Review

Cholangiopathies – Towards a molecular understanding

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A B S T R A C T

Liver diseases constitute an important medical problem, and a number of these diseases, termed cholangiopathies, affect the biliary system of the liver. In this review, we describe the current understanding of the causes of cholangiopathies, which can be genetic, viral or environmental, and the few treatment options that are currently available beyond liver transplantation. We then discuss recent rapid progress in a number of areas relevant for decoding the disease mechanisms for cholangiopathies. This includes novel data from analysis of transgenic mouse models and organoid systems, and we outline how this information can be used for disease modeling and potential development of novel therapy concepts. We also describe recent advances in genomic and transcriptomic analyses and the importance of such studies for improving diagnosis and determining whether certain cholangiopathies should be viewed as distinct or overlapping disease entities.

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Keywords:
Liver
Transplant
Cholangiocyte
Hepatocyte
Bile duct
Organoid
Alagille syndrome
Biliary atresia
Primary sclerosing cholangitis (PSC)
Primary biliary cholangitis (PBC)
Cystic fibrosis

1. Liver development and function

The liver originates from the ventral foregut endoderm and the hepatoblasts - cells that will give rise to cholangiocytes (a.k.a. biliary epithelial cells, BEC) and hepatocytes - emerge around embryonic day 8.5 in the mouse (Fig. 1A, B). The liver bud grows and at E9.5 envelops the vitelline, umbilical and posterior cardinal veins, leading to a close association between venous endothelial cells and hepatoblasts [1] (Fig. 1C–F). The veins undergo extensive branching and once surrounded by hepatoblasts, vasculogenesis creates a network of hepatic sinusoids. In humans, it is unclear whether the vitelline veins contribute to the hepatic venous system, and instead it has been suggested that the left umbilical vein is the origin of the human hepatic venous system [2]. Importantly, the vasculature plays a key role in biliary development, and portal mesenchyme surrounding the portal vein and portal

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sinus signals to hepatoblasts to initiate intrahepatic bile duct formation via transforming growth factor-β (Tgfb-2 and Tgfb-3) [3,4] and Notch signaling (via the ligand Jagged1) [5] (Fig. 1G–J). Next, bile ducts and hepatoblasts secrete angiogenic factors that induce hepatic artery formation (Fig. 1K) [6], demonstrating a reciprocal relationship between the vascular and biliary systems in inducing one another’s formation and maintenance.

The mechanisms controlling hepatoblast differentiation to the hepatocyte or cholangiocyte lineages are incompletely understood, but a number of signaling pathways including Wnt, FGF, TGFβ, and Notch have emerged as important regulators of cholangiocyte differentiation. The fact that dysregulated Notch signaling causes Alagille syndrome demonstrates the importance of these pathways for human health. Recently, the transcriptomic signature for the mouse hepatoblast lineage choice towards a hepatocyte or cholangiocyte fate was derived [7], showing that protein kinase C/mitogen-activated protein kinase (PKC/MAPK) signaling enhances cholangiocyte maturation. For early human hepatic differentiation, an analysis of in vitro differentiation of pluripotent cells to the hepatocytic lineage identified VEGF signaling as a driver of endothelial vascularization and hepatoblast differentiation [8].

The bile duct system is composed of intra- and extrahepatic ducts. The intrahepatic bile ducts are generated when cholangiocytes surrounding the portal vein first form the ductal plate, a layer of cholangiocytes surrounding the portal vein, in a process that initiates near the hilum and progresses towards the periphery. Small lumina form, with cholangiocytes on the portal side and hepatoblast-like cells on the parenchymal side that subsequently differentiate into cholangiocytes. In mice, bile ducts then induce formation of the hepatic artery, while in humans the inductive signal is thought to come from the ductal plate itself.

The bile duct system is important for transport of bile, which the liver produces to facilitate digestion of lipids and bilirubin excretion. Hepatocytes secrete bile into the canalicular space and further into the canals of Hering, where it is then transported to the bile ducts. The bile ducts then convey bile to the duodenum for further processing and elimination from the body.
canals of Hering, which are lined jointly by hepatocytes and cholangiocytes (Fig. 2). Cholangiocytes contribute to the bile composition by secretion of fluids and electrolytes. The bile is then further transported via the bile ducts to the gall bladder for storage. The intrahepatic biliary tree is formed by convergence of small bile ductules into larger bile ducts towards the hilum, ending up in the left and right hepatic ducts. The extrahepatic biliary system resides outside the liver and includes the common hepatic duct, common bile duct and gallbladder.

Biliary Atresia (BA) (see below) is a cholangiopathy that mostly affects the extrahepatic biliary tree. The transcription factors Pdx1, Hes1 and Sox17 are important for development of the extrahepatic biliary tree [2] and Sox17 expression is downregulated in experimental models for BA. It has long been established that cholangiocytes are a heterogeneous cell population, and can, for example, be subdivided into large and small cholangiocytes, which differ in terms of expression of certain markers such as the secretin receptor and CFTR (for review see [10,11]). The extent of cholangiocyte heterogeneity is however not well understood. Recent studies provide evidence for at least two major cholangiocyte populations but how they relate to morphologically distinguishable cholangiocyte subtypes is not clear. Cholangiocytes immunoreactive for MIC1-IC3 and expressing high levels of ST14 (suppression of tumorigenicity 14), are far more clonogenic than ST14-low cells, but express similar levels of Sox9, EpCAM, Krt19 and Hnf1β. On the other hand, ST14-high cholangiocytes express higher levels of Pkhd11, Bmp4, Vim and Rspo1 [12,13], and can engraft when transplanted into mice. Importantly, the MIC1-IC3 monoclonal antibody, from Novus Biologicals, is raised against nonparenchymal cells from DDC-treated mice, and is suggested to react with oval cells/hepatic proliferating duct cells, which means these experiments enrich for cells present or arising in ductular regenerative processes. The organization of possible subclasses of cholangiocytes along the biliary tree still needs to be established, and it will be interesting to learn whether there are for example hilar–peripheral zonation principles similar to the recently established portal-central zonation of hepatocytes [14].

