts. In this regard, a link between centrosome amplification and renal cystogenesis was reported, emphasizing the importance of centrosome abnormality in hepatorenal cystogenesis.⁵⁹

The absence of PCLD-related proteins (fibrocystin,^{54,55,60} polycystin-1 and polycystin-2^{53,61,62}) and cAMP-associated G-protein-coupled receptor TGR5,⁶³ or the overexpression of the calcium channel TRPV4,^{64,65} causes functional abnormalities in cholangiocyte cilia and enhances proliferation and fluid secretion, which contributes to progressive cyst growth.^{1,25,66–68} Interestingly, a strong piece of evidence that might implicate cilia in the pathogenesis of cyst growth comes from outstanding research carried out in animal models of PCLDs. Polycystin-1, polycystin-2, fibrocystin, hepatocystin and SEC63 seem to interact together in a complex network; hepatocystin and SEC63 are necessary for the adequate expression of polycystin-1 and polycystin-2 functional complex, and importantly, polycystin-1 was defined as the rate-limiting component that determines cyst formation.⁶⁹ Thus, polycystin-1 expression levels seem to be involved in determining the severity of ADPKD, ARPKD and ADPLD phenotypes, providing a direct link between cyst growth and the primary cilium.⁷⁰ In addition, the use of proteasome inhibitors has been suggested as a therapy, which might inhibit cystogenesis in patients with ADPLD by increasing the steady-state levels of polycystin-1 and also by inducing apoptosis due to increased toxic levels of unfolded proteins in cyst-lining cells.⁷⁰ On the other hand, studies carried out in experimental animal models of ADPKD suggest that, in the absence of polycystin-1 or polycystin-2, signalling mechanism present in the remaining cilium are required to promote cyst growth, whereas total absence of the cilium results in inhibition of

cystogenesis.⁷¹ Evidence supporting the concept that ciliary dysfunctions underlie hepatic cystogenesis is summarized in Box 2.

Proliferation and angiogenesis—Normal cholangiocytes are quiescent with almost no expression of proliferating cell nuclear antigen (PCNA) detectable by immunohistochemistry,⁷² whereas cystic cholangiocytes show intense PCNA staining.^{72–75} However, proliferation of cystic cholangiocytes in patients with ADPLD is insignificant, with few Ki67 positive cells,⁷⁶ which suggests that other events such as secretion, cell–matrix interactions and ductal plate malformations could have a major role in ADPLD pathogenesis. The hyperproliferative phenotype of cystic cholangiocytes is regulated by growth factors and hormones present in the cystic fluid and/or secreted by cystic cholangiocytes (Figure 1c).^{72,77–79}

The growth factors epidermal growth factor (EGF), vascular endothelial growth factor (VEGF) and insulin-like growth factor 1 (IGF1) all participate in autocrine and/or paracrine loops that stimulate cellular proliferation. Cholangiocytes from the PCK rat, an animal model of ARPKD, show more pronounced hyperproliferative features in response to Egf than normal rat cholangiocytes.⁸⁰ Egf-stimulated hyperproliferation was linked to an overexpression of mitogen-activated protein kinase kinase 5 (Map2k5 also known as Mek5) and subsequent phosphorylation of Erk5, which was abolished by molecular or pharmacological targeting of either Mek5 or the Egf receptor with gefitinib.⁸⁰ However, the role of EGF in hepatic cystogenesis is uncertain because a different study demonstrated that chronic administration of EGF receptor inhibitors (EKI-785 or EKB-569) had no effect on hepatic cystogenesis in PCK rats.⁸¹

VEGF and angiopoietin 1 are pleiotropic growth factors that have key roles in hepatic cystogenesis promoting cyst growth and their vascular supply. The expression of VEGF and angiopoietin 1 and their respective receptors (that is, VEGFR1, VEGFR2 and TIE2) are upregulated in cholangiocytes of patients with ADPKD.⁷⁸ In addition, VEGF is found in liver cyst fluids of patients with ADPKD.⁸² VEGF stimulates proliferation of cystic cholangiocytes in patients with ADPKD, in the *Pkd2*^{WS25/-} and *Pkd2*^{fllox}:*pCxCreER* (*Pkd2cKO*) mouse models of ADPKD^{74,78,82} and promotes liver cyst growth in *Pkd2cKO* mice but not in *Pkd1*^{fllox}:*pCxCreER* (*Pkd1cKO*) mice.⁷⁴ The effects of VEGF secretion and VEGFR2 signalling are dependent on protein kinase A (PKA) and/or extracellular signal regulated kinase 1/2 (ERK1/2).⁷⁴ In addition, the effect of VEGF is synergized by angiopoietin 1.⁷⁸ Biliary cysts are surrounded by vascular networks that are critical for hepatic cystogenesis. Different intracellular signalling pathways and growth factors present in the cystic fluid, such as VEGF and IL-8, promote proliferation of endothelial cells (Figure 1c),⁸³ which suggests that autocrine and paracrine mechanisms could be involved in neovascularization.⁷⁸ The potential therapeutic value of targeting VEGF in patients with PCLDs is supported by the fact that the VEGFR2 inhibitor, SU5416, blunts liver cyst growth in animal models.^{74,82}

Another potential therapeutic target is IGF1, a promitotic factor present in the cystic fluid of patients with ADPKD.⁷² IGF1, its main receptor IGF1R and their downstream effectors phosphorylated AKT and phosphorylated mammalian target of rapamycin (mTOR) are all

overexpressed in the cystic epithelium.⁷² Moreover, the proliferation of cholangio cytes stimulated by cystic fluid, 17 β -estradiol or IGF1 was inhibited by an IGF1R antagonist.⁷²

As well as being downstream of IGF1 signalling, AKT is an activator of mTOR, through which it regulates the expression of hypoxia inducible factor 1 α (encoded by *HIF1 α*), a major transcriptional activator of *VEGF*. As mTOR is involved in both VEGF and IGF1 signalling pathways it was thought that mTOR could be a useful therapeutic target for treating patients with PCLD. The expression of phosphorylated mTOR is increased in the liver cystic epithelium of *Pkd2cKO* mice;⁷³ inhibiting mTOR *in vivo* with sirolimus decreased IGF1-stimulated HIF1 α accumulation, VEGF secretion in cystic cholangiocytes and cyst growth.⁷³ Accordingly, sirolimus and SU5416 (VEGFR2 inhibitor) inhibited the proliferation of cholangiocytes stimulated by IGF1 in *Pkd2cKO* mice.⁷³ However, the inhibition of mTOR by sirolimus did not attenuate hepatic and renal cystogenesis in PCK rats,⁸⁴ highlighting the necessity to clarify in clinical trials the potential therapeutic role of mTOR inhibitors in the different forms of PCLDs. Interestingly, a reduction in liver volume was observed in a clinical trial using sirolimus as an immunosuppressant after renal transplantation in 16 patients with ADPKD (Table 3).⁸⁵ However, in two independent clinical trials, chronic everolimus (an mTOR inhibitor derived from sirolimus) treatment in patients with ADPKD did not slow the progression of renal impairment and kidney growth.^{86,87} Unfortunately, effects of everolimus on liver volume were not examined in these trials. In a 2013 clinical trial, the efficacy of combining everolimus and octreotide (a somatostatin analogue) was compared with octreotide monotherapy. Everolimus did not enhance the beneficial effect of octreotide in reducing liver volume in patients with PCLDs (Table 3).⁸⁸ Overall, the role of mTOR inhibitors in the treatment of patients with PCLDs has not fulfilled expectations and is not clinically recommended.

The potential therapeutic value of sorafenib, a tyrosine kinase inhibitor that might block the action of all EGF, VEGF and IGF1 receptors, has been tested for its ability to attenuate the proliferation of cystic cholangiocytes. However, evidence indicates that, paradoxically, sorafenib transactivates Raf1 thereby promoting cystogenesis in *Pkd2cKO* mice.⁸⁹

Peroxisome proliferator-activated receptor γ (PPAR- γ) is also involved in hepatic cystogenesis by inhibiting genes involved in proliferation, inflammation and fibrosis. The PPAR- γ agonist, pioglitazone, inhibited kidney and liver disease in PCK rats,⁹⁰ but its use in clinical practice is discouraged because of the increased risk of bladder cancer associated with this drug.^{90,91} However, telmisartan—an angiotensin II type 1 receptor antagonist that has off-target PPAR- γ agonist properties but fewer associated risks—reduced liver cystogenesis in PCK rats,⁹² which suggests that PPAR- γ might be a potential therapeutic target in PCLDs.

