Review

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# Polycystic liver disease: ductal plate malformation and the primary cilium

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**Polycystic livers are found in autosomal dominant polycystic kidney disease (ADPKD), caused by *polycystic kidney disease* (*PKD*)*1* and *PKD2* mutations in virtually all cases, and in isolated polycystic liver disease (PCLD), where 20% of cases are caused by mutations in *Protein kinase C substrate 80K-H* (*PRKCSH*) or *SEC63*. Loss of heterozygosity in single hepatoblasts leads to underlying cystogenic ductal plate malformations. Crucially, actual components driving this development remain elusive. Recent advances have unraveled the roles of transforming growth factor (TGF)-β, Notch and Wnt signaling, transcriptional regulators such as hepatocyte nuclear factor (HNF)6 and HNF1β, as well as cilium function in hepatobiliary organogenesis. In polycystic liver disease, mutation or defective co-translational processing of key elements required for primary cilium formation have been implicated. This review recapitulates liver patterning factors in hepatobiliary development and extracts molecular players in hepatic cystogenesis.**

## A brief introduction to polycystic liver disorders

Liver cysts are a relatively common finding on routine abdominal imaging, being present in approximately 2.5–18.0% of the population [1]. Polycystic livers are defined by the presence of >20 cysts and are estimated to be present in 0.05–0.53% of the population [2]. Polycystic liver disease (PLD; see Glossary) is part of the phenotype of two inherited disorders: autosomal dominant polycystic kidney disease (ADPKD) and isolated polycystic liver disease (PCLD). PCLD is distinguished from ADPKD by the absence of polycystic kidneys. Cystic livers, together with hepatic fibrosis, may also be found in the spectrum of diseases that have dysfunction of the primary cilium (Box 1).

ADPKD, caused by mutations in *polycystic kidney disease* (*PKD*) *1* and *PKD2*, is the most common cause for end-stage kidney disease, and is seen in approximately 0.10–0.25% of the population [3]. *PKD1* encodes the transmembrane protein polycystin-1 and is mutated in 85% of ADPKD cases. *PKD2* encodes the transmembrane protein polycystin-2, which is responsible for 15% of ADPKD cases. Although polycystic kidneys are the primary presentation, liver cysts occur in 83–94% of patients as an extrarenal manifestation [4,5]. PCLD is rare and is estimated to be present in 1:158 000 people [6]. There is considerable genetic heterogeneity as *Protein kinase C substrate 80K-H* (*PRKCSH*) and *SEC63* mutations have been identified only in approximately 20% of cases [7], leaving over 80% of cases currently without a genetic diagnosis.

Cyst development is driven by somatic second hit mutations, which affect wild type alleles of biliary type cells during early hepatic organogenesis [8,9]. This process, termed loss of heterozygosity (LOH), occurs in most cysts associated with *PRKCSH*, *PKD1*, or *PKD2* mutations, and at a lower proportion in *SEC63* mutated cysts [10]. The key processes that lead to development of cysts start early in life. In PCLD it is thought that subsets of cells behave abnormally during maturation of the ductal plate [11]. Unlike normal biliary plate hepatoblasts, these cyst initiator cells do not undergo regression after disconnection from the biliary tree but continue to proliferate.

The molecular disease mechanism leading from focal LOH to a defective response during ductal plate remodeling represents a key event for cystogenesis, but actual components that drive the development remain elusive. In normal hepatobiliary development, the important roles of Notch, transforming growth factor (TGF)-β, and Wnt signaling have recently been unraveled and have afforded better insight into transcriptional regulation by the involved transcription factors [i.e., hepatocyte nuclear factor (HNF)6 and HNF1β]. The common denominator in cystogenesis is the mutation of individual genes that are part of these signal transduction routes, and the disruption of many of these encoded proteins affects the primary cilium. This review aims to recapitulate molecular players in cystogenesis by focusing on known liver patterning factors in hepatobiliary development.

## Normal hepatobiliary development

An overview of the process of normal hepatobiliary development and the roles of liver patterning factors therein is critical to understand the molecular mechanisms of hepatic cyst development. Hepatic organogenesis starts at week

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## Glossary

**Adenomatosis polyposis coli (APC):** is a member of the β-catenin destruction complex of the canonical Wnt signaling pathway. Loss of APC leads to constitutive Wnt pathway activation.
**Autophagy/autophagosome:** catabolic mechanism involving breakdown of unnecessary protein components in lysosome organelles of cells, following transport by autophagosomes. Autophagosome induction and cilium formation have recently been found to be interdependent processes.
**Autosomal dominant polycystic kidney disease (ADPKD):** a common genetic nephropathy, in which PLD is found in 83–94% of patients.
**Canonical Wnt signaling:** is involved in cellular differentiation and proliferation in embryonic and adult tissues. Following activation by Wnt ligands, Frizzled and LRP5/6 receptors initiate signaling through the β-catenin transcription factor by induction of a destruction complex.
**Cholangiocytes/biliary cells:** epithelial cells that line the biliary tract.
**Ciliopathy:** genetic disease caused by dysfunction of the cilium or cilium-anchoring structures. Examples include ADPKD, ARPKD, MKS, and, most probably, PCLD. Ciliopathies are as phenotypically diverse as the cells and tissues on which primary cilia are present; cystic kidneys, fibrocystic liver disease, diabetes, retinal degeneration, anosmia, situs inversus, skeletal involvement, mental retardation, obesity, and infertility are all part of the ciliopathy phenotype spectrum.
**Congenital disorders of glycosylation (CDG):** type I CDG has defects in synthesis and transfer of glycans, whereas type II CDG has defective glycan processing. CDG often presents with ductal plate malformation.
**cyclic AMP (cAMP):** a second messenger important in many biological processes. The cAMP signaling pathway is one of many disturbed in PLD but is important as the pathway is inhibited by SAs.
**Cytokeratins (CKs):** are protein markers commonly used to identify cell types. For example, CK19 marks biliary cells.
**Ductal plate:** layer of hepatoblasts enveloping the portal vein/mesenchyme, bound to become biliary tract cholangiocytes and periportal hepatocytes during hepatobiliary development.
**Ductal plate malformation (DPM):** defects in remodeling of the ductal plate during biliary tract development.
**Extracellular matrix (ECM):** includes the interstitial matrix and basement membrane of multicellular tissues. It functions in cell adhesion, cell-to-cell communication, and cellular differentiation.
**Hepatoblast:** bipotent cell that may differentiate towards biliary or hepatocyte cells. Normally present during embryology, but bipotent liver progenitors persist in the biliary tract during adulthood.
**Hepatocyte:** main epithelial cell of the liver parenchyma.
**Notch signaling:** notch signaling is involved in cellular differentiation and proliferation in embryonic and adult tissues. Following activation by Notch or Jagged ligands, Notch receptors initiate signaling through Rbpj and Hes/Hey transcription factor families.
**Polycystic kidney disease (PKD):** polycystic kidneys are the primary presentation of PKD, but polycystic livers are found in most patients. *PKD1* and *PKD2* are the genes responsible for ADPKD in 85% and 15% of cases, respectively. *PKHD1* is the gene responsible for virtually all incidences of ARPKD.
**Polycystic liver disease (PLD):** PLDs are defined as presence of >20 cysts in the liver.
**Polycystic liver disease (PCLD):** a rare form of PLD, which presents without polycystic kidneys.
**Primary cilia:** solitary, non-motile organelles on the apical cell membrane surface of most mammalian cells involved in mechano-, osmo-, and chemoreception. In addition, primary cilia are implicated in planar cell polarity, cell cycle control, and numerous essential signaling pathways.
***Protein kinase C substrate 80K-H (PRKCSH)*:** is one of two genes known to cause PCLD, and *PRKCSH* mutation is found in more than 10% of cases. It encodes the protein hepatocystin that serves as the non-catalytic β-subunit of GII and is involved in N-glycan processing.
***SEC63*:** is one of two genes known to cause PCLD. *SEC63* mutation is found in more than 5% of cases. It encodes the Sec63p protein, which is part of the Sec61 complex involved in co- and post-translational protein transport.
**Somatostatin analogs (SAs):** include lanreotide, octreotide, and pasireotide. cAMP regulation can be orchestrated by SAs through SSTRs. Lanreotide, octreotide, and pasireotide have different binding affinities for SSTR subtypes as well as different half-lives, which affects clinical effectivity.
**Transforming growth factor (TGF)-β signaling:** is involved in cellular differentiation and proliferation in embryonic and adult tissues. Following activation by TGF-β ligands, TGF-β receptors initiate signaling through SMAD complex transcription factors.

4, or embryonic day (E) 8.5 in mice [12], when the liver bud arises from the cephalic part of the primitive foregut near the yolk sac [11]. At that point the pars hepatica, one of two liver bud divisions, initiates liver precursor cells to grow into mesenchymal separation between the pericardial and peritoneal cavities. These cells start to occupy endothelium-lined spaces of the septum transversum. In this manner, the basal liver structure is formed, with parenchymal cords and plates separated by hepatic sinusoids. At week 8, or E10.5 in mice [13], the first intrahepatic bile duct (IHBD) precursors develop. Cytokeratin (CK)8, CK18, and CK19 positive hepatoblasts start organizing in a ductal plate [11], enveloping the portal mesenchyme. This ductal plate induction is tightly controlled by a signaling environment involving several key signaling pathways [12].

### *TGF-β, Notch, and Wnt signaling pathways*

One of the signaling pathways that drives ductal plate development is TGF-β [13]. A signaling gradient of increased TGF-β/activin near the portal vein is implicated in ductal plate development at mouse E12.5. The TGF-β antagonist chordin is present in liver parenchyma at E16.5, whereas the presence of TGF-β transcription factor SMAD5 is limited to portal hepatoblasts [14]. The TGF-β gradient occurs in combination with HNF6-induced repression of the TGF-β receptor II (TGF-βRII) in the liver parenchyma. TGF-βRII positive prebiliary cells shape a primary layer near the portal vein, a process that is associated with subsequent loss of TGF-βRII expression. Subsequently, a second layer of TGF-βRII positive hepatoblasts is created at the parenchymal side of the primary layer [13]. This allows development of a lumen between the asymmetric layers. Maturation of these layers expands in a wave starting at the largest portal veins of the liver hilum propelling towards the periphery, inducing the prebiliary hepatoblasts of the second layer to become cholangiocytes.

Notch signaling is required for the processes of differentiation and tubulogenesis, as well as bile duct density [12,15]. Notch receptors are activated by ligands such as Jagged1 and Notch2 from the periportal mesenchyme and biliary cells, which signal through (Recombining binding protein suppressor of hairless) Rbpj and hairy and enhancer of split-1 (Hes)/Hairy/enhancer-of-split related with YRPW motif (Hey) family members. Both TGF-β and Notch signaling have consistently been implicated in *in vitro* murine biliary fate specification of bipotent adult liver stem cells [16].

Similarly, a gradient of canonical Wnt signaling appears to regulate the ductal plate [17]. Effects of Wnt signaling are time- and context-dependent during liver development. In the ductal plate of wild type mice, the Wnt signal transducer β-catenin is strongly present at the membrane, whereas hepatoblasts elsewhere have a much weaker membranous β-catenin staining. This stabilization of β-catenin is transiently induced at mouse E15.5, reaching a peak membranous intensity on E17.5 up to adulthood. It is therefore hypothesized that Wnt, generated by the portal vein or mesenchyme, may help induce ductal plate formation. This concept is supported by *in vitro* studies that show that early embryonic liver cultures lack $CK19^+$ biliary cells following β-catenin silencing [18], whereas culture in Wnt3a conditioned media leads to biliary differentiation [19]. Fibroblast growth factor (FGF) and bone morphogenetic protein (BMP) signaling pathways, together with