Single cell RNA-sequencing has provided higher-resolution insight into liver cell populations, as well as into the various differentiation steps (Table 1). Sequencing of different organs during mouse embryonic development (E9.5-E11.5) confirmed a transient hybrid epithelial/ mesenchymal cell state [15] previously identified in a small subset of liver cells by single cell RNA-sequencing [16], and also suggested by experiments transplanting mesenchymal cells into liver via intrasplenic injection, wherein the mesenchymal cells adjacent to intrahepatic vascular structures took on a hepatic fate [17]. Single cell RNA-sequencing of developing liver also suggests a self-regulating transcription factor network including Hnf4α, Hnf1β and Grhl2 [15], and both Hnf1β and Grhl2-regulated networks are enriched for target genes regulating tube development. Future work to dissect apart the regulatory networks controlling cholangiocyte differentiation and bile duct morphogenesis will improve our understanding of embryonic development, as well as providing crucial guidance to develop therapeutics or improve stem cell differentiation protocols for cell replacement therapy. As an example, when differentiated induced pluripotent stem (iPSC) cells, mesenchymal stem cells (MSCs) and human umbilical vein endothelial cells (HUVECs) were co-cultured, hypoxia was shown to regulate hepatic vs cholangiocyte differentiation via suppression of

![Fig. 2. The biliary system of the liver.](image-url)
| Species, stage | Number of cells sequenced | Method used and Read depth | Main findings related to cholangiocytes | Additional notes | Reference |
|---------------|---------------------------|---------------------------|----------------------------------------|------------------|-----------|
| Mouse         | >50 mouse tissues         | Microwell-Seq             | Adult liver scRNA seq identified (in addition to several other cell types) 4 types of hepatocytes: pericentral, periportal, Fabp1-high, and mt-Nd4 high; and identified two types of epithelial (biliary) cells: undefined, and Spp1-high. | Including cell lines/cultures, ~400,000 cells were sequenced in this paper. Liver not explicitly discussed in main text, some data in supplementary figures and data available and explorable at http://bis.zju.edu.cn/MCA. Fetal liver is mainly immune cells, as well as AFP-high hepatocytes and stem/progenitor cells. | [138] |
| Adult female liver 6–10 weeks and fetal liver E14.5 | 60,000 cells total; 3730 cells from fetal liver; 6426 cells from adult liver | Proof of principle in cell lines shows saturated sequencing yields 6,500 genes from 55,000 transcripts per cell. Sequencing depth used for tissues not stated. | Adult liver scRNA seq identified (in addition to several other cell types) 4 types of hepatocytes: pericentral, periportal, Fabp1-high, and mt-Nd4 high; and identified two types of epithelial (biliary) cells: undefined, and Spp1-high. | Including cell lines/cultures, ~400,000 cells were sequenced in this paper. Liver not explicitly discussed in main text, some data in supplementary figures and data available and explorable at http://bis.zju.edu.cn/MCA. Fetal liver is mainly immune cells, as well as AFP-high hepatocytes and stem/progenitor cells. | [138] |
| Mouse         | E11.5-P2.5 dissociated and randomly picked on a C1 RNA-Seq IFC (Fluidigm), P3.25 FACS sorted for Epcam | C1 Fluidigm chip | Cholangiocytes isolated as Epcam positive cells showed high Spp1 expression, and higher expression of Jag1/Notch2 and Hes1 than hepatoblasts. Comparison of embryonic hepatoblasts with Epcam+ cholangiocytes at P3.25 showed that the two E11.5 hepatoblasts (but not later embryonic hepatoblasts) clustered with the cholangiocytes, suggesting hepatoblasts may commit to this fate earlier than previously thought. | Hepatoblast/mesenchymal hybrid cells co-express Dlk1 and Vimentin. Cdh1 is proposed as a highly specific and sensitive marker for isolation of embryonic hepatoblasts. | [16] |
| E11.5, 12.5, 13.5, 14.5, 16.5, 18.5, P2.5 whole liver and P3.25 Epcam-sorted cells | 557 cells from dissociated liver, 52 from Epcam-sorted P3.25 | For dissociated liver: unique mapped reads 1.1 - 3.8 million per cell. 3000-6000 genes per cell with FPKM >1. For Epcam-sorted cells, 2000 genes per cell at same sequencing depth and mapping rate. | Cholangiocytes isolated as Epcam positive cells showed high Spp1 expression, and higher expression of Jag1/Notch2 and Hes1 than hepatoblasts. Comparison of embryonic hepatoblasts with Epcam+ cholangiocytes at P3.25 showed that the two E11.5 hepatoblasts (but not later embryonic hepatoblasts) clustered with the cholangiocytes, suggesting hepatoblasts may commit to this fate earlier than previously thought. | Hepatoblast/mesenchymal hybrid cells co-express Dlk1 and Vimentin. Cdh1 is proposed as a highly specific and sensitive marker for isolation of embryonic hepatoblasts. | [16] |
| Mouse         | E9.5, E10.5 & E11.5 liver. | Organs dissected and trypsinized, individual cells mouth pipetted to lysis buffer. | E9.5-E11.5 liver possibly contains multiple clusters of mesoderm-derived cells, one clear cluster of epithelial cells and possibly several clusters of hematopoietic cells. Epithelial cells with mesenchymal features: some Epcam/Cdh1 positive cells in liver also express Vimentin. Dlk1 expression not described. | 1916 cells in total sequenced. Cells with fewer than 2000 genes/cell removed -- 1819 were used in analyses, from embryonic mouse including forebrain, hindbrain, skin, heart, somite, lung, liver, and intestine. | [15] |
| Human         | Liver bud organoid cells: Liver bud organoids, different constellations of cells: 177 cells dissociated, no selection. Isolation of adult human liver cells: 256 cells from human adult liver. Protocol of hepatocyte or other cell isolation from adult liver published in [82]; liver is dissociated and cell types separated using centrifugation steps. Isolation of fetal human cells: 238 cells from fetal stages, dissociated and briefly cultured (12h) on laminin-coated plates to remove red blood cells, followed re-dissociation of cells. Isolation of mouse hepatoblasts: 92 cells from mouse liver, dissociated, erythrocytes were lysed, and magnetic | C1 Fluidigm chip | This manuscript does not explicitly identify cholangiocytes, but provides valuable insight into which culture systems better support in vitro differentiation faithful to in vivo hepatoblast growth. | IPSC-derived hepatoblasts undergoing culture in liver bud organoids more closely resemble fetal liver hepatic cells than do 2D cultured IPSC-derived hepatoblasts. Ligand-receptor pair analyses of co-cultured cells in organoids showed a KDR/VEGFA signaling pair in which VEGFA secreted by immature hepatocytes stimulates KDR on endothelial cells, which in turn support hepatoblast growth. | [8] |
| In vitro: 2D culture of iPSCs (TKDA3–4, University of Tokyo) undergoing hepatic differentiation and 3D culture of liver bud organoids derived from hepatic cells differentiated from the iP5 cell line, cocultured with HUVECs (Lonza) and MSCs (Lonza) | 332 sequenced cells from liver, 320 used after QC for further analyses | Modified STRT protocol An average of 6361 genes per cell from 0.43 million UMI transcripts. | This manuscript does not explicitly identify cholangiocytes, but provides valuable insight into which culture systems better support in vitro differentiation faithful to in vivo hepatoblast growth. | IPSC-derived hepatoblasts undergoing culture in liver bud organoids more closely resemble fetal liver hepatic cells than do 2D cultured IPSC-derived hepatoblasts. Ligand-receptor pair analyses of co-cultured cells in organoids showed a KDR/VEGFA signaling pair in which VEGFA secreted by immature hepatocytes stimulates KDR on endothelial cells, which in turn support hepatoblast growth. | [8] |
| Mouse         | E14.5, E15.5, and E16.5 | Liver bud organoid cells: Liver bud organoids, different constellations of cells: 177 cells dissociated, no selection. Isolation of adult human liver cells: 256 cells from human adult liver. Protocol of hepatocyte or other cell isolation from adult liver published in [82]; liver is dissociated and cell types separated using centrifugation steps. Isolation of fetal human cells: 238 cells from fetal stages, dissociated and briefly cultured (12h) on laminin-coated plates to remove red blood cells, followed re-dissociation of cells. Isolation of mouse hepatoblasts: 92 cells from mouse liver, dissociated, erythrocytes were lysed, and magnetic | This manuscript does not explicitly identify cholangiocytes, but provides valuable insight into which culture systems better support in vitro differentiation faithful to in vivo hepatoblast growth. | IPSC-derived hepatoblasts undergoing culture in liver bud organoids more closely resemble fetal liver hepatic cells than do 2D cultured IPSC-derived hepatoblasts. Ligand-receptor pair analyses of co-cultured cells in organoids showed a KDR/VEGFA signaling pair in which VEGFA secreted by immature hepatocytes stimulates KDR on endothelial cells, which in turn support hepatoblast growth. | [8] |
TGFB2 is expressed in mesenchymal cells (MCs) while both TGFB1 and TGFB3 are expressed in ECs and MCs. TGFB receptor 1 (TGFBR1) is expressed in fetal hepatocytes and MCs.

4822 cells sequenced in total that passed quality control, of which 2636 were embryoid body cells. Day 8 embryoid bodies included liver-like cells characterized by APOA1, TTR, FGB and AFP.

Hypoxia induces hepatic differentiation. Epithelial cell cluster is SOX9 and FOXP1 positive, and differentiation is regulated by Hippo and AMPK pathways. This could be a liver epithelial (biliary) population, or other epithelial cells.

Human

Epithelial cell cluster is SOX9 and FOXP1 positive, and differentiation is regulated by Hippo and AMPK pathways. This could be a liver epithelial (biliary) population, or other epithelial cells.

Cells allowed to differentiate into embryoid bodies in vitro and dissociated for analysis. Naïve-like H9 iPSCs, primed iPSCs, and embryoid bodies.

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Cells identified as liver cells. 498 cells identified as epithelial.

Reanalysis of cells in [8] is expectant and depends on clinical features. Localized forms can be characterized by saccular dilatations of the intrahepatic bile ducts. Treatment is transplantation. Caroli disease (CD) is a rare hereditary disorder characterized by innumerable small ducts in the liver, heart, skeleton, kidneys and eyes. The most common symptom is prolonged neonatal jaundice caused by progressive ductal paucity.

Cholangiopathies may be caused by genetic, viral, and environmental insults, as well as unknown stimuli. All cholangiopathies are associated with obstructed bile flow, immune responses and cholangiocyte proliferation. They are chronic diseases affecting the biliary epithelium which can proceed to biliary fibrosis, liver parenchymal damage, and further to endstage liver disease, requiring liver transplantation. Cholangiopathies can be classified into primary and secondary cholangiopathies, depending on whether the bile ducts are directly targeted in a disease (primary) or whether the bile ducts degrade as a consequence of injury or other pathological processes in the biliary tree (secondary). The salient features of the primary cholangiopathies, which are the main focus of this review, with regard to prevalence, genetics and current therapy possibilities, are summarized in Table 2 and Suppl File 1 (for a complete list of primary and secondary cholangiopathies, see [20]).

Briefly, biliary atresia (BA) is a devastating, progressive, inflammatory, fibro-obliterating cholangiopathy and the predominant surgical cause for prolonged neonatal jaundice. The standard treatment is timely diagnosis and performance of Kasai portoenterostomy: jaundice clearance is however achieved in only 60–70% of treated patients. Recurrent cholangitis, portal hypertension and cirrhosis remain life-long risks and 50% of patients eventually require liver transplantation. Alagille syndrome (ALGS) is a rare inherited genetic multi-organ disorder affecting the liver, heart, skeleton, kidneys and eyes. The most common symptom is prolonged neonatal jaundice caused by progressive ductal paucity. Currently, apart from liver transplantation, treatment modalities are supportive. Primary biliary cholangitis (PBC) is a chronic, progressive, immune-mediated cholestatic liver disease characterized by inflammatory damage of the intrahepatic bile ducts of small to intermediate sizes. Patients may present with fatigue and pruritus, and eventually develop cirrhosis and liver failure. Currently, the only FDA-approved medical treatment is ursodeoxycholic acid which improves liver function and delays disease progression. Some potential therapeutic agents from clinical trials are promising especially for non-responders to ursodeoxycholic acid. Primary sclerosing cholangitis (PSC) is a chronic, progressive cholestatic fibroinflammatory disease causing multifocal strictures and segmental dilatations of the intrahepatic and extrahepatic bile ducts. PSC is associated with inflammatory bowel disease (IBD), particularly ulcerative colitis (UC) in 80% of patients. Without a known cause, the only current curative treatment modality is liver transplantation. Caroli disease (CD) is a rare hereditary disorder characterized by saccular dilatations of the intrahepatic bile ducts. Treatment is expectant and depends on clinical features. Localized forms can be...
treated by hepatic resections but diffuse disease ultimately requires liver transplantation. Up to 30% of Cystic Fibrosis patients develop cystic fibrosis-associated liver disease (CFLD). Viscous and reduced bile flow result in cholangiocyte injury, periductal inflammation, abnormal bile duct proliferation and perportal fibrosis. Clinical features appear late and are related to damage of the hepatobiliary system. Current treatment is expectant. Improved understanding of the pathophysiology is the key to developing more disease-specific therapeutics. Polycystic liver diseases (PLD) are autosomal dominant disorders characterized by embryonic ductal plate malformation of the intrahepatic biliary tree. Initial treatment is conservative, with the use of somatostatin analogues to halt cyst growth. Surgical decompression and liver transplantation may eventually be required. Some primary cholangiopathies, including primary sclerosing cholangitis (PSC), cholelithiasis, Caroli disease and Caroli syndrome, and cirrhosis itself are risk factors for development of malignant cholangiocarcinoma, a liver cancer with poor prognosis [21].

The genetic contribution to cholangiocarcinopathy differs extensively between the different disease forms, ranging from diseases with a clear-cut monogenic cause, to diseases which are largely idiopathic, with only susceptibility genes identified. Monogenic diseases include Notch pathway mutations in ALGS [4,22], and claudin mutations in neonatal sclerosing cholangitis [2]. While diseases which are largely idiopathic, with only susceptibility genes identified, include BA (which is associated with ADD3 mutations in a small fraction of patients [23,24], and with additional susceptibility loci defined), and PSC, in which 23 susceptibility loci have been reported [25,26].

### 3. Modelling cholangiopathies in vivo and in vitro

In vivo and in vitro modeling increasingly contribute to unraveling disease mechanisms and providing platforms for exploring new therapies. With regard to cholangiopathies, an important step was the development of protocols that direct stem or progenitor cells to differentiate into cholangiocytes. Protocols for deriving cholangiocytes from human embryonic stem cells (ES cells) and iPS cells have been established [27–30], which open up new vistas for disease modeling, as iPS cells can be derived directly from cholangiopathic patients and retain the genetic configuration of the patient. The ability to develop organoids, i.e. mini-organs, from various organs is another important technological development of protocols that direct stem or progenitor cells to differentiate into cholangiocytes. With regard to cholangiopathies, an important step was the development of protocols that direct stem or progenitor cells to differentiate into cholangiocytes. Protocols for deriving cholangiocytes from human embryonic stem cells (ES cells) and iPS cells have been established [27–30], which open up new vistas for disease modeling, as iPS cells can be derived directly from cholangiopathic patients and retain the genetic configuration of the patient. The ability to develop organoids, i.e. mini-organs, from various organs is another important technological development, and this approach has recently been applied also to the liver. In one liver organoid system, EpCam+ ductal cells produce cholangiocytes, but can, upon R-spondin withdrawal, switch to produce arterial endoderm, abnormal bile duct proliferation and perportal fibrosis. Clinical features appear late and are related to damage of the hepatobiliary system. Current treatment is expectant. Improved understanding of the pathophysiology is the key to developing more disease-specific therapeutics. Polycystic liver diseases (PLD) are autosomal dominant disorders characterized by embryonic ductal plate malformation of the intrahepatic biliary tree. Initial treatment is conservative, with the use of somatostatin analogues to halt cyst growth. Surgical decompression and liver transplantation may eventually be required. Some primary cholangiopathies, including primary sclerosing cholangitis (PSC), cholelithiasis, Caroli disease and Caroli syndrome, and cirrhosis itself are risk factors for development of malignant cholangiocarcinoma, a liver cancer with poor prognosis [21].

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### Table 2

| Cholangiopathy                  | Prevalence: Sex preponderance | Current therapy                                                                 | Genetic cause                             | Ref. |
|---------------------------------|------------------------------|--------------------------------------------------------------------------------|-------------------------------------------|------|
| Genetic                         |                              |                                                                                |                                           |      |
| Alagille syndrome (ALGS)        | 2.2–3.3 in 100,000 live births; no sex preponderance | Medical: supportive; Surgical: liver transplantation | JAG1 (majority), NOTCH2                  | [83] |
| Caroli disease (CD) and         | 0.1 in 100,000 live births; no sex preponderance | Medical: supportive; Surgical: portosystemic shunting, liver transplantation | PRHD1                                     | [84] |
| Caroli syndrome (CS) with       |                              | Medical: Ursodeoxycholic acid (UDCA), supportive; Surgical: liver transplantation | CFTR                                      |      |
| congenital hepatic fibrosis     |                              |                                                                                |                                           |      |
| Cystic fibrosis-associated      | 12.5 in 100,000 live births | Medical: supportive; Surgical: aspiration of cyst fluid, liver transplantation (uncommon indication) | ADPLD: PKCSH, SECE2, ADPKD: PRK2, PRK1, PRK2, CANAB, ARPKD: PRKD1 | [85]; [86] |
| liver disease                   |                              |                                                                                |                                           |      |
| Polycystic liver disease        | ADPLD: 1–9 in 100,000 live births; | Medical: supportive; Surgical: aspiration of cyst fluid, liver transplantation (uncommon indication) | ADPLD: PKCSH, SECE2, ADPKD: PRK2, PRK1, PRK2, CANAB, ARPKD: PRKD1 | [84]; [87] |
| (autosomal dominant polycystic  | ADPKD: 100–250 in 100,000 live births; | Medical: supportive; Surgical: aspiration of cyst fluid, liver transplantation (uncommon indication) | ADPLD: PKCSH, SECE2, ADPKD: PRK2, PRK1, PRK2, CANAB, ARPKD: PRKD1 | [84]; [87] |
| liver disease (ADPKD, autosomal | ARPKD: 5 in 100,000 live births | Medical: supportive; Surgical: aspiration of cyst fluid, liver transplantation (uncommon indication) | ADPLD: PKCSH, SECE2, ADPKD: PRK2, PRK1, PRK2, CANAB, ARPKD: PRKD1 | [84]; [87] |
| dominant polycystic kidney       |                              | Medical: supportive; Surgical: aspiration of cyst fluid, liver transplantation (uncommon indication) | ADPLD: PKCSH, SECE2, ADPKD: PRK2, PRK1, PRK2, CANAB, ARPKD: PRKD1 | [84]; [87] |
| disease ADPKD, autosomal recessive polycystic kidney disease ARPKD |                              | Medical: supportive; Surgical: aspiration of cyst fluid, liver transplantation (uncommon indication) | ADPLD: PKCSH, SECE2, ADPKD: PRK2, PRK1, PRK2, CANAB, ARPKD: PRKD1 | [84]; [87] |
| Idiopathic/multifactorial        |                              | Medical: supportive; Surgical: aspiration of cyst fluid, liver transplantation (uncommon indication) | ADPLD: PKCSH, SECE2, ADPKD: PRK2, PRK1, PRK2, CANAB, ARPKD: PRKD1 | [84]; [87] |
| Biliary atresia                 | 5–14.3 in 100,000 live births; higher prevalence in Asia; female: male ratio 1:4-1 | Medical: post-operative systemic corticosteroids, choleretic (agent stimulating bile flow) | Surgical: Kasai portoenterostomy, liver transplantation | [88] |
| Primary biliary cholangitis     | 35 in 100,000; female: male ratio 9:1 | Medical: UDCA, supportive; Surgical: liver transplantation | Medical: UDCA, supportive; Surgical: liver transplantation | [89] |
| (formerly, primary biliary cirrhosis) |                              | Medical: supportive; Surgical: liver transplantation | Medical: UDCA, supportive; Surgical: liver transplantation | [89] |
| Primary sclerosing cholangitis  | 4 in 100,000; female: male ratio 1:2 | Medical: supportive; Surgical: therapeutic endoscopic retrograde cholangiopancreatography (ERCP), biliary reconstruction, liver transplantation | Medical: UDCA, supportive; Surgical: liver transplantation | [89] |
| Autoimmune cholangitis          | Not well-defined. Considered as autoimmune hepatitis-PBC/PSC overlaps | Medical: post-operative systemic corticosteroids, choleretic (agent stimulating bile flow) | Surgical: Kasai portoenterostomy, liver transplantation | [91] |
| Idiopathic childhood/adolescent | 0.5 in 100,000; male preponderance | Medical: supportive; Surgical: liver transplantation | Medical: supportive; Surgical: liver transplantation | [92] |
| ductopenia and IgG4-related cholangitis | 4.6 in 100,000 (Japan); male preponderance | Medical: supportive; Surgical: liver transplantation | Medical: supportive; Surgical: liver transplantation | [93] |
| Malignant                       | 1–2 in 100,000 live births (North America) | Non-surgical: transarterial chemoembolization, transarterial radioembolization, radiofrequency ablation (for unresectable tumors) | Surgical: complete resection, liver transplantation | [94] |

[Ref. 83]: Tam et al. / EBioMedicine 35 (2018) 381–393
Organoids derived from iPS cells have also been used to model some cholangiopathies including ALGS, polycystic liver disease and cystic fibrosis [30]. More recently, organoids from the extrahaepatic biliary tree have been developed, and, as discussed in further detail below, show promise in replacing failing or lost biliary tissue in a mouse model for biliary injury [32].

Animal models are increasingly important in disease research. Rodent-based models have yielded valuable insights into cholangiopathies, although it should be remembered that there are important differences between humans and rodents in terms of liver function, which may limit the extent to which rodent data can be extrapolated to humans. Bile duct ligation models have been available for half a century [33] and recapitulate important aspects of cholangiopathies, such as cholangioyte proliferation and fibrosis, although at a much more rapid pace than in the human equivalent. To mimic xenobiotic-induced cholangiopathies, feeding rodents toxic substances such as 3,5-diethoxy carbonyl-1,4-dihydrocollidine (DDC) and alpha-naphthy-isothiocyanate (ANT) has been extensively deployed, and these models provide a more slowly developing fibrosis, accompanied by bile duct proliferation, inflammation and infiltration of immune cells (for review see [33]). To study BA, infection of mice by Rhesus rotavirus type A (RRV) immediately after birth has proven useful to mimic the disease process [34]. An interesting recent addition to BA modeling is the plant toxin biliratrese, which disrupts the extrahaepatic biliary system in zebrafish and causes disrupted cell polarity in cholangioyte organoids [35]. An intriguing aspect of biliratrese is its reduction of the transcription factor Sox17, which, as discussed above, is a critical factor for biliary development.

Transgenic mouse models have significantly contributed to an improved understanding of cholangiopathies, notably for diseases in which specific monogenic mutations are prevalent (Table 3). Mutations in the human MDR3 gene, which encodes a transport protein important for phosphatidylcholine excretion into bile, leads to cholestasis and biliary cirrhosis due to bile toxicity [36], and is also associated with cholelithiasis [37,38]. In keeping with this, the Mdr2 knockout (KO) mouse develops peribiliary inflammation as a result of breakdown of cholangioytes in the biliary barrier [39]. An important but sometimes neglected aspect of cystic fibrosis, more generally considered a lung disorder, is the development of peribiliary fibrosis. While mice deficient for Cfr, encoding a transmembrane chloride channel, do not spontaneously develop cholangiopathies [40] until the age of 1 year [41], a liver phenotype can be provoked with oral dextran [33,42]. This work has more recently been extended to also define the proto-oncogene Src as an effector for a cholangioyte phenotype in Cfr-deficiency [43,44]. Hepatic fibrosis and Caroli disease, which are caused by mutations in the PKHD1 gene [33], have also been assessed in transgenic mouse models, and disruption of the mouse Phkd1 gene leads to aberrant bile duct development with cyst formation [45]. Mice heterozygous for the transcription factor gene Sox17 on specific genetic backgrounds recapitulate some aspects of BA [46], which is interesting in the light of the observed downstream effects of biliratrese, which includes downregulation of Sox17 levels (see above).

Research on the pathomechanisms for ALGS, which in the majority of cases is caused by mutations in the Notch ligand Jagged1 (and with a minority of patients instead carrying NOTCH2 mutations), has benefitted hugely from analysis of transgenic mouse models. A conditional knock out of the Notch ligand Jag1 in portal vein mesenchyme [5] as well as Jag1/Notch2 double heterozygous mice [48] generate a bile duct phenotype resembling ALGS. Interestingly, a heterozygous Jag1 mouse model on a C57B6 genetic background generates an ALGS phenotype, and deletion of the Notch glycosyltransferase Puglut1 ameliorates the phenotype [50], arguing that the dosage of Notch signaling is important for development of ALGS. A recent transgenic model demonstrates that a missense mutation in Jag1 (Jag1H309R), which lies in a hotspot for ALGS missense mutations, in homozygous form is sufficient to recapitulate most of the symptoms seen in patients including jaundice and ductopenia [51]. An interesting feature of these models is that cholestasis is generally transient in early postnatal mice, while adults display no cholestasis. This suggests that Notch-independent compensatory mechanisms can rescue ductopenia, and indeed, while in the majority of patients biliary breakdown continues [52,53], some patients with ALGS recover from cholestasis with time and even display regenerating liver nodules [51]. Recent work has taken ALGS mouse models one step further, identifying TGFβ signaling as a driver of adult Notch-independent regeneration of the biliary system, inducing hepatocytte transdifferentiation [54]. Collectively these studies suggest that there may be a therapeutic window for ALGS therapy and provide targets for intervention.

4. The importance of cell polarity for bile duct integrity and function

The disease processes leading to cholangiopathies are complex and multifactorial. Biliary fibrosis is a cardinal feature of most cholangiopathies and an area of intense research. Progress has been made in a number of areas, including elucidating the role of integrins and prominin 1-positive progenitor cells in fibrosis [55,56] and how biliary tissue is remodeled during liver regeneration [57]. How different cell types, such as hepatic stellate cells, portal fibroblasts and so called reactive ductular cells (RDCs) contribute to fibrosis has, however, been subject to a number of excellent recent reviews [58,59] and will for space reasons not be further discussed in this review. Similarly, the importance of the immune system and infiltration of inflammatory cells has been the subject of recent reviews [60,61]. Here, we will instead focus on another important facet of the disease process, where considerable progress recently has been made: dysregulation of cholangioyte cell polarity and barrier function in the bile ducts.

A hallmark of the bile duct system is epithelial cell polarization, and both hepatocytes and cholangioytes display strong apical-basal polarity (Fig. 2). In cholangioytes, a number of proteins are specifically localized to the apical (luminal) side, such as CFTR, aquaporin 1 (AQP1) and the anion exchange protein 2 (AE2). Conversely, AQP4 and the secretin receptor are specifically localized to the basal side [62] (Fig. 2). Lumen formation and cell polarization are, as discussed above, an integral part of early bile duct tubulogenesis and are disrupted in ALGS. A recent transcriptomic analysis of ALGS patients and an ALGS mouse model revealed that although cholangioyte markers per se are not downregulated, instead genes encoding proteins with apical localization in cholangioytes show reduced expression, including CFTR, SLC5A1 and CHST4 [51], suggesting morphogenesis defects rather than differentiation defects alone.

It will be interesting to explore how dysfunctional Notch signaling in ALGS links to the molecular programs setting up apical-basal polarity. Disruption of the primary cilia, a signaling center located at the apical side of cholangioytes, leads to biliary fibrosis and macrophage infiltration in a mouse model for hepatorenal fibrocystic disease [63], and in line with this, reduction in the frequency of primary cilia has been observed in BA [64]. Similarly, a number of ciliopathies affect cholangioyte and ductal plate differentiation [65]. Furthermore, BA is characterized by decreased levels of beta-1-integrin, laminin b1 and nidogen [66], indicating that cell-matrix interactions at the basal side may also be important contributors to cholangiopathies.

An important part of the epithelial polarization process is the formation of tight junctions between cholangioytes, necessary to maintain barrier function, to confine bile to the bile ducts and to avoid inflammatory cell invasion of the liver parenchyma, which may otherwise trigger or accelerate the fibrotic process [67]. Barrier integrity is disrupted in neonatal sclerosing cholangitis, which is caused by claudin mutations [2]. Claudin is a key protein in the tight junctions and perturbation of claudin function in zebrafish leads to aberrant bile duct development [68]. The transcription factor grainyhead-like 2 may be a key regulator of establishing the barrier function, as it regulates expression of claudins
| Disease | Gene | Phenotype | Ref |
|---------|------|-----------|-----|
| Alagille syndrome | Jag1<sup>dDSL/+</sup> | Jag1<sup>dDSL/+</sup> pups were recovered at lower than expected frequencies (35% rather than 50%). No jaundice at any stage. | [50] |
|  | Jag1<sup>dDSL/+</sup> Rumi<sup>−/−</sup> | Large decrease in Sox9+ ductal plate cells (~95%) at E18, a 75% reduction in bile ducts at P3-P7, and ductular reaction at P30, which is partially rescued in Jag1<sup>dDSL/+</sup> Rumi<sup>−/−</sup> (Poglut1) mice. | [95] |
|  | Jag1<sup>dDSL/+</sup> Lfng<sup>−/−</sup> | No phenotype at birth, though all double heterozygous mice and Jag1<sup>dDSL/+</sup> alone were recovered at lower than expected frequencies. | [50] |
|  | Jag1<sup>dDSL/+</sup> Mfng<sup>−/−</sup> | Massive bile duct proliferation in adult Jag1<sup>dDSL/+</sup> Lfng<sup>−/−</sup> and Jag1<sup>dDSL/+</sup> Mfng<sup>−/−</sup> mice. | [11] |
|  | Jag1<sup>dDSL/+</sup> Notch2<sup>−/−</sup> | Small but significant increase in number of bile ducts in adult Jag1<sup>dDSL/+</sup> Mfng<sup>−/−</sup> mice. | [95] |
|  | Jag1<sup>Ndr/Ndr</sup> | Half of Jag1<sup>Ndr/Ndr</sup> mice die the first week after birth. Jaundice at P3. Absence of bile ducts. | [47] |
|  | Jag1<sup>lox/lox</sup>;SM22-Cre | Defective ductal plate remodeling, biliary cells present, but absence of bile ducts. Portal inflammation, bile duct proliferation, and liver cysts. | [103] |
|  | Rbpj<sup>−/−</sup> Foxa3<sup>−/−</sup> | Delayed ductal plate remodeling. Normal bile ducts by the age of 5 weeks. | [100] |
|  | Sox9<sup>lox/lox</sup>;Alfp-cre | Liver cysts in aged heterozygous mice (~19 months). Homozygous mice are embryonic lethal. | [104] |
| Arthrogryposis, renal dysfunction and cholestasis (ARC) syndrome | Pkd1<sup>−/−</sup> | Liver cysts by 4 weeks of age. | [110] |
|  | ApoE<sup>−/−</sup>;Fibrocystin/polyductin | Liver cysts by 4 weeks of age. | [107] |
| Autosomal recessive polycystic kidney disease & Caroli syndrome | Phd1<sup>−/−</sup> | Smaller liver, inflammation, extrahepatic bile duct stenosis and atresia. | [109] |
| PLD-ADPKD: Polycystic liver disease associated with autosomal dominant polycystic kidney disease | Pkd1<sup>−/−</sup> | Liver cysts by 4 weeks of age. | [107] |
|  | Pkd1<sup>−/−</sup> Defective ductal plate remodeling, biliary cysts present, but absence of bile ducts. Portal inflammation, fibrosis, bile duct dilation, and proliferation. | [108] |
|  | Pkd1<sup>−/−</sup> | Liver cysts by 4 weeks of age. | [107] |
| Biliary atresia | Sox17<sup>−/−</sup> | Liver cysts by 4 weeks of age. | [111] |
| Autosomal dominant polycystic liver disease | SRY-related HMG-box 17 | Liver cysts by 4 weeks of age. | [111] |
| Primary biliary cholangitis | Dominant negative TGF-βRII (driven by CD4 promoter lacking the CDS silence) | Liver fibrosis and bile duct destruction. | [112] |
|  | Dn TGF-βRII II-12p35<sup>−/−</sup> | Onset is delayed by IL-12p35 deletion. | [113] |
|  | IL-12p40 deletion protects against liver inflammation in Dn TGF-βRII mice. | IL-12p40 deletion protects against liver inflammation in Dn TGF-βRII mice. | [114] |
| Primary biliary cholangitis | NOD.c3c4 mice | Portal inflammation and biliary ductal damage. | [115] |
| Primary biliary cholangitis/ SJögrens syndrome | IL-2Rα<sup>-/−</sup> IL-12-p40<sup>−/−</sup> | Compared to IL-2Rα<sup>−/−</sup> mice alone, worsened portal inflammation and bile duct damage, but reduced colitis in IL-2Rα<sup>−/−</sup> IL-12-p40<sup>−/−</sup> mice. | [116] |
|  | Autoimmune polyolycystic destructive cholangitis, granuloma formation, and eosinophilic infiltration | Autoimmune polyolycystic destructive cholangitis, granuloma formation, and eosinophilic infiltration | [117]; |
in addition to extrahepatic bile duct effects. Partially penetrant portal inflammation and bile ducts destruction (4/11 mice with severe or moderate inflammation).

Portal inflammation and bile duct destruction. Portal inflammation and cholangitis of small intrahepatic bile ducts. 

Ae2a,b−/−, Cl(−)/HCO3(−) anion exchanger 2 (AE2) Scurfy mice (Foxp3mutant) Faslpr/lpr Portal inflammation and cholangitis of small intrahepatic bile ducts. 

MRL (genetic background)/lpr (lymphoproliferation) mice Mdr2−/− Sex-dependent liver disease. Inflammation and ductular reaction in large portal tracts. Fibrosis and bile duct destruction. 

Cl(−)/HCO3(−) anion exchanger 2 (AE2) Scurfy mice (Foxp3mutant) Portal inflammation and bile duct destruction. Periportal inflammation and periductal fibrosis leading to liver tumors. E-cad is required primarily in bile ducts rather than hepatocytes to avoid cholestasis. 

Faslpr/lpr MRL (genetic background)/lpr (lymphoproliferation) mice 

CDH1-loxp/loxp; Alb-Cre (CDH1ΔL, Liver-specific E-cadherin knockout) Adenovirus-Cre; CDH1-loxp/loxp Progressive Familial Intrahepatic Cholestasis (PFIC2) Abcb11 (ATP-binding cassette, sub-family B (MDR/TAP), member 11, aka sister of P-glycoprotein (Pgp) or bile salt export pump (BSEP)) 

Krt19-Cre; CDH1-loxp/loxp E-cad is required primarily in bile ducts rather than hepatocytes to avoid cholestasis. Progressive Familial Intrahepatic Cholestasis (PFIC2) PFIC-like inherited cholestasis Atp11c ATPase Phospholipid Transporting 11C Cystic fibrosis liver disease Cfr−/− Cystic fibrosis transmembrane conductance regulator 

Abcb11 (ATP-binding cassette, sub-family B (MDR/TAP), member 11, aka sister of P-glycoprotein (Pgp) or bile salt export pump (BSEP)) 

Cystic fibrosis transmembrane conductance regulator 

E-cad is required primarily in bile ducts rather than hepatocytes to avoid cholestasis. PFIC-like inherited cholestasis Atp11c ATPase Phospholipid Transporting 11C Cystic fibrosis liver disease Cfr−/− Cystic fibrosis transmembrane conductance regulator 

Bile duct proliferation and biliary fibrosis. Bile duct proliferation and biliary fibrosis. Fibrosis was attenuated but not completely rescued by Rag2 deletion. Decrease in overall liver size and bile duct paucity. Decreased bile flow but no liver injury or cholestasis. However, 3,5-diethoxycarbonyl-1,4-dihydrocollidine (DCC) feeding induced more severe liver injury with obstruction of bile ducts by porphyrin plugs. 

Erythropoietic protoporphyria (fch/fch (ferrochelatase mutation)) General liver inflammation and liver fibrosis Fra-1 overexpression driven by histocompatibility complex class I antigen H2-Kb (H2) promoter (Fra-1mutant) mice & Fra-1mutant−/− mice Role of bile duct innervation M3-R−/− (muscarinic 3 receptor) 

Canaliculi and bile duct development defects Lkb1lox/lox, Alb-Cre Ctnnb1lox/lox, Foxa3-Cre Role of bile duct innervation M3-R−/− (muscarinic 3 receptor) 

Zellweger spectrum disorder (includes liver fibrosis) Pex1G844D (peroxisomal biogenesis factor 1) 

Bile duct proliferation and biliary fibrosis. Bile duct proliferation and biliary fibrosis. Fibrosis was attenuated but not completely rescued by Rag2 deletion. Decrease in overall liver size and bile duct paucity. Decreased bile flow but no liver injury or cholestasis. However, 3,5-diethoxycarbonyl-1,4-dihydrocollidine (DCC) feeding induced more severe liver injury with obstruction of bile ducts by porphyrin plugs.
and Rab25, which is important for localizing claudins to the tight junctions [69].

5. Towards improved diagnosis and therapy development for cholangiopathies

Diagnosis is still far from perfect for a number of cholangiopathies, and this may result in failure to treat even when options are available (Table 2, Suppl File 1), or that an incorrect type of treatment is chosen. For example, the current treatment of BA (Kasai portoenterostomy (KPE)), in which all bile duct tissue up to the liver capsule is excised and a loop of jejunum is attached creating a portoenterostomy) relies on early diagnosis (within 60–100 days) and timely performance of KPE. Missed or late diagnosis of BA results in rapid progression to end-stage liver disease, rendering KPE futile and leaving liver transplantation as the only and last resort. Misdiagnosing ALGS as BA can lead to children erroneously receiving KPE, which in ALGS appears to result in higher rates of liver transplantations than when children with ALGS do not receive KPE [70,71]. From this, it is obvious that more precise biomarkers for BA and ALGS would be useful. Bulk transcriptomes (i.e. from a whole biopsy) from ALGS, PSC and progressive familial intrahepatic cholestasis type 2 biopsies have begun to reveal differentially expressed genes [51], which could provide biomarkers where genetic diagnosis is difficult, as well as provide mechanistic insight into disease processes and identify therapeutically amenable pathways. As bulk transcriptomes capture an average transcriptome for all cell types present in a biopsy, single cell RNAsequencing is however likely to be more successful for identifying cholangiocyte-specific markers, and in particular if this information can be transformed into new serum biomarkers, it is likely to become more clinically useful. An improved biomarker portfolio would allow us to address whether BA and ALGS may in fact represent extremes of a continuous disease spectrum that can pose ambiguity in the context of clinical diagnosis and management. Proteomics-based approaches may also be a valuable complement to improve diagnosis, and matrix metalloprotease 7 (MMP7) was recently identified as a novel BA marker using this strategy [72].

Apart from understanding the causes of cholangiopathies, understanding the mechanisms of disease progression is equally important. As discussed above, there are currently limited curative options for cholangiopathies, other than liver transplantation, which is a high-risk procedure incurring high morbidity and post-transplantation issues with lifelong immunosuppression and post-transplant malignancies. The development of new therapies to ameliorate or reverse progressive cholangiocyte damage is therefore a prioritized research area. Success depends both on appropriate patient selection (with relevant and possibly new biomarkers) and availability of novel target therapies. Ursodeoxycholic acid (UDCA) is a promising potential therapy for PBC patients with inadequate response to the FDA-approved first-line treatment ursodeoxycholic acid (UDCA) [73,74]. The efficacy and safety of OCA were demonstrated in two phase 2 studies and a phase 4 study is now under way (Supplementary File 2). Another potential therapeutic treatment for PSC is all-trans retinoic acid (ATRA), which demonstrated improvement in liver enzyme function in a phase 1 study, and a phase 2 study to evaluate its efficacy against fibrogenesis in PSC is currently ongoing (Supplementary File 2). A list of completed and current (May 2018) clinical trials for primary cholangiopathies (PBC, PSC and BA) is provided in Supplementary File 2.

In addition to pharmacological approaches, there is an increasing interest in cell-based therapeutic strategies and approaches harnessing the liver’s own endogenous repair potential. For endogenous repair, an important question is which cells would be best suited to replace the lost or ailing cells. Research in liver disease has thus far mostly focused on replacing hepatocytes, and some research groups propose a cholangiocyte origin of cells taking part in the relevant repair processes in animal models [75,76], while other groups advocate hepatocytes as the cellular source [54,77–79]. A potential stem cell population expressing Lgr5, a hallmark for stem cells in different tissues, was observed in response to liver injury [80] and represents an interesting candidate cell type for endogenous repair. The replacement of cholangiocytes is yet less explored, but mouse models for ALGS, given their bile duct paucity, may be a suitable test platform to learn if new cholangiocytes can be generated in vivo. The report that new cholangiocytes are transdifferentiated from hepatocytes in an ALGS mouse model, in a TGFβ1-dependant manner, is encouraging in this regard [54].

An alternative approach is to generate cells for transplantation in vitro. As discussed above, cholangiocytes can be in vitro differentiated by the organoid technology [31] or from pluripotent cells (ES and iPSC cells) [28–30], and could be interesting sources of cells for transplantation. The recent report that the extrahepatic biliary tree can be partially reconstructed in animal models is a very exciting development [32].

6. Outstanding questions

Cholangiopathies are rare diseases, but collectively they constitute a major clinical problem and a considerable burden for the healthcare system. Current challenges include the lack of functional therapies beyond liver transplantation as well as suboptimal methods for diagnosis. In this review, we have focused on describing recent progress especially in the molecular understanding of the diseases. Information from areas such as transgenic models, organoid technology and transcriptomics can now be used to make progress for diagnosis, and, in the long term, for therapy. An important outstanding question is how diagnosis can become more precise, and we envisage that the rapid technology development in the area of transcriptomics, and in particular in single cell RNA-sequencing, will contribute to identify new biomarkers for early and unambiguous diagnosis, and outcome prediction. This could lead to more timely and effective interventions, and improved outcomes. Currently, disease modeling using organoids and in vitro differentiation of iPSCs cells has mostly been used for monogenic cholangiopathies, notably ALGS, and it will be interesting to see if these technologies can also be applied to cholangiopathies with a more complex genetic makeup.

Finally, novel organoid and in vitro culture systems open new vistas for accelerated testing of new drug candidates, which may help identify novel pharmacological principles that can be moved forward to animal experiments and clinical testing. Ultimately, it is hoped that a cellular and molecular understanding of biliary pathologies will enable accurate and rapid diagnosis, ensuring patients receive correct management and treatment.

Acknowledgments

PT is supported by Innovation and Technology Commission, Hong Kong, UICP project UIM/300, and Dr. Li Dak-Sum Research Centre, The University of Hong Kong – Karolinska Institutet Collaboration in Regenerative Medicine. E.R.A. acknowledges support from the Center of Innovative Medicine (CIMED) Grant, the Daniel Alagille Award, KI Funding, the Heart and Lung Foundation, and the Alex and Eva Wallström Foundation.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jebiom.2018.08.024.

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