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Biliary Atresia – emerging diagnostic and therapy opportunities*

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ABSTRACT

Biliary Atresia is a devastating pediatric cholangiopathy affecting the bile ducts of the liver. In this review, we describe recent progress in the understanding of liver development with a focus on cholangiocyte differentiation and how use of technical platforms, including rodent, zebrafish and organoid models, advances our understanding of Biliary Atresia. This is followed by a description of potential pathomechanisms, such as autoimmune responses, inflammation, disturbed apical-basal cell polarity, primary cilia dysfunction as well as beta-amyloid accumulation. Finally, we describe current and emerging diagnostic opportunities and recent translation breakthroughs for Biliary Atresia in the area of emerging therapy development, including immunomodulation and organoid-based systems for liver and bile duct repair.

1. Introduction

Cholangiopathies are diseases that primarily affect the bile ducts in the liver and are responsible for nearly 80% of all pediatric liver transplantations [1]. The biliary system takes the bile from the liver to the gall bladder and eventually into the small intestine. Biliary Atresia (BA) is a cholangiopathy which affects both the extra- and intrahepatic bile ducts of the liver (Figure 1). The incidence is particularly high in Asia (100-500 per 100,000 live births in Taiwan and Japan [2] as compared to Europe (5-25 per 100,000 live births) [3], for review see [4]. The current treatment options are limited to a surgical procedure called Kasai portoenterostomy (KPE), but KPE fails to improve the condition in nearly 50% of the patients and leaves the intrahepatic cholangiopathy unresolved. Furthermore, BA leads to fibrosis, portal hypertension and liver failure in many cases. Ultimately, liver transplantation is often needed, requiring life-long immunosuppression, which affects the quality of life for BA patients.

Genetics in BA is complex, and most cases are nonsyndromic. PKD1L1 mutations have been linked to syndromic form of BA [5], while common variants of a small number of genes (ADD3, CRIPTO, NODAL, LEFTY, GPC, EFEMP1 and ARF6) have been shown to be associated with increased risk of nonsyndromic BA [6–10]. Most recently, our trio-based whole exome sequencing study identified mutations of ciliary genes including PCNT, KIF3B and TTC17 in 31.5% of non-syndromic BA patients [11] (Figure 1). The etiology of BA is complex with both virus and hepatotoxin exposure appearing to be important factors, and the fact that elevated bilirubin levels can be detected at birth in children that will proceed to develop BA may suggest that exposure occurs at the fetal stage [12]. A number of viruses, including Cytomegalovirus (CMV), Epstein-Barr virus (EBV) and Human papillomavirus (HPV) have been linked to BA [13], and hepatotoxins such as the plant toxin biliatresone, can induce BA-like pathology in various species [14] (Figure 1). BA can be classified into different subcategories. One classification scheme divides BA into isolated BA (70-80%), syndromic BA (10-15% and including Biliary Atresia Splenic Malformation (BASM)), cystic BA (5-10%) and CMV IgM$^+$ BA (approximately 10%) [15]. An alternative classification scheme reports approximately 80% isolated BA cases, a variable incidence of CMV BA, a congenital malformation form (10-20%), the BASM variant (1-10%), BA with major malformation variant (5-10%) and cystic variant (8%) [16]. Proposed aetiologies for BA and their potential relationships are also discussed by Tam et al., (2017) [17].