Although PCLDs affect both men and women, the latter usually exhibit a more severe phenotype. A number of clinical observations suggest that oestrogens are key regulators of hepatic cystogenesis.^{93–95} Cholangiocytes that line bile ducts of healthy individuals and unaffected bile ducts of patients with ADPKD do not express the oestrogen receptors α or β .⁷² By contrast, cystic cholangiocytes in patients with ADPKD are positive for oestrogen receptors α and β . Oestrogens exert promitotic effects on cystic cholangiocytes either

directly or by inducing IGF1 secretion (Figure 1d);⁹⁶ these effects can be partially inhibited by oestrogen receptor antagonists.⁷² All these data support the rationale for studying the potential therapeutic value of antioestrogen therapies (such as tamoxifen) for the treatment of female patients with PCLDs.

cAMP and calcium levels—Intracellular signalling abnormalities associated with increased cAMP levels and decreased $[Ca^{2+}]_i$ levels underlie the hyperproliferative phenotype of cystic cholangiocytes and represent potential therapeutic targets (Figure 1e). Raised cAMP levels in PCK rat cholangiocytes⁹⁷ stimulates proliferation via two intracellular effectors involved in the Mek/Erk pathway—rap guanine nucleotide exchange factor 3 (Rapgef3, also known as Epac) and Pka.⁹⁸ Octreotide treatment decreased these raised cAMP levels in the cholangiocytes and serum of PCK rats, reducing liver weight, cyst volume, hepatic fibrosis and mitotic indices.⁹⁷ A study has shown that pasireotide, a more potent somatostatin analogue than octreotide with broader receptor specificity and a longer half-life, is more effective than octreotide in reducing hepatorenal cystogenesis in PCLD rodent models.⁹⁹ Several clinical trials have evaluated the effects of octreotide^{100–102} and lanreotide^{103–105} in patients with PCLDs (Table 3). Both drugs moderately decreased liver volume (by ~5%) in patients with ADPLD and ADPKD, and improved quality of life.^{61,101,106} In addition, a clinical trial (NCT01670110¹⁰⁷) to evaluate the therapeutic potential of pasireotide in patients with ADPKD and ADPLD is now underway at the Mayo Clinic. For these reasons, current pharmacological strategies are based on the chronic administration of somatostatin analogues, which are clinically recommended but might result in gastrointestinal adverse effects such as diarrhoea, abdominal cramps, flatulence, bloating, gas and injection site granulomas.^{101,105}

Cholangiocytes from both PCK rats and *Pkd2cKO* mice are also characterized by diminished levels of $[Ca^{2+}]_i$.^{98,108} Restoration of intracellular calcium by a calcium ionophore inhibited both the basal and cAMP/Pka-stimulated proliferation of PCK rat cholangiocytes via the Pi3k/Akt pathway, whereas cAMP/Epac-stimulated proliferation was not affected.⁹⁸ Thus, cAMP might regulate the proliferation of cystic cholangiocytes through calcium-dependent (Pka) and calcium-independent (Epac) mechanisms.⁹⁸ Restoration of intracellular calcium could be a useful therapeutic approach in treating patients with PCLD. Pharmacological activation of the calcium-entry channel Trpv4 (which is overexpressed in PCLDs) inhibited the proliferation of PCK rat cholangiocytes *in vitro*, but did not affect hepatic cystogenesis *in vivo*.⁶⁵ The *in vivo* study was limited by the low sublethal dose of the Trpv4 activator used (GSK1016790A), as it induces acute circulatory collapse when administered at higher doses. Therefore, we believe that new pharmacological strategies are needed to evaluate *in vivo* the potential therapeutic role of intracellular calcium restoration in PCLDs.

Secretion—Cholangiocytes have a key role in the fluidization and alkalization of the primary bile generated by hepatocytes, which are controlled by nucleotides, bile acids and hormones, such as secretin.^{109–110} Secretin interacts with its receptor (localized to the basolateral membrane of cholangiocytes), which leads to increased cAMP levels and further activation of PKA. PKA then mediates the exocytosis of intracellular vesicles containing the

chloride channel, cystic fibrosis transmembrane conductance regulator (CFTR), the chloride/bicarbonate exchanger, anion exchanger 2 (AE2) and the water channel, aquaporin 1 (AQ1), which results in bicarbonate-rich choleresis.^{109–111} Increased fluid secretion is one of the contributing mechanisms of bile duct dilatation and cyst expansion in PCLDs (Figure 1f). PCK rat cholangiocytes cultured in a 3D collagen matrix showed enhanced expansion under basal conditions in response to secretin and hypotonicity. Observed alterations were associated with abnormal expression and location of Cftr, anion exchanger 2 and aquaporin 1.⁶⁸ These carriers are preferentially localized to the apical membrane of normal rat cholangiocytes, but they were mainly found overexpressed and mislocalized at the basolateral membrane of PCK rat cholangiocytes. The basolateral presence of Cftr or anion exchanger 2 inhibitors blocked secretin-stimulated hypersecretion in cysts of PCK rats but not in normal cystic structures,⁶⁸ which suggests that targeting these aberrant mechanisms could have potential therapeutic value in treating patients with PCLDs.

However, the role of secretin and flux proteins in cyst progression is more complex than expected. In contrast to the aforementioned data, human cholangiocytes from patients with ADPKD exhibit decreased anion exchanger activity due to diminished expression of mature glycosylated anion exchanger 2 polypeptide and decreased membrane-localized anion exchanger 2.¹¹² The secretory differences between PCK rat cholangiocytes and cholangiocytes from patients with ADPKD could be linked to differences related to the particular form of PCLD. Thus, as cysts from PCK rats flow into the bile ducts, cysts in patients with ADPKD might be disconnected from the biliary tree,⁵⁵ which could result in different pathological or adaptive secretory processes. Of note, chronic administration of secretin had negligible effects on hepatic cystogenesis of PCK rats and *Pkd2*^{WS25/-} mice and the absence of the secretin receptor in *Pkd2*^{WS25/-}:*SCTR*^{-/-} double mutant mice did not alter the severity of PCLD.¹¹³ Together, these data suggest that secretin could have a modest role in the pathogenesis of PCLDs.

Cell–matrix interactions—Interactions between cells and the extracellular matrix are involved in normal ductal plate formation but also in the development and progression of PCLDs. The extracellular matrix is a complex structure of glycoproteins and is remodelled by matrix metalloproteases, tissue inhibitors of metalloproteases and hormones. Free space for cyst growth is generated by overexpression and hyper secretion of matrix metalloproteases by cholangiocytes, which is regulated by oestrogens and cytokines in an autocrine and paracrine fashion (Figure 1g).¹¹⁴ IL-6 and IL-8 present in the cystic fluid interact with their plasma membrane receptors (IL-6R and CXCR1, respectively) localized in cholangiocytes, which enhances the expression and secretion of matrix metalloproteases. Oestrogens, present in the cystic fluid of female patients, have the same effect.¹¹⁴ By contrast, other factors present in the cystic fluid such as VEGF, EGF, hepatocyte growth factor, epithelial neutrophil attractant 78 and growth-related oncogene α did not alter the matrix metalloprotease activity of normal cholangiocytes or cholangiocytes from patients with ADPKD.¹¹⁴ Altered cell–extracellular matrix interactions detailed above are in agreement with the observation that the expression of basement membrane proteins such as laminin and collagen type IV around bile ducts is degraded in CHF, Caroli disease and in PCK rats.¹¹⁵ Finally, the importance of matrix metalloprotease hyperactivity in PCLDs is

highlighted by the fact that chronic pharmacological inhibition of matrix metalloproteases with marimastat halts hepatic cystogenesis in PCK rats, decreasing fibrosis and inflammation.¹¹⁴ Importantly, the dose of marimastat used was reported as nontoxic in clinical trials for cancer treatment.^{116,117} Thus, inhibition of matrix metalloprotease hyperactivity in cystic cholangio cytes has emerged as a potential therapeutic tool for the treatment of patients with PCLDs and needs to be validated in future clinical trials.

Epigenetic abnormalities—In addition to genetic mutations, epigenetic alterations such as abnormal expression of microRNAs (miRNAs) and histone deacetylases have been associated with the hyperproliferative phenotype of cystic cholangiocytes (Figure 1h). Global changes in miRNA levels were reported in cholangiocytes isolated from PCK rats compared with control animals. Out of 109 differentially expressed miRNAs analysed by microarray, 97 (~89%) were downregulated and 12 (~11%) were overexpressed in PCK rats.¹¹⁸ Downregulation of the majority of miRNAs in PCK rat cholangiocytes was associated with overexpression of their predicted target proteins involved in proliferation, secretion and cell–extracellular matrix interactions.¹¹⁸ In particular, miRNA-15a was highly underexpressed in cultured PCK rat cholangiocytes, as well as in cystic tissue from PCK rats and patients with PCLDs. Downregulation of miRNA-15a led to overexpression of the cell division cycle 25A (Cdc25A) protein.¹¹⁸ Experimental upregulation of miRNA-15a in PCK rat cholangiocytes decreased levels of Cdc25A, which halted cholangiocyte hyperproliferation and cyst growth. By contrast, inhibition of miRNA-15a in normal rat cholangiocytes promoted cell proliferation, increased Cdc25A levels and accelerated cyst growth.¹¹⁸ To determine the potential therapeutic value of targeting Cdc25A in patients with PCLDs, *Cdc25A*^{+/-} mice (with decreased Cdc25A expression but normal liver morphology) were cross-bred with *Pkhd1*^{del2/del2} mice (which overexpress Cdc25A and develop hepatic cysts). As expected, liver weights, hepatic cystogenesis and fibrosis were reduced in double mutants compared with *Pkhd1*^{del2/del2} mice.⁷⁵ In addition, pharmacological inhibition of Cdc25A with vitamin K3 or PM-20 decreased hepatorenal cystogenesis and fibrosis in PCK rats and *Pkd2*^{WS25/-} mice by affecting cell cycle progression and proliferation.⁷⁵

Histone deacetylase 6, which is involved in the regulation of the cell cycle and ciliary disassembly, has also been associated with the pathogenesis of PCLDs. Histone deacetylase 6 is overexpressed in cystic cholangiocytes from both PCK rats and patients with PCLDs,¹¹⁹ and pharmacological inhibition of this complex with tubastatin-A, tubacin or ACY-1215 all decreased proliferation of PCK rat cholangiocytes *in vitro* in a dose-dependent and time-dependent manner. Moreover, treatment of PCK rats with ACY-1215 attenuated hepatic cystogenesis and fibrosis, which indicates that targeting histone deacetylase 6 might be a useful therapeutic approach.¹¹⁹

Conclusions

Despite advances in our understanding of the mechanisms of hepatic cystogenesis and the discovery of potential therapeutic targets, the available treatment options (conservative management, surgery and medical therapies) are limited.^{1,22,25,120,121} Novel approaches are mainly focused on the cAMP signalling pathway^{1,61,120,122} and evaluating the effect of somatostatin analogues on hepatic cystogenesis. However, given that the benefits of using

somatostatin analogues are modest, identification of new targets for therapeutic intervention is urgently needed. Several new targets (for example, histone deacetylase 6, Cdc25A phosphatase, PPAR- γ and matrix metalloproteases)^{75,90,92,114,119} have already been evaluated in preclinical studies and need to be tested clinically (Table 4). Moreover, downregulation of the intracellular calcium levels in cholangiocytes seems to be a central event in hepatic cystogenesis. Therefore, restoration of these levels in cystic cholangiocytes seems to be a promising therapeutic strategy. Advances in the field of PCLD research and the discovery of new mutations in genes involved in disease susceptibility, such as *LRP5* in ADPLD, has highlighted new signalling pathways, such as Wnt signalling, that could be pharmacologically important to target. Finally, the possibility exists that combined therapeutic strategies might have an additive or synergistic effect.

References

1. Gevers TJ, Drenth JP. Diagnosis and management of polycystic liver disease. *Nat Rev Gastroenterol Hepatol.* 2013; 10:101–108. [PubMed: 23296249]
2. Drenth JP, te Morsche RH, Smink R, Bonifacino JS, Jansen JB. Germline mutations in *PRKCSH* are associated with autosomal dominant polycystic liver disease. *Nat Genet.* 2003; 33:345–347. [PubMed: 12577059]
3. Li A, et al. Mutations in *PRKCSH* cause isolated autosomal dominant polycystic liver disease. *Am J Hum Genet.* 2003; 72:691–703. [PubMed: 12529853]
4. Davila S, et al. Mutations in *SEC63* cause autosomal dominant polycystic liver disease. *Nat Genet.* 2004; 36:575–577. [PubMed: 15133510]
5. Cnossen WR, et al. Whole-exome sequencing reveals *LRP5* mutations and canonical Wnt signaling associated with hepatic cystogenesis. *Proc Natl Acad Sci USA.* 2014; 111:5343–5348. [PubMed: 24706814]
6. The polycystic kidney disease 1 gene encodes a 14 kb transcript and lies within a duplicated region on chromosome 16. The European Polycystic Kidney Disease Consortium. *Cell.* 1994; 77:881–894. [No authors listed]. [PubMed: 8004675]
7. Mochizuki T, et al. *PKD2*, a gene for polycystic kidney disease that encodes an integral membrane protein. *Science.* 1996; 272:1339–1342. [PubMed: 8650545]
8. Ward CJ, et al. The gene mutated in autosomal recessive polycystic kidney disease encodes a large, receptor-like protein. *Nat Genet.* 2002; 30:259–269. [PubMed: 11919560]
9. Strazzabosco M, Somlo S. Polycystic liver diseases: congenital disorders of cholangiocyte signaling. *Gastroenterology.* 2011; 140:1855–1859. [PubMed: 21515270]
10. Rossetti S, et al. Comprehensive molecular diagnostics in autosomal dominant polycystic kidney disease. *J Am Soc Nephrol.* 2007; 18:2143–2160. [PubMed: 17582161]
11. Waanders E, te Morsche RH, de Man RA, Jansen JB, Drenth JP. Extensive mutational analysis of *PRKCSH* and *SEC63* broadens the spectrum of polycystic liver disease. *Hum Mutat.* 2006; 27:830. [PubMed: 16835903]
12. Waanders E, et al. Secondary and tertiary structure modeling reveals effects of novel mutations in polycystic liver disease genes *PRKCSH* and *SEC63*. *Clin Genet.* 2010; 78:47–56. [PubMed: 20095989]
13. Ong AC, Harris PC. Molecular pathogenesis of ADPKD: the polycystin complex gets complex. *Kidney Int.* 2005; 67:1234–1247. [PubMed: 15780076]
14. Geng L, et al. Syntaxin 5 regulates the endoplasmic reticulum channel-release properties of polycystin-2. *Proc Natl Acad Sci USA.* 2008; 105:15920–15925. [PubMed: 18836075]
15. Al-Bhalal L, Akhtar M. Molecular basis of autosomal recessive polycystic kidney disease (ARPKD). *Adv Anat Pathol.* 2008; 15:54–58. [PubMed: 18156813]
16. Janssen MJ, Salomon J, te Morsche RH, Drenth JP. Loss of heterozygosity is present in *SEC63* germline carriers with polycystic liver disease. *PLoS ONE.* 2012; 7:e50324. [PubMed: 23209713]

17. Janssen MJ, et al. Secondary, somatic mutations might promote cyst formation in patients with autosomal dominant polycystic liver disease. *Gastroenterology*. 2011; 141:2056–2063. [PubMed: 21856269]
18. Watnick TJ, et al. Somatic mutation in individual liver cysts supports a two-hit model of cystogenesis in autosomal dominant polycystic kidney disease. *Mol Cell*. 1998; 2:247–251. [PubMed: 9734362]
19. Pei Y, et al. Somatic *PKD2* mutations in individual kidney and liver cysts support a “two-hit” model of cystogenesis in type 2 autosomal dominant polycystic kidney disease. *J Am Soc Nephrol*. 1999; 10:1524–1529. [PubMed: 10405208]
20. Watnick T, et al. Mutations of *PKD1* in ADPKD2 cysts suggest a pathogenic effect of trans-heterozygous mutations. *Nat Genet*. 2000; 25:143–144. [PubMed: 10835625]
21. Banales JM, Munoz-Garrido P, Bujanda L. Somatic second-hit mutations leads to polycystic liver diseases. *World J Gastroenterol*. 2013; 19:141–143. [PubMed: 23326178]
22. Chandok N. Polycystic liver disease: a clinical review. *Ann Hepatol*. 2012; 11:819–826. [PubMed: 23109444]
23. Raynaud P, Carpentier R, Antoniou A, Lemaigre FP. Biliary differentiation and bile duct morphogenesis in development and disease. *Int J Biochem Cell Biol*. 2011; 43:245–256. [PubMed: 19735739]
24. Wills ES, Roepman R, Drenth JP. Polycystic liver disease: ductal plate malformation and the primary cilium. *Trends Mol Med*. 2014; 20:261–270. [PubMed: 24506938]
25. Temmerman F, et al. Systematic review: the pathophysiology and management of polycystic liver disease. *Aliment Pharmacol Ther*. 2011; 34:702–713. [PubMed: 21790682]
26. Gunay-Aygun M. Liver and kidney disease in ciliopathies. *Am J Med Genet C Semin Med Genet*. 2009; 151C:296–306. [PubMed: 19876928]
27. Godlewski G, Gaubert-Cristol R, Rouy S, Prudhomme M. Liver development in the rat and in man during the embryonic period (Carnegie stages 11–23). *Microsc Res Tech*. 1997; 39:314–327. [PubMed: 9407542]
28. Carpentier R, et al. Embryonic ductal plate cells give rise to cholangiocytes, periportal hepatocytes, and adult liver progenitor cells. *Gastroenterology*. 2011; 141:1432–1438. [PubMed: 21708104]
29. Desmet VJ. Ludwig symposium on biliary disorders—part I. Pathogenesis of ductal plate abnormalities. *Mayo Clin Proc*. 1998; 73:80–89. [PubMed: 9443684]
30. Raynaud P, et al. A classification of ductal plate malformations based on distinct pathogenic mechanisms of biliary dysmorphogenesis. *Hepatology*. 2011; 53:1959–1966. [PubMed: 21391226]
31. Crawford JM. Development of the intrahepatic biliary tree. *Semin Liver Dis*. 2002; 22:213–226. [PubMed: 12360416]
32. Ezratty EJ, et al. A role for the primary cilium in Notch signaling and epidermal differentiation during skin development. *Cell*. 2011; 145:1129–1141. [PubMed: 21703454]
33. Lopes SS, et al. Notch signalling regulates left-right asymmetry through ciliary length control. *Development*. 2010; 137:3625–3632. [PubMed: 20876649]
34. Shih HP, et al. A Notch-dependent molecular circuitry initiates pancreatic endocrine and ductal cell differentiation. *Development*. 2012; 139:2488–2499. [PubMed: 22675211]
35. Sato Y, et al. Cholangiocytes with mesenchymal features contribute to progressive hepatic fibrosis of the polycystic kidney rat. *Am J Pathol*. 2007; 171:1859–1871. [PubMed: 18055542]
36. Hassane S, et al. Elevated TGF β -Smad signalling in experimental Pkd1 models and human patients with polycystic kidney disease. *J Pathol*. 2010; 222:21–31. [PubMed: 20549648]
37. Decaens T, et al. Stabilization of β -catenin affects mouse embryonic liver growth and hepatoblast fate. *Hepatology*. 2008; 47:247–258. [PubMed: 18038450]
38. May-Simera HL, Kelley MW. Cilia, Wnt signaling, and the cytoskeleton. *Cilia*. 2012; 1:7. [PubMed: 23351924]
39. Benzing T, Simons M, Walz G. Wnt signaling in polycystic kidney disease. *J Am Soc Nephrol*. 2007; 18:1389–1398. [PubMed: 17429050]
40. Yanai M, et al. FGF signaling segregates biliary cell-lineage from chick hepatoblasts cooperatively with BMP4 and ECM components in vitro. *Dev Dyn*. 2008; 237:1268–1283. [PubMed: 18393311]

41. Neugebauer JM, Amack JD, Peterson AG, Bisgrove BW, Yost HJ. FGF signalling during embryo development regulates cilia length in diverse epithelia. *Nature*. 2009; 458:651–654. [PubMed: 19242413]
42. Pavik I, et al. Patients with autosomal dominant polycystic kidney disease have elevated fibroblast growth factor 23 levels and a renal leak of phosphate. *Kidney Int*. 2011; 79:234–240. [PubMed: 20944552]
43. Clotman F, et al. The onecut transcription factor HNF6 is required for normal development of the biliary tract. *Development*. 2002; 129:1819–1828. [PubMed: 11934848]
44. Hunter MP, et al. The homeobox gene *Hhex* is essential for proper hepatoblast differentiation and bile duct morphogenesis. *Dev Biol*. 2007; 308:355–367. [PubMed: 17580084]
45. Pierreux CE, et al. The transcription factor hepatocyte nuclear factor-6 controls the development of pancreatic ducts in the mouse. *Gastroenterology*. 2006; 130:532–541. [PubMed: 16472605]
46. Guay-Woodford LM, Green WJ, Lindsey JR, Beier DR. Germline and somatic loss of function of the mouse *cpk* gene causes biliary ductal pathology that is genetically modulated. *Hum Mol Genet*. 2000; 9:769–778. [PubMed: 10749984]
47. Hou X, et al. Cystin, a novel cilia-associated protein, is disrupted in the *cpk* mouse model of polycystic kidney disease. *J Clin Invest*. 2002; 109:533–540. [PubMed: 11854326]
48. Hiesberger T, et al. Mutation of hepatocyte nuclear factor-1 β inhibits *Pkhd1* gene expression and produces renal cysts in mice. *J Clin Invest*. 2004; 113:814–825. [PubMed: 15067314]
49. Gresh L, et al. A transcriptional network in polycystic kidney disease. *EMBO J*. 2004; 23:1657–1668. [PubMed: 15029248]
50. Yamasaki H, et al. Suppression of C/EBP α expression in periportal hepatoblasts may stimulate biliary cell differentiation through increased *Hnf6* and *Hnf1b* expression. *Development*. 2006; 133:4233–4243. [PubMed: 17021047]
51. Hand NJ, et al. The microRNA-30 family is required for vertebrate hepatobiliary development. *Gastroenterology*. 2009; 136:1081–1090. [PubMed: 19185580]
52. Masyuk AI, Masyuk TV, LaRusso NF. Cholangiocyte primary cilia in liver health and disease. *Dev Dyn*. 2008; 237:2007–2012. [PubMed: 18407555]
53. Masyuk AI, et al. Cholangiocyte cilia detect changes in luminal fluid flow and transmit them into intracellular Ca²⁺ and cAMP signaling. *Gastroenterology*. 2006; 131:911–920. [PubMed: 16952559]
54. Masyuk TV, et al. Defects in cholangiocyte fibrocystin expression and ciliary structure in the PCK rat. *Gastroenterology*. 2003; 125:1303–1310. [PubMed: 14598246]
55. Masyuk TV, et al. Biliary dysgenesis in the PCK rat, an orthologous model of autosomal recessive polycystic kidney disease. *Am J Pathol*. 2004; 165:1719–1730. [PubMed: 15509540]
56. Stroope A, et al. Hepato-renal pathology in *Pkd2^{ws25/-}* mice, an animal model of autosomal dominant polycystic kidney disease. *Am J Pathol*. 2010; 176:1282–1291. [PubMed: 20093497]
57. Alvaro D, Mancino MG. New insights on the molecular and cell biology of human cholangiopathies. *Mol Aspects Med*. 2008; 29:50–57. [PubMed: 18230407]
58. Masyuk TV, et al. Centrosomal abnormalities characterize human and rodent cystic cholangiocytes and are associated with *Cdc25A* overexpression. *Am J Pathol*. 2014; 184:110–121. [PubMed: 24211536]
59. Battini L, et al. Loss of polycystin-1 causes centrosome amplification and genomic instability. *Hum Mol Genet*. 2008; 17:2819–2833. [PubMed: 18566106]
60. Woollard JR, et al. A mouse model of autosomal recessive polycystic kidney disease with biliary duct and proximal tubule dilatation. *Kidney Int*. 2007; 72:328–336. [PubMed: 17519956]
61. Torres VE, Harris PC. Strategies targeting cAMP signaling in the treatment of polycystic kidney disease. *J Am Soc Nephrol*. 2014; 25:18–32. [PubMed: 24335972]
62. Torrice A, et al. Polycystins play a key role in the modulation of cholangiocyte proliferation. *Dig Liver Dis*. 2010; 42:377–385. [PubMed: 19897428]
63. Masyuk AI, et al. Ciliary subcellular localization of TGR5 determines the cholangiocyte functional response to bile acid signaling. *Am J Physiol Gastrointest Liver Physiol*. 2013; 304:G1013–G1024. [PubMed: 23578785]

64. Gradilone SA, et al. Cholangiocyte cilia express TRPV4 and detect changes in luminal tonicity inducing bicarbonate secretion. *Proc Natl Acad Sci USA*. 2007; 104:19138–19143. [PubMed: 18024594]
65. Gradilone SA, et al. Activation of Trpv4 reduces the hyperproliferative phenotype of cystic cholangiocytes from an animal model of ARPKD. *Gastroenterology*. 2010; 139:304–314. [PubMed: 20399209]
66. Onori P, et al. Polycystic liver diseases. *Dig Liver Dis*. 2010; 42:261–271. [PubMed: 20138815]
67. Muchatuta MN, Gattone VH 2nd, Witzmann FA, Blazer-Yost BL. Structural and functional analyses of liver cysts from the BALB/c-cpk mouse model of polycystic kidney disease. *Exp Biol Med (Maywood)*. 2009; 234:17–27. [PubMed: 18997107]
68. Banales JM, et al. Hepatic cystogenesis is associated with abnormal expression and location of ion transporters and water channels in an animal model of autosomal recessive polycystic kidney disease. *Am J Pathol*. 2008; 173:1637–1646. [PubMed: 18988797]
69. Fedeles SV, et al. A genetic interaction network of five genes for human polycystic kidney and liver diseases defines polycystin-1 as the central determinant of cyst formation. *Nat Genet*. 2011; 43:639–647. [PubMed: 21685914]
70. Fedeles SV, Gallagher AR, Somlo S. Polycystin-1: a master regulator of intersecting cystic pathways. *Trends Mol Med*. 2014; 20:251–260. [PubMed: 24491980]
71. Ma M, Tian X, Igarashi P, Pazour GJ, Somlo S. Loss of cilia suppresses cyst growth in genetic models of autosomal dominant polycystic kidney disease. *Nat Genet*. 2013; 45:1004–1012. [PubMed: 23892607]
72. Alvaro D, et al. Morphological and functional features of hepatic cyst epithelium in autosomal dominant polycystic kidney disease. *Am J Pathol*. 2008; 172:321–332. [PubMed: 18202196]
73. Spirli C, et al. Mammalian target of rapamycin regulates vascular endothelial growth factor-dependent liver cyst growth in polycystin 2 defective mice. *Hepatology*. 2010; 51:1778–1788. [PubMed: 20131403]
74. Spirli C, et al. ERK1/2-dependent vascular endothelial growth factor signaling sustains cyst growth in polycystin-2 defective mice. *Gastroenterology*. 2010; 138:360–371. [PubMed: 19766642]
75. Masyuk TV, et al. Inhibition of *Cdc25A* suppresses hepato-renal cystogenesis in rodent models of polycystic kidney and liver disease. *Gastroenterology*. 2012; 142:622–633. [PubMed: 22155366]
76. Waanders E, Van Krieken JH, Lameris AL, Drenth JP. Disrupted cell adhesion but not proliferation mediates cyst formation in polycystic liver disease. *Mod Pathol*. 2008; 21:1293–1302. [PubMed: 18587325]
77. Nichols MT, et al. Secretion of cytokines and growth factors into autosomal dominant polycystic kidney disease liver cyst fluid. *Hepatology*. 2004; 40:836–846. [PubMed: 15382115]
78. Fabris L, et al. Effects of angiogenic factor overexpression by human and rodent cholangiocytes in polycystic liver diseases. *Hepatology*. 2006; 43:1001–1012. [PubMed: 16628643]
79. Amura CR, et al. CXCR2 agonists in ADPKD liver cyst fluids promote cell proliferation. *Am J Physiol Cell Physiol*. 2008; 294:C786–C796. [PubMed: 18199703]
80. Sato Y, et al. Activation of the MEK5/ERK5 cascade is responsible for biliary dysgenesis in a rat model of Caroli's disease. *Am J Pathol*. 2005; 166:49–60. [PubMed: 15631999]
81. Torres VE, et al. Epidermal growth factor receptor tyrosine kinase inhibition is not protective in PCK rats. *Kidney Int*. 2004; 66:1766–1773. [PubMed: 15496147]
82. Amura CR, et al. VEGF receptor inhibition blocks liver cyst growth in *pkd2^{WS25/-}* mice. *Am J Physiol Cell Physiol*. 2007; 293:C419–C428. [PubMed: 17475663]
83. Brodsky KS, McWilliams RR, Amura CR, Barry NP, Doctor RB. Liver cyst cytokines promote endothelial cell proliferation and development. *Exp Biol Med (Maywood)*. 2009; 234:1155–1165. [PubMed: 19596832]
84. Renken C, Fischer DC, Kundt G, Gretz N, Haffner D. Inhibition of mTOR with sirolimus does not attenuate progression of liver and kidney disease in PCK rats. *Nephrol Dial Transplant*. 2011; 26:92–100. [PubMed: 20615907]
85. Qian Q, et al. Sirolimus reduces polycystic liver volume in ADPKD patients. *J Am Soc Nephrol*. 2008; 19:631–638. [PubMed: 18199797]

86. Walz G, et al. Everolimus in patients with autosomal dominant polycystic kidney disease. *N Engl J Med.* 2010; 363:830–840. [PubMed: 20581392]
87. Serra AL, et al. Sirolimus and kidney growth in autosomal dominant polycystic kidney disease. *N Engl J Med.* 2010; 363:820–829. [PubMed: 20581391]
88. Chrispijn M, et al. Everolimus does not further reduce polycystic liver volume when added to long acting octreotide: results from a randomized controlled trial. *J Hepatol.* 2013; 59:153–159. [PubMed: 23499726]
89. Spirli C, et al. Cyclic AMP/PKA-dependent paradoxical activation of Raf/MEK/ERK signaling in polycystin-2 defective mice treated with sorafenib. *Hepatology.* 2012; 56:2363–2374. [PubMed: 22653837]
90. Yoshihara D, et al. PPAR- γ agonist ameliorates kidney and liver disease in an orthologous rat model of human autosomal recessive polycystic kidney disease. *Am J Physiol Renal Physiol.* 2011; 300:F465–F474. [PubMed: 21147840]
91. Yoshihara D, et al. Global gene expression profiling in PPAR- γ agonist-treated kidneys in an orthologous rat model of human autosomal recessive polycystic kidney disease. *PPAR Res.* 2012; 2012:695898. [PubMed: 22666229]
92. Yoshihara D, et al. Telmisartan ameliorates fibrocystic liver disease in an orthologous rat model of human autosomal recessive polycystic kidney disease. *PLoS ONE.* 2013; 8:e81480. [PubMed: 24324698]
93. Chapman AB. Cystic disease in women: clinical characteristics and medical management. *Adv Ren Replace Ther.* 2003; 10:24–30. [PubMed: 12616460]
94. Gabow PA, et al. Risk factors for the development of hepatic cysts in autosomal dominant polycystic kidney disease. *Hepatology.* 1990; 11:1033–1037. [PubMed: 2365280]
95. Sherstha R, et al. Postmenopausal estrogen therapy selectively stimulates hepatic enlargement in women with autosomal dominant polycystic kidney disease. *Hepatology.* 1997; 26:1282–1286. [PubMed: 9362373]
96. Alvaro D, et al. Estrogens and the pathophysiology of the biliary tree. *World J Gastroenterol.* 2006; 12:3537–3545. [PubMed: 16773710]
97. Masyuk TV, Masyuk AI, Torres VE, Harris PC, Larusso NF. Octreotide inhibits hepatic cystogenesis in a rodent model of polycystic liver disease by reducing cholangiocyte adenosine 3', 5'-cyclic monophosphate. *Gastroenterology.* 2007; 132:1104–1116. [PubMed: 17383431]
98. Banales JM, et al. The cAMP effectors Epac and protein kinase A (PKA) are involved in the hepatic cystogenesis of an animal model of autosomal recessive polycystic kidney disease (ARPKD). *Hepatology.* 2009; 49:160–174. [PubMed: 19065671]
99. Masyuk TV, et al. Pasireotide is more effective than octreotide in reducing hepatorenal cystogenesis in rodents with polycystic kidney and liver diseases. *Hepatology.* 2013; 58:409–421. [PubMed: 23172758]
100. Caroli A, et al. Reducing polycystic liver volume in ADPKD: effects of somatostatin analogue octreotide. *Clin J Am Soc Nephrol.* 2010; 5:783–789. [PubMed: 20185596]
101. Hogan MC, et al. Randomized clinical trial of long-acting somatostatin for autosomal dominant polycystic kidney and liver disease. *J Am Soc Nephrol.* 2010; 21:1052–1061. [PubMed: 20431041]
102. Hogan MC, et al. Somatostatin analog therapy for severe polycystic liver disease: results after 2 years. *Nephrol Dial Transplant.* 2012; 27:3532–3539. [PubMed: 22773240]
103. van Keimpema L, et al. Lanreotide reduces the volume of polycystic liver: a randomized, double-blind, placebo-controlled trial. *Gastroenterology.* 2009; 137:1661–1668. [PubMed: 19646443]
104. Chrispijn M, et al. The long-term outcome of patients with polycystic liver disease treated with lanreotide. *Aliment Pharmacol Ther.* 2012; 35:266–274. [PubMed: 22111942]
105. Temmerman F, et al. Safety and efficacy of different lanreotide doses in the treatment of polycystic liver disease: pooled analysis of individual patient data. *Aliment Pharmacol Ther.* 2013; 38:397–406. [PubMed: 23799922]
106. Gevers TJ, et al. Young women with polycystic liver disease respond best to somatostatin analogues: a pooled analysis of individual patient data. *Gastroenterology.* 2013; 145:357–365. [PubMed: 23665274]

107. US National Library of Medicine. ClinicalTrials.gov [online]. 2012. <http://www.clinicaltrials.gov/ct2/show/NCT01670110>
108. Spirli C, et al. Altered store operated calcium entry increases cyclic 3', 5'-adenosine monophosphate production and extracellular signal-regulated kinases 1 and 2 phosphorylation in polycystin 2 defective cholangiocytes. *Hepatology*. 2012; 55:856–868. [PubMed: 21987453]
109. Banales JM, Prieto J, Medina JF. Cholangiocyte anion exchange and biliary bicarbonate excretion. *World J Gastroenterol*. 2006; 12:3496–3511. [PubMed: 16773707]
110. Banales JM, et al. Bicarbonate-rich choleresis induced by secretin in normal rat is taurocholate-dependent and involves AE2 anion exchanger. *Hepatology*. 2006; 43:266–275. [PubMed: 16440368]
111. Tietz PS, et al. Agonist-induced coordinated trafficking of functionally related transport proteins for water and ions in cholangiocytes. *J Biol Chem*. 2003; 278:20413–20419. [PubMed: 12660234]
112. Perrone RD, et al. Autosomal dominant polycystic kidney disease decreases anion exchanger activity. *Am J Physiol*. 1997; 272:C1748–C1756. [PubMed: 9176168]
113. Wang X, et al. Insignificant effect of secretin in rodent models of polycystic kidney and liver disease. *Am J Physiol Renal Physiol*. 2012; 303:F1089–F1098. [PubMed: 22811488]
114. Urribarri, AD., et al. Inhibition of metalloprotease hyperactivity in cystic cholangiocytes halts the development of polycystic liver diseases. *Gut*. <http://dx.doi.org/10.1136/gutjnl-2013-305281>
115. Yasoshima M, et al. Matrix proteins of basement membrane of intrahepatic bile ducts are degraded in congenital hepatic fibrosis and Caroli's disease. *J Pathol*. 2009; 217:442–451. [PubMed: 19025978]
116. Nemunaitis J, et al. Combined analysis of studies of the effects of the matrix metalloproteinase inhibitor marimastat on serum tumor markers in advanced cancer: selection of a biologically active and tolerable dose for longer-term studies. *Clin Cancer Res*. 1998; 4:1101–1109. [PubMed: 9607566]
117. Rosenbaum E, et al. Marimastat in the treatment of patients with biochemically relapsed prostate cancer: a prospective randomized, double-blind, phase I/II trial. *Clin Cancer Res*. 2005; 11:4437–4443. [PubMed: 15958628]
118. Lee SO, et al. MicroRNA15a modulates expression of the cell-cycle regulator Cdc25A and affects hepatic cystogenesis in a rat model of polycystic kidney disease. *J Clin Invest*. 2008; 118:3714–3724. [PubMed: 18949056]
119. Gradilone SA, et al. *HDAC6* is overexpressed in cystic cholangiocytes and its inhibition reduces cystogenesis. *Am J Pathol*. 2014; 184:600–608. [PubMed: 24434010]
120. Gevers TJ, Drenth JP. Somatostatin analogues for treatment of polycystic liver disease. *Curr Opin Gastroenterol*. 2011; 27:294–300. [PubMed: 21191289]
121. Takiar V, Caplan MJ. Polycystic kidney disease: pathogenesis and potential therapies. *Biochim Biophys Acta*. 2011; 1812:1337–1343. [PubMed: 21146605]
122. Abu-Wasel B, Walsh C, Keough V, Molinari M. Pathophysiology, epidemiology, classification and treatment options for polycystic liver diseases. *World J Gastroenterol*. 2013; 19:5775–5786. [PubMed: 24124322]

Key points

- Proteins encoded by the genes that cause polycystic liver diseases are predominantly localized in the primary cilium, plasma membrane and/or the endoplasmic reticulum of cholangiocytes
- Current treatments are based on surgical procedures and/or pharmacological management; however, their beneficial effects are modest, leaving liver transplantation as the only definitive remedy
- Elucidating the molecular mechanisms involved in the pathogenesis of these disorders is crucial in order to identify new potential targets for therapy
- Hepatic cystogenesis is characterized by ductal plate malformation, abnormalities of the cholangiocyte primary cilium, centrosome amplification, hyperproliferation, hypersecretion, matrix-metalloprotease hyperactivity, angiogenesis, epigenetic alterations and atypical levels of key intracellular mediators
- Preclinical studies have revealed new potential therapeutic targets that need to be validated in future clinical trials

Box 1**Regulators of ductal plate remodelling****Signaling pathways**

- Notch receptors and Notch-processing enzymes are expressed in primary cilia and regulate their length.^{32–34}
- TGF- β signalling also regulates biliary commitment of hepatoblasts.²³ TGF- β and its receptor are overexpressed in cystic cholangiocytes and renal epithelial cells.^{35,36}
- Wnt signalling is implicated in both ciliary sensation and cystogenesis.^{37–39}
- FGF signalling regulates cilia length and hepatoblast differentiation towards the biliary lineage. Patients with ADPKD have raised FGF23 levels.^{40–42}

Transcription factors^{23,24}

- HNF1 β , 4 and 6
- HHEX
- C/EBP α
- Smad2 and Smad3, Onecut 2
- FOXM1b
- SALL4
- TBX3

Examples include deletion of *Hnf6* and *Hhex* in mice results in accumulation of ductal plate remnants, cyst development and cilia absence in cystic cholangiocytes.^{23,43,44} In addition, in the pancreas, *Hnf6* stimulates the expression of *Pkhd1* and *Cys1* genes, mutations of which cause hepatic cystogenesis and affect ciliary length.⁴⁵ In a further example, mice deficient in *Cys1*,^{46,47} *Hnf-1 β* ^{48,49} or *C/EBP α* ⁵⁰ exhibit ductal plate malformation.

miRNAs

miRNA-30 family. miR-30a depletion in zebrafish affects bile duct morphogenesis.⁵¹

Abbreviations: ADPKD, autosomal dominant polycystic kidney disease; C/EBP α , CCAAT/enhancer binding protein α ; FGF, fibroblast growth factor; FOXM1b, forkhead box factor M1b; HHEX, hematopoietically expressed homeobox; HNF, hepatocyte nuclear factor; miRNA, microRNA; SALL4, spalt-like transcription factor 4; TBX3, T-box transcription factor 3; TGF, transforming growth factor.

Box 2**Ciliary dysfunction underlies hepatic cystogenesis**

- Disappearance of fibrocystin (PKHD1) from cholangiocyte cilia leads to ciliary malformations and accelerated cyst expansion.^{54,55,60}
- Somatic inactivation of *Pkd2* in mice results in hepato-cystic phenotype.⁵⁶
- Polycystin-1 and polycystin-2 function as ciliary sensors of cell injury activating the cholangiocyte proliferation as a reparative mechanism.⁶²
- Ciliary structural malformations (shortened, unusually long or entirely absent cilia) are present in cystic cholangiocytes of PCLD animal models^{47,54–56} and patients with ADPKD.⁵⁷
- Ciliary defects are linked to basal body abnormalities (that is atypical centrosome positioning, supernumerary centrosomes, multipolar spindles and extra cilia).⁵⁸
- *Hnf1β* deficient mice lack cholangiocyte cilia due to mispositioning of the basal body resulting in ductal plate malformation and biliary dysgenesis that resembles the lesions observed in patients with ARPKD.^{30,49,50}
- Abnormal ciliary structure is associated with enhanced fluid secretion and ion transport.^{62,66–68}
- Absence of polycystin-1 in cholangiocyte cilia decreases levels of $[Ca^{2+}]_i$ and increases cAMP production via a Ca^{2+} -inhibitable adenylyl cyclase 6 (which is also localized within the cilium) increasing cell proliferation and accelerating fluid secretion.^{53,61}
- TGR5, the G-protein coupled receptor linked to cAMP signalling, is overexpressed in cystic cholangiocytes and is mislocalized from cilia.⁶³
- Decreased levels of $[Ca^{2+}]_i$ in hepatic cysts are linked to aberrantly expressed calcium channel TRPV4 in cholangiocyte cilia.⁶⁵
- Glucosidase 2 subunit β and Sec63 are required in mice for adequate expression of a functional complex of polycystin-1 and polycystin-2. Polycystin-1 is the rate-limiting component of this complex.^{69,70}
- In the absence of polycystin-1 or polycystin-2, the resulting cilium is required to promote cyst growth, whereas the total absence of the cilium results in inhibition of cystogenesis.⁷¹

Abbreviations: ADPKD, autosomal dominant polycystic kidney disease; ARPKD, autosomal recessive polycystic kidney disease; cAMP, cyclic adenosine monophosphate; *Hnf1β*, hepatocyte nuclear factor 1β; PCLD, polycystic liver disease; PKDH1, polycystic kidney and hepatic disease 1; Sec63, Sec63 homolog; TGR5, g-protein coupled bile acid receptor-1; TRPV4, transient receptor potential cation subfamily V member 4.

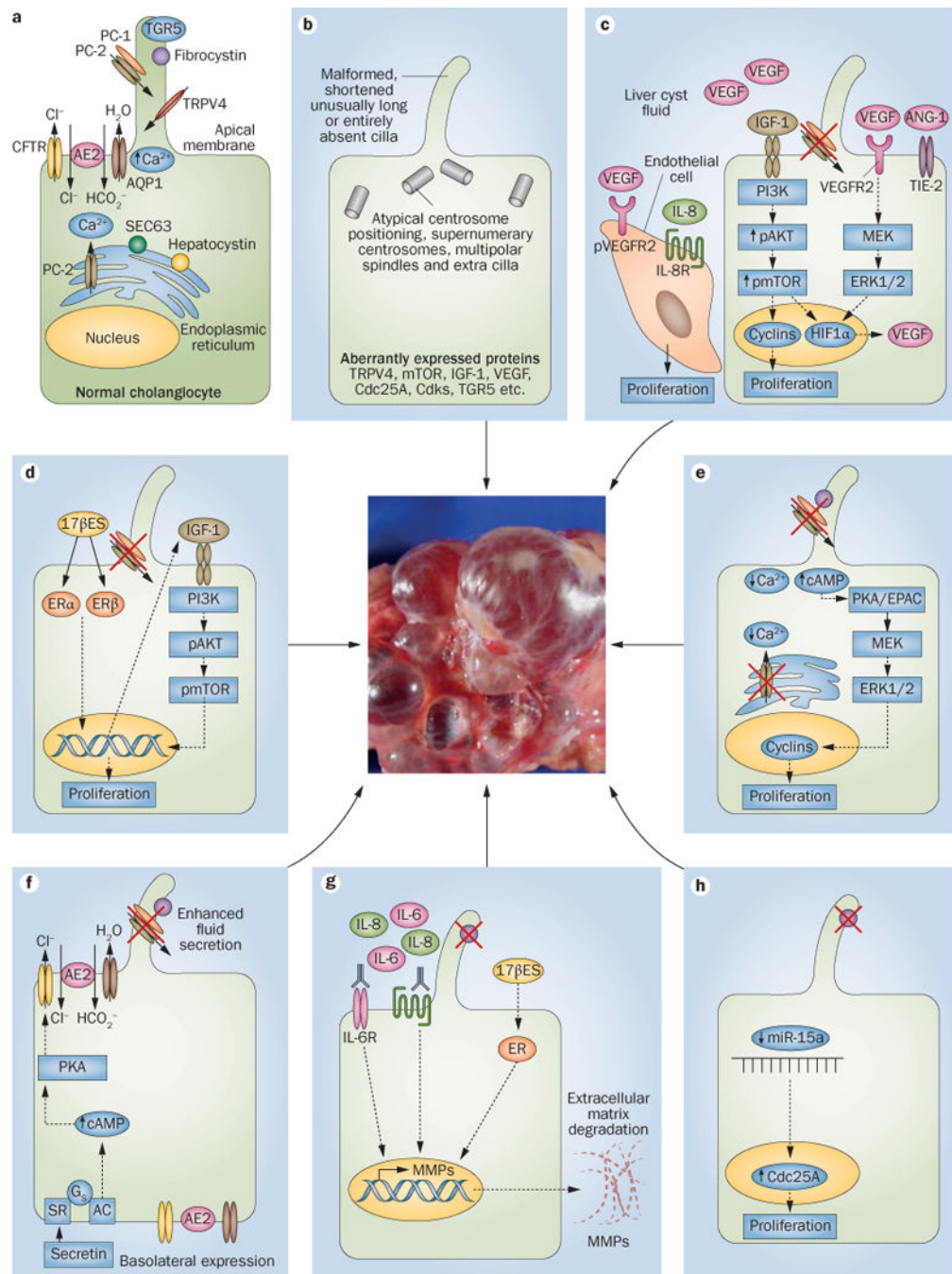


Figure 1.

Cellular alterations and molecular mechanisms involved in hepatic cystogenesis. **a** | The normal structure of both a cholangiocyte and a primary cilium. **b** | The primary cilium and gene expression levels of key intracellular mediators can be altered in polycystic liver diseases. **c** | Different growth factors and cytokines stimulate the proliferation of cystic cholangiocytes and endothelial cells in an autocrine and/or paracrine fashion. Moreover, **d** | oestrogens and **e** | changes in intracellular calcium and cAMP levels might also induce the proliferation of cystic cholangiocytes. Hepatic cystogenesis is associated with **f** | alterations

in fluid secretion and **g** | extracellular matrix remodelling. **h** | Global downregulation of microRNAs occurs in cystic cholangiocytes, which facilitates the proliferation of cystic cholangiocytes. The relative involvement of each pathway in different forms of hepatic cystogenesis has been highlighted in the main body text. The central image is of human liver tissue with cysts. Abbreviations: 17 β ES, 17 β oestradiol; AC, adenylate cyclase; AE2, anion exchanger 2; ANG-1, angiopoetin-1; AQP1, aquaporin 1; cAMP, cyclic adenosine monophosphate; Cdc25A, cell division cycle 25A; Cdks, cyclin dependent kinase; CFTR, cystic fibrosis transmembrane conductance regulator; EPAC, rap guanine nucleotide exchange factor 3; ER, oestrogen receptor; ER α , oestrogen receptor α ; ER β , oestrogen receptor β ; ERK1/2, extracellular signal regulated kinase 1/2; Gs, Gs protein; HIF1 α , hypoxia inducible factor 1 α ; IGF1, insulin-like growth factor 1; MEK, mitogen-activated protein kinase kinase 1; miR-15a, microRNA 15a; MMP, matrix metalloproteinase; mTOR, mammalian target of rapamycin; pAKT, phosphorylated v-akt murine thymoma viral oncogene homolog 1; PC-1, polycystin-1; PC-2, polycystin-2; PI3K, phosphatidylinositol 4,5-bisphosphate 3-kinase; PKA, protein kinase A; pmTOR, phosphorylated mTOR; pVEGFR2, phosphorylated vascular endothelial growth factor receptor 2; SEC63, SEC63 homolog; SR, serotonin receptor; TGR5, g-protein coupled bile acid receptor-1; TIE-2, TEK tyrosine kinase; TRPV4, transient receptor potential cation subfamily V member 4; VEGF, vascular endothelial growth factor.

Table 1

Genes, proteins and animal models of polycystic liver diseases

Mutated genes	Protein	Localization	Function	Animal models
ADPLD (~1:100,000)				
<i>PRKCSH</i>	Glucosidase 2 subunit β , protein kinase C substrate 80K-H or hepatocystin	ER	N-linked glycan-processing enzyme in the endoplasmic reticulum	<i>Prkcs^h</i> ^{flox/flox} ;pCXCreER mice and zebrafish
<i>SEC63</i>	Translocation protein SEC63 homologue	ER	Translocation of proteins in the endoplasmic reticulum	<i>Sec63</i> ^{flox/flox} ;pCXCreER mice and zebrafish
<i>LRP5</i>	Low density lipoprotein receptor-related protein 5	Plasma membrane	Canonical Wnt signalling	<i>Lrp5</i> KO mouse
ADPKD (~1:500–1:1,000)				
<i>PKD1</i>	Polycystin-1	Primary cilium, plasma membrane and cell junctions	Mechanoreceptor involved in calcium signalling	<i>Pkd1</i> ^{flox/} ;pCxCreER TM (<i>Pkd1c</i> KO) and zebrafish
<i>PKD2</i>	Polycystin-2	Primary cilium and endoplasmic reticulum	Nonselective calcium channel	<i>Pkd2</i> ^{flox/} ;pCxCreER TM (<i>Pkd2c</i> KO) and <i>Pkd2</i> ^{WS25/-}
ARPKD, CHF or CD (~1:20,000)				
<i>PKHD1</i>	Fibrocystin or polyductin	Primary cilium	Tubulogenesis and/or maintenance of bile duct architecture	PCK rat, and <i>Pkhd1</i> ^{del2/del2} mouse

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

Table 2

Mutations in genes causing polycystic liver diseases resulting in change of function with clinical significance

Gene	Locus	Nucleotide change	Amino acid change	OMIM number	Phenotype MIM number
<i>PRKCSH</i>	19p13.2	IVS16, A-G, -2	-	177060.0001	174050
		IVS4, G-C, +1	-	177060.0002	
		2-bp del, IVS16GT, +1	-	177060.0003	
		c.1240C>T	p.Gln414Ter	177060.0004	
		c.1269C>G	p.Tyr423Ter	177060.0005	
		1-bp ins, 216A	-	177060.0006	
<i>SEC63</i>	6q21	c.173G>A	p.Trp58Ter	608648.0001	174050
		1-bp ins, 442A	-	608648.0002	
		IVS8ds, G-A, +1	-	608648.0003	
		c.1702_1704del	p.Glu568del	608648.0004	
<i>LRP5</i>	11q13.2	c.1360G>A	p.Val454Met	Not assigned	Not assigned
		c.3562C>T	p.Arg1188Trp	Not assigned	
		c.4587G>C	p.Arg1529Ser	Not assigned	
		c.4651G>A	p.Asp1551Asn	Not assigned	
<i>PKD1</i>	16p13.3	IVSds, G-C, +1	-	601313.0001	173900
		c.12124C>T	p.Gln4042Ter	601313.0002	
		15-bp del	-	601313.0003	
		c.12682C>T	p.Arg4228Ter	601313.0004	
		c.11512C>T	p.Gln3838Ter	601313.0005	
		c.12261T>A	p.Cys4087Ter	601313.0006	
		c.11457C>A	p.Tyr3819Ter	601313.0007	
		12036G-A	-	601313.0008	
		28-bp del, nt6434	-	601313.0009	
		IVS14, G-A, -1	-	601313.00010	
c.971G>T	p.Arg324Leu	601313.00011			
c.2534T>C	p.Leu845Ser	601313.00012			
c.5764C>T	p.Gln1922Ter	601313.00013			

Gene	Locus	Nucleotide change	Amino acid change	OMIM number	Phenotype/MIM number
		2-bp del, 5224AG	-	601313.00014	
		c.12420G>A	p.Trp4140Ter	601313.00015	
		Gly>2579del, 8-bp del	-	601313.00016	
<i>PKD2</i>	4q22.1	c.1139G>A	p.Trp380Ter	173910.0001	613095
		c.2224C>T	p.Arg742Ter	173910.0002	
		c.1213C>T	p.Gln405Ter	173910.0003	
		1-bp ins, 693C	-	173910.0004	
		c.1390C>T	p.Arg464Ter	173910.0005	
		1-bp ins, 2160A	-	173910.0006	
		1-bp ins, 197-203C	-	173910.0007	
		c.1532A>T	p.Asp511Val	173910.0008	
		2-bp del/1-bp ins, nt1934	-	173910.0009	
		Ex3dup	-	173910.00010	
		c.305_306insGAG	p.Glu102_Val1103insArg	173910.00011	
<i>PKHD1</i>	6p12.2	c.107C>T	p.Thr36Met	606702.0001	263200
		c.4991C>T	p.Ser1664Phe	606702.0002	
		c.9053C>T	p.Ser3018Phe	606702.0003	
		c.5221G>A	p.Val1741Met	606702.0004	
		c.8011C>T	p.Arg2671Ter	606702.0005	
		c.10658T>C	p.Ile3553Thr	606702.0006	
		c.1486C>T	p.Arg496Ter	606702.0007	
		c.10412T>G	p.Val3471Gly	606702.0008	
		IVS46ds, A-G, +653	-	606702.0009	

Data were obtained in June 2014 from the Online Mendelian Inheritance in Man (OMIM) of the National Center for Biotechnology Information (NCBI). Four new mutations in *LRP5* have been described but the OMIM number and the phenotype (MIM number) have not yet been assigned. The mutations listed consist of single nucleotide polymorphisms resulting in an amino acid change, exon duplications, small deletions and insertions, and splice site mutations. Abbreviations: bp, base pair; del, small deletion; ds, donor splice site; ER, endoplasmic reticulum; Ex3dup, exon 3 duplication; ins, insertion; IVS, intervening sequence. Permission obtained from the Online Mendelian Inheritance in Man, OMIM®. McKusick-Nathans Institute of Genetic Medicine, John Hopkins University (Baltimore, MD), September 2014. World Wide Web URL: <http://omim.org/>.

Table 3

Clinical trials in polycystic liver diseases

Study	Patient randomization		Treatment		Liver volume reduction
	Dose	Duration	Route of administration		
Octreotide (targets cAMP)					
Caroli <i>et al.</i> (2010) ¹⁰⁰	ADPKD, n = 12 (5 treated, 7 placebo)	40 mg every 28 days	6 months	Intramuscular	4.5% (vs 0.9% increase in placebo)
Hogan <i>et al.</i> (2010) ¹⁰¹	ADPKD and ADPLD, n = 42 (28 treated, 14 placebo)	40 mg every 28 (± 5) days	12 months	Intramuscular	4.95% (vs 0.92% increase in placebo)
Hogan <i>et al.</i> (2012) ¹⁰²	Extension study: ADPKD and ADPLD, n = 41/42 (all received treatment)	40 mg every 28 (± 5) days	Additional 12 months (24 months total)	Intramuscular	No significant changes (−0.77%) after an additional year of therapy
Lanreotide (targets cAMP)					
Tenmerman <i>et al.</i> (2013) ¹⁰⁵	ADPKD and ADPLD, n = 132 (106 treated, 26 placebo)	90 mg (n = 55) Or 120 mg (n = 51) every 28 days	6 months	Subcutaneous	1.4% (LAN 90 mg) 2.8% (LAN 120 mg) (vs 1.1% increase in placebo) LAN 90 mg had fewer adverse effects
van Keimpema <i>et al.</i> (2009) ¹⁰³	ADPKD and ADPLD, n = 54 (28 treated, 27 placebo)	120 mg every 28 days	24 weeks	Subcutaneous	2.9% (vs 1.6% increase in placebo)
Chrispijn <i>et al.</i> (2012) ¹⁰⁴	Extension study: ADPKD and ADPLD, n = 41/54 (all received treatment)	120 mg every 28 days	12 months	Subcutaneous	4%
Sirolimus vs tacrolimus (targets mTOR)					
Qian <i>et al.</i> (2008) ⁸⁵	ADPKD (sirolimus n = 7, tacrolimus n = 9)	5–10 mg daily (sirolimus) 3 mg twice day (tacrolimus)	Retrospective analysis 19.4 months	Oral	11.9% decrease with sirolimus vs 14.1% increase with tacrolimus
Everolimus alone or in combination with octreotide (targets cAMP and mTOR)					
Chrispijn <i>et al.</i> (2013) ⁸⁸	ADPKD and ADPLD, n = 44 (23 received octreotide and 21 received octreotide + everolimus)	40 mg octreotide every 4 weeks, 2.5 mg everolimus daily	48 weeks	Intramuscular (octreotide) and oral (everolimus)	3.5 ± 5.2% octreotide monotherapy vs 3.8 ± 4.7% in the octreotide/everolimus group (in response to octreotide, everolimus does not further reduce liver volume)

Clinical trial NCT01670110 testing pasireotide in severe polycystic liver disease is underway (Mayo Clinic).¹⁰⁷ Abbreviations: ADPKD, autosomal dominant polycystic kidney disease; ADPLD, autosomal dominant polycystic liver disease; cAMP, cyclic adenosine monophosphate; mTOR, mammalian target of rapamycin.

Table 4

Preclinical studies in animal models of polycystic liver diseases

Study	Drug	Animal model	Treatment	Results
cAMP				
Masyuk <i>et al.</i> (2007) ⁹⁷	Octreotide	PCK rat	Intraperitoneally (10µg/kg daily) for 4, 8, 12, and 16 weeks or 100µg/kg daily for 4 weeks	Reduction of liver weight, cyst volume, hepatic fibrosis, and mitotic indices
Masyuk <i>et al.</i> (2013) ⁹⁹	Pasireotide	PCK rat and <i>Pkd2</i> ^{WS25/-} mouse	Osmotic mini-pumps (20 µg/kg daily) for 6 weeks	Reduction of liver weight, cyst volume, hepatic fibrosis and mitotic indices
Raf kinase				
Spirli <i>et al.</i> (2012) ⁸⁹	Sorafenib	<i>Pkd2</i> ^{fox/-} ;pCxCreER TM (<i>Pkd2cKO</i> mouse)	Orally (20–60 mg/kg daily) for 8 weeks	Increase in liver cyst area, cell proliferation and phosphorylated Erk
mTOR				
Spirli <i>et al.</i> (2010) ⁷³	Sirolimus	<i>Pkd2</i> ^{fox/-} ;pCxCreER TM (<i>Pkd2cKO</i> mouse)	Intraperitoneally (1.5 mg/kg daily) for 8 weeks	Reduction of liver cyst area, liver weight and Pcnα expression
Renken <i>et al.</i> (2011) ⁸⁴	Sirolimus	PCK rat	In drinking water (2 mg/kg daily) for 4, 8 or 12 weeks	Failed to attenuate hepatorenal cystogenesis
Cdc25A				
Masyuk <i>et al.</i> (2012) ⁷⁵	Vitamin K3	<i>Pkd2</i> ^{WS25/-} mouse and PCK rat	In drinking water (0.15 g/l) for 4 and 8 weeks	Reduction of both liver and kidney weights and hepatorenal cystic and fibrotic areas
Masyuk <i>et al.</i> (2012) ⁷⁵	PM-20	<i>Pkd2</i> ^{WS25/-} mouse	Intraperitoneally (1 mg/kg) for 4 weeks	Reduction of hepatorenal cystogenesis
MMPs				
Urribarri <i>et al.</i> (2014) ¹¹⁴	Marimastat	PCK rat	Orally twice a day (0.29 mg/kg daily) for 8 weeks	Reduction of hepatic cystogenesis, fibrosis and inflammation
Hdac6				
Gradilone <i>et al.</i> (2014) ¹¹⁹	ACY-1215	PCK rat	Intraperitoneally (30 mg/kg daily) for 4 weeks	Reduction of hepatic cystogenesis and fibrosis
Ppar-γ				
Yoshihara <i>et al.</i> (2011) ⁹⁰	Pioglitazone (full agonist)	PCK rat	Orally (10 mg/kg daily) for 16 weeks	Reduction of liver weight, liver cystic area and fibrotic index
Yoshihara <i>et al.</i> (2013) ⁹²	Telmisartan (partial agonist)	PCK rat	Orally (3 mg/kg daily) for 16 weeks	Reduction of liver weight, cystic and fibrotic areas
Vegfr2				

Study	Drug	Animal model	Treatment	Results
Spirli <i>et al.</i> (2010) ⁷⁴	SU5416	<i>Pkd1KO</i> and <i>Pkd2KO</i> mice	Intraperitoneally (12.5 mg/kg) twice a week for 8 weeks	Reduction of hepatic cystogenesis, liver weight and levels of phosphorylated Erk and PcnA in <i>Pkd2KO</i> but not <i>Pkd1KO</i> mice
Amura <i>et al.</i> (2007) ⁸²	SU5416	<i>Pkd2</i> ^{WS25/} mouse	Subcutaneously (0.75 mg) every 2 weeks for 4 and 8 months	Reduction of liver cystic area
<i>Egfr</i>				
Torres <i>et al.</i> (2004) ⁸¹	EKI-785 and EKB-569	PCK rat	Intraperitoneally (EKI-785: 90 mg/kg) every 3 days Intraperitoneally (EKB-569: 20 mg/kg) every 3 days Orally (EKB-569: 5, 10 or 20 mg/kg) every 3 days All administered for 7 weeks	No effects on fibrocystic liver disease
<i>Trpv4</i>				
Gradilone <i>et al.</i> (2010) ⁶⁵	Trpv4 activator GSK1016790A	PCK rat	Intraperitoneally (0.01 mg/kg daily) for 8 weeks	Trpv4 activation induced a significant decrease in renal cystic area and a nonsignificant decrease in liver cysts

Abbreviations: cAMP, cyclic adenosine monophosphate; Cdc25A, cyclin division cycle 25A; Egfr, epidermal growth factor receptor; Erk, extracellular-regulated kinase; Hdac6, histone deacetylase 6; MMP, matrix metalloprotease; mTOR, mammalian target of rapamycin; PcnA, proliferating cell nuclear antigen; PPAR- γ , peroxisome proliferator-activated receptor gamma; Raf, rapidly accelerated fibrosarcoma; Trpv4, transient receptor potential cation channel, subfamily V, member 4; Vegfr2, vascular endothelial growth factor receptor 2.