2. Molecular and cellular mechanisms regulating normal development of the extra- and intrahepatic bile ducts and aberrations in Biliary Atresia

BA affects both extra- and intrahepatic bile ducts, which have different developmental origins. The intrahepatic bile ducts, along with

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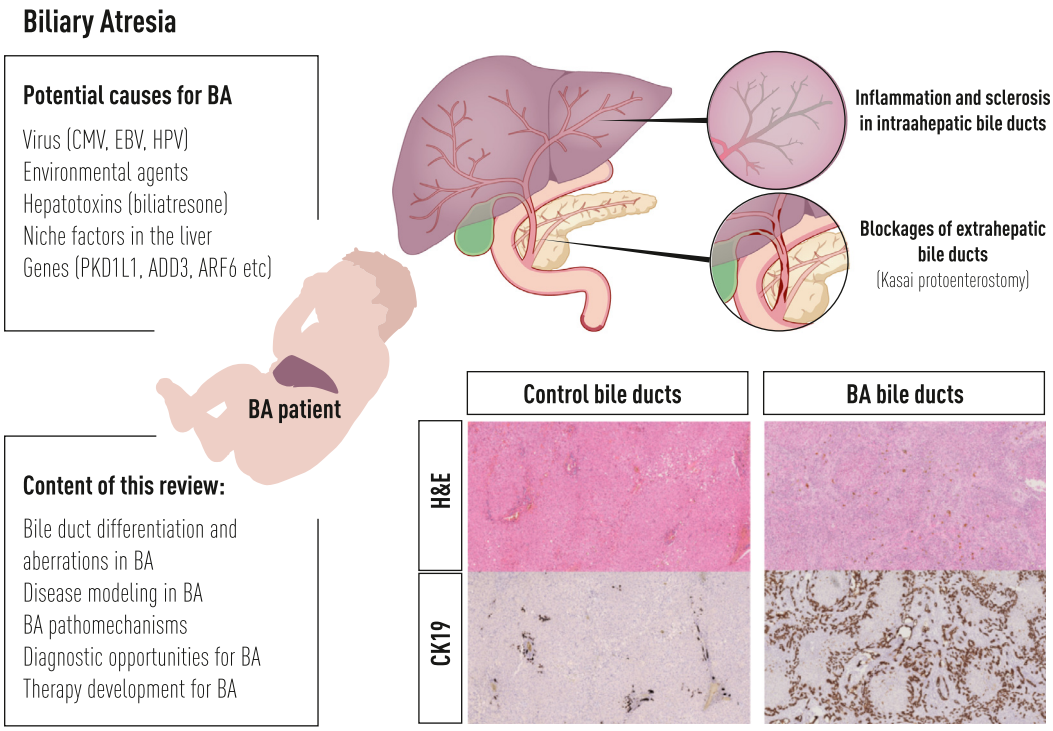

Fig. 1. Schematic depiction of Biliary Atresia. Potential causes for BA are described, as well as the major pathological features of BA, including obstruction of the extrahepatic bile ducts and deterioration of intrahepatic bile ducts. The gallbladder (green) pancreas (beige) and duodenum (light pink) are also shown. Hematoxylin and eosin (H&E) stained and cytokeratin 19 (CK19)-immunostained liver sections from a BA patient and a non-BA control are displayed. At the bottom of the figure, the main topics of the review article are described.

the cranial part of the common hepatic duct which represents a part of the extrahepatic bile ducts, originally stem from the cranial (proximal) part of the liver bud (pars hepatica) and the cholangiocytes forming the intrahepatic bile ducts are derived from hepatoblasts along the portal vein (Figure 2). The other major parts of the extrahepatic system, i.e., the caudal (distal) part of the common hepatic duct along with the gallbladder and cystic duct, instead originate from the caudal part of the liver bud (pars cystica) (Figure 2). The architecture of the intrahepatic ducts is complex, with probably at least 18 branchings. The branching process and the reciprocal interactions between the portal vein and intrahepatic bile ducts are yet poorly understood, but Notch signalling regulates branching after biliary injury [18]. A novel microCT method allows the simultaneous visualization of both the intrahepatic bile duct system and the vasculature [19], allowing the reciprocal interactions to be studied in further detail. The extrahepatic bile ducts appear earlier [15], pouching out from the foregut around 20 days of gestation, while the intrahepatic bile ducts appear around 45 days of gestation (see [20] for review) (Figure 2).

Cholangiocytes in the extra- and intrahepatic bile ducts follow different developmental trajectories (for review see [18]). For the intrahepatic bile ducts, cholangiocytes and hepatocytes differentiate from a common progenitor, the hepatoblast. Whether all hepatoblasts are bipotent and can give rise to both hepatocytes and cholangiocytes is not clear, but recent observations indicate that an Lgr5-positive cell pool remains bipotential throughout life [21]. We are gaining insights into the factors regulating the hepatoblast's choice to become a cholangiocyte or a hepatocyte, and this information is relevant for BA, as an instability in the cholangiocyte fate has been observed in BA organoids (see below). We are also beginning to identify transcriptional and epigenetic factors controlling hepatoblast fate choices, including TGF-beta, Wnt, Notch and Hippo/YAP signalling [20]. Cholangiocytes can furthermore be generated by transdifferentiation from hepatocytes in a TGF-beta-dependent manner [22], indicating that the

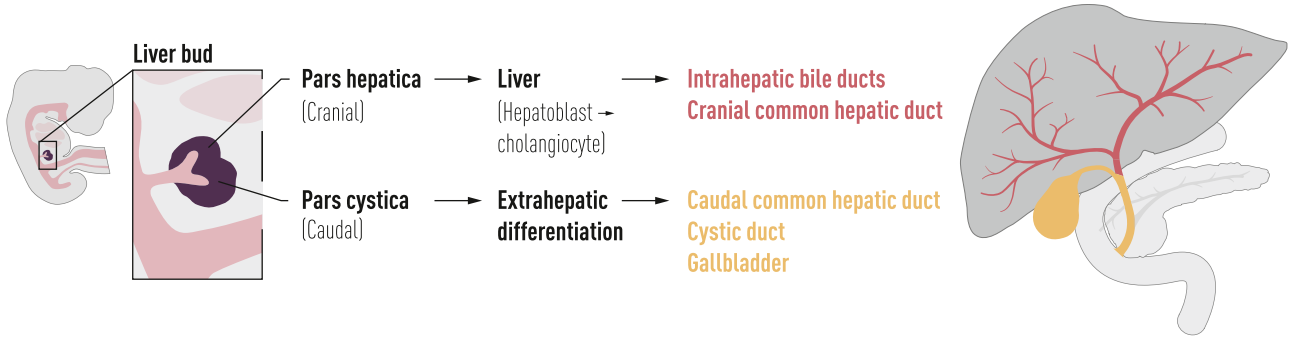

Fig. 2. Overview of the liver, with the extra- and intrahepatic bile ducts and their different developmental origins. The intrahepatic bile ducts and the cranial (proximal) common bile duct (shown in red) are derived from one portion of the liver bud (pars hepatica). The extrahepatic bile ducts, including the caudal (distal) common bile duct, as well as the gallbladder and cystic duct connecting the gallbladder to the common bile duct (shown in yellow), are derived from the pars cystica in the liver bud.

differentiated hepatocyte state is not carved in stone but may be to some extent plastic.

During the early steps of differentiation of the extrahepatic bile ducts, i.e., when they appear as a diverticulum from the foregut, the transcription factor Sox17 plays an important role, and its absence leads to conversion to pancreatic tissue [23]. Downstream of Sox17 are other transcription factors, including Hfn6, Hnf1b and Hhex [24,25] and Fgf signalling contributes to the patterning of the extrahepatic biliary tree [26]. Collectively, these observations point to the existence of distinct molecular programs for cholangiocyte differentiation in the extra- and intrahepatic bile ducts.

Decoding the molecular landscapes of extra- and intrahepatic cholangiocytes by single cell transcriptomics reinforces the notion that the two types of cholangiocytes are different. Molecular profiles from human intrahepatic cholangiocytes have been obtained [27], and recently a comparison between intra- and extrahepatic cholangiocyte transcriptomes was conducted [28]. While there is a core transcriptional profile across cholangiocytes from different locations, different parts of the biliary tree show transcriptomic heterogeneity at the single cell level [28]. Interestingly, specific gene expression patterns for intra- and extrahepatic cholangiocytes were revealed, where for example the Notch ligand JAGGED1, which is mutated in Alagille Syndrome (ALGS), was found to be expressed in intrahepatic cholangiocytes, which may explain the intrahepatic bile duct paucity in ALGS. Furthermore, the transcriptomic profiling provided evidence for zonation along the biliary tree [28], and zonation has also been observed along the porto-central axis for hepatocytes and endothelial cells [29,30] as well as for hepatic stellate cells [31].

3. Technology platforms underpinning Biliary Atresia research

Considerable progress has been made in our understanding of BA through the use of different technology platforms for disease modeling, including rodents, zebrafish and liver organoids (see Table 1 for a list of studies using these systems).

3.1. Rodents

An initial breakthrough in experimental BA research was the development of a mouse model, where mouse pups are infected with rhesus rotavirus (RRV) [32]. This results in phenotypes which in many ways resemble the human pathology [32]. Although it is still debated whether rotavirus infection leads to BA in humans [33,34], work in the RRV mouse model has been very important to shed light on BA disease mechanisms (Table 1). A modified version of the RRV mouse model, based on a reengineered rotavirus was recently described and yields improved survival rates [35]. Success of RRV mouse experiments relies on genetic background, time of infection, virus dosage and the immunological status of the mouse. Balb/c mice are the most susceptible mouse strain, high dosage of RRV is still the only known agent that induces definite extrahepatic bile duct atresia, and the first 24 hours postpartum is the optimal time point for intraperitoneal injection of the virus. Modulation of the mouse immune system, for example by depletion of granulocyte receptor-1 (Gr-1) expressing myeloid cells attenuated the BA symptoms [36], further underscoring the importance of the immunological status. A surgical rat BA model was recently established, based on ligation of the common bile duct [37], and biliatresone induces BA-like effects in standard laboratory mice [38].

3.2. Zebrafish

Zebrafish (*Danio rerio*) is gaining ground as a model system for analysis of various organs because genetic and chemical manipulations can readily be conducted, transparent larval development allows organ development to be visualized in vivo, maintenance cost is low, and fecundity is high. Zebrafish is increasingly used also in early stages of cholestasis research, and liver development and function are in many aspects similar to that of humans, with zebrafish hepatocytes secreting bile salts through a bile canaliculus on the apical side, and the bile fluid is drained to a network of intrahepatic bile ducts that is connected to an extrahepatic bile duct. There are however some differences, including a different organization of the liver vasculature and the portal triads [39,40], and fibrosis and inflammation has been difficult to assess, possibly linked to that the adaptive immune system does not mature until after 4–6 weeks post-fertilization [41].

These differences however do not detract from the importance of zebrafish as a model system for BA, and zebrafish has significantly contributed to our understanding of BA, notably in terms of working

Table 1
List of publications using model systems (rodents, zebrafish, organoids) to study Biliary Atresia.

Rodent (mouse & rat)	Main findings	References
Virus-induced. Inoculation of *Balb/c* mouse pups (1-2 days) with rhesus rotavirus (RRV)	Intraperitoneally RRV-infected neonatal mice developed jaundice and liver fibrosis, with a role for the RRV Spike Protein VP4	Riebentoff-Talty et al., (1993). REF 32 Wang et al., (2011). REF 64 Mohanty et al., (2020). REF 35
Surgery-induced. Ligation of the common bile duct in 21-30 days old *Sprague-Dawley* rats	Operated rats developed jaundice, choluria and acholia, progressive cholestatic injury, leading to massive liver fibrosis	Garrido et al., (2017). REF 37
Toxin-induced. Biliatresone treatment of *Balb/c* mouse pups (0-3 days)	Neonatal mice injected with biliatresone developed biliary obstruction and dysplasia or absence of extrahepatic bile ducts	Yang et al., (2020). REF 38
Morpholino-gene knockdown or CRISPR/Cas9 genome editing	Knockdown or knockout of BA candidate genes resulted in biliary developmental defects in zebrafish larvae	Cui et al., (2013). REF 6 Tang et al., (2016). REF 43 Cheung et al., (2021). REF 44
Biliatresone treatment	Zebrafish larvae treated with biliatresone displayed morphological defects of the gallbladder and extrahepatic bile ducts	Lorent et al., (2015). REF 14 Zhao et al., (2016). REF 42 Yang et al., (2020). REF 38
Epigenetics	Analysis of DNA methylation status using zebrafish	Matthews et al., (2011). REF 46 Cofer et al., (2016). REF 45
Liver organoids in BA research	BA-liver tissue-derived and IPSCs-derived organoids from BA patients displayed biliary developmental defects resembling those observed in BA	Tian et al., (2019). REF 58 Babu et al., (2020). REF 54 Amarachintha et al., (2021). REF 55
CRISPR/Cas9 gene knockouts in IPSC-derived organoids	Knockout of *ADD3* and *GPC1* in organoids resulted in reduced formation of ductal structures	Tian et al., (2019). REF 58
Biliatresone treatment	Biliatresone treatment led to a disruption of apical-basal polarity in liver spheroids/spheroids	Waisbourd-Zinman et al., (2016). REF 56 Fried et al., (2020). REF 57

out the effects of biliatresone (see above) [14,42] and validating candidate genes from genome wide association studies (GWAS) [6,43,44] (Table 1). Furthermore, pharmacological and genetic manipulations of DNA methylation in zebrafish revealed the role of DNA hypomethylation in BA [45,46].

3.3. Organoids

Liver organoids, i.e., 3D-cultured mini organs, are increasingly used to study different aspects of disease processes, including liver diseases [47–49]. Liver organoids can be differentiated towards both cholangiocyte and hepatocyte fates and have been used to study an increasing number of liver diseases, including a1-antitrypsin (A1AT) deficiency and ALGS [49,50], for review see [51]. Organoids can be generated from induced pluripotency cells (iPS) or embryonic stem cells (ES cells), hepatic progenitor cells, as well as directly from tissue-derived cells [51]. In one study, differences between intra- and extrahepatic organoids, notably with regard to their response to Wnt signaling, were observed [52]. Primary human extrahepatic cholangiocyte organoids were used to construct the mouse extrahepatic biliary tree [53] and repair human intrahepatic ducts [28].

In terms of BA research, organoids from BA patients or RRV-infected mice showed a breakdown in apical-basal polarity [54], a phenotype which was corroborated in a recent study [55], which also showed delayed epithelial differentiation in BA organoids. Furthermore, biliatresone led to a similar apical-basal disruption in control liver spheroids, accompanied by decreased glutathione and Sox17 levels combined with elevated Hey2 levels, indicating activation of the Notch pathway [56,57] (Table 1). Tian et al., (2019) used CRISPR/Cas9 genome editing to remove ADD3 and GPC1 in organoids [58], which resulted in reduced formation of ductal structures. Human cholangiocyte organoids (ICOs) were recently shown to be susceptible to rotavirus infection and mirror BA development, with a disrupted organoid morphology, combined with the induction of an immune response [59].

The recapitulation of at least some BA phenotypes in organoids opens up new vistas to use organoids both to screen for novel drugs that may ameliorate the disease process and to identify potential cholangiotoxic agents. While this may warrant cautious optimism, it should however also be recognized that organoids from different locations in the extrahepatic tree take on a similar transcriptomic profile after in vitro culturing [28], suggesting that some of the in vivo properties may be lost upon in vitro culturing. To identify niche conditions that keep their “originality” would be important and shed light on niche factors in general. As an alternative strategy, Du et al., (2020) reported the construction of a cholangiocyte epithelial layer with barrier function and apical-basal polarization on a chip [60].

4. Novel insights into Biliary Atresia pathomechanisms

4.1. Virus, inflammation and immune responses as pathomechanisms for BA

Virus infections have long been suspected to play a role as a cause for BA, and several viruses, such as EBV, CMV and HPV have indeed been found in BA livers [13]. Notably, 60% of BA cases in China contained CMV [61], and Davenport (2012) proposed CMV-associated BA as a BA subgroup, defined by liver biopsies staining positive for immunoglobulin M (IgM) antibodies against CMV [62]. CMV has also been used to recapitulate BA in mice depleted for regulatory T cells [63]. Progress has been made regarding the underpinning mechanisms for viral infections. In the RRV mouse model, the SRL peptide in the VP4 protein has been shown to be responsible for pathogenesis and interacts with heat shock protein 70 (Hsp70) on cholangiocytes [64]. Interestingly, the SRL peptide is not unique to proteins in RRV, but is also found on CMV proteins, and it may therefore be asked whether the SRL-Hsp70 axis is a common node for all viral cholangiocyte infections. If true, this may facilitate more generic therapy development covering different types of viral infections, but additional research is required to settle this question.

Viral infections may lead to autoimmune responses, which indeed are observed in BA patients, with accumulation of circulating macrophages and CD4/CD8 positive T-cells and autoantibodies [65]. Furthermore, autoantibodies targeting cholangiocytes were observed in BA patients [66]. Interestingly, in a recent report, Wang et al., (2020) conducted an analysis of the immune profiles of BA patients, and revealed an imbalance in the immune response, with expansion of cytotoxic T-cells and a reduction of CXC3RA T effector and natural killer cells [67]. These changes were accompanied by a continued hepatic B lymphocyte production in BA patients, along with accumulation of autoantibodies. In the same study, the use of rituximab to reduce B cell numbers was successful and in line with the findings in humans, the RRV mouse model exhibited a decrease in the CXC3RA T cell effector and NK cells [67]. Transfer of T-reg cells to newborn mice prior to RRV exposure blocked the extrahepatic bile duct obstruction [68,69], see [70] for review).

Inflammation is intimately associated with BA. In a recent report, RRV infection in mice was shown to cause increased expression and release of the protein HMGB1, which has been implicated in mediating inflammation [71]. HMGB1 induction was mediated via the p38 MAPK signalling pathway, which offers potential therapeutic opportunities. An inflammatory response, with elevated Toll like- and cytokine receptor expression was also observed in RRV-infected liver organoids [59]. Elevation of IL-17, produced by $\gamma\delta$ T cells, was suggested to contribute to hepatic inflammation in the RRV mouse model of BA and in the livers of BA infants [72]. Treatment by AM80, a synthetic retinoid with superior pharmacological properties, suppressed the IL-17 production of $\gamma\delta$ T cells and ameliorated hepatic inflammation in RRV mice [73]. In conclusion, there are a number of recent exciting findings that link viral infection to autoimmune responses and inflammation, which provide interesting novel ideas for future therapy development.

4.2. Disruption of apical-basal polarization in BA cholangiocytes

Acquisition of apical-basal polarity is fundamental for proper development and function of bile ducts [20]. Cholangiocytes have an apical and basal (basolateral) side, and are joined together by tight junctions, with expression of different claudins [74,75]. There are several cholangiocyte proteins that exhibit apical-basal polarization including CFTR, TGRS and AE2 at the apical side (facing the lumen) and the secretin receptor (SCTR) at the basal side [76]. Lysosomes and multivesicular bodies are present close to the apical side [77]. In BA livers and organoids, the apical-basal localization of a number of proteins including ZO-1, CFTR and SCTR, is disrupted [54], which corroborates earlier reports on apical-basal disruption and lumen obstruction in liver spheroids in response to exposure to biliatresone [56,57]. The altered distribution of CFTR may impact bile flow, as it plays a role in bile duct secretion. A recent report extends these findings by demonstrating that ZO-1, β-catenin, E-cadherin and claudin-3 are mislocalized in BA livers [78]. The same study also revealed a downregulation of Cdc42, a regulator of cell polarity, in BA livers, and knocking out Cdc42 in mouse cholangiocytes, which resulted in extra- and intrahepatic bile duct obstruction, further underscoring the importance of apical-basal polarization in cholangiocytes. Perturbation of the apical-basal axis is not restricted only to BA but also observed in ALGS [50] and may thus be common to a number of diseases affecting cholangiocytes [20,79]. To further explore the underpinning molecular principles for apical-basal polarity, including the role of Rho/Rac signalling and its dysregulation in BA, remains an important topic for future studies.

4.3. Primary cilia dysfunction

A special aspect of apical-basal dysregulation in BA is the effect on localization and abundance of primary cilia. Primary cilia, localized at the apical side of cholangiocytes [80–82], are important for cholangiocyte polarization and for sensing flow in the bile ducts through the mechanoreceptors PC-1 and PC-2. A reduced number of primary cilia was observed on BA cholangiocytes [83]. The importance of primary cilia as a BA pathomechanism is further corroborated by the fact a number of genes important for ciliogenesis, including PDK1L1, are mutated in BA (see below), and that laterality abnormalities are observed in BA [84,85]. Importantly, our recent whole genome sequencing study detected rare, deleterious de novo or biallelic variants of liver-expressed ciliary genes, including PCNT, KIF3B and TTC17, in 28 of 89 nonsyndromic BA patients [11]. Primary cilia play an important role for Hedgehog signalling [86] and it will be interesting to explore to what extent Hedgehog signalling is perturbed in BA. This may also shed light on cilia dysfunction in other liver diseases, such as neonatal sclerosing cholangitis and polycystic liver disease [16,87,88].

4.4. Beta-amyloid accumulation in BA

Beta-amyloid deposits in the form of neural plaques is a hallmark of Alzheimer's disease, but recently, Babu et al. (2020) showed that beta-amyloid also accumulates in livers in BA patients as well as in the RRV mouse model and in BA organoids (see above) [54]. BA thus joins the ranks of diseases with beta-amyloid deposits, such as Alzheimer's disease and cerebral amyloid angiopathy (CAA), but with the notable difference that beta-amyloid deposition appears to be intracellular in BA, in contrast to the extracellular deposits in other amyloid diseases. To explore the consequences of the intracellular beta-amyloid aggregates in BA, and what type of cleavage products of amyloid precursor protein (APP) they represent, remains to be explored. It also remains to be elucidated whether BA beta-amyloid deposition is caused by other viruses than RRV. We also do not yet know whether strategies to abrogate beta-amyloid accumulation, which have been explored in Alzheimer research, will prove beneficial, or whether beta-amyloid accumulation in BA is more of a bystander effect. It is of note that the γ-secretase inhibitor DAPT, which blocks cleavage of APP to beta-amyloid, led to amelioration of the BA phenotype in the RRV mouse model [89], but as DAPT also blocks Notch signalling [90], improvement may also be related to perturbing Notch signaling. Additional work is required to specifically address the effects of APP processing and beta-amyloid deposition and its role in the BA disease process.

4.5. Alterations in extracellular matrix and the cholangiocyte niche

Extracellular matrix (ECM) is critical for providing a niche for several cell types, such as intestinal stem cells [91], and there is an increasing interest in exploring the role of ECM also for cholangiocytes in BA, as progressive fibrosis is frequently observed in BA patients. Pathway enrichment analysis of BA patients with low survival after KPE revealed that the top 10 most dysregulated pathways were ECM-related [92] and Kyrönlahti et al., (2021) observed an increase in ECM gene expression after KPE [93]. Furthermore, elevated expression of matrix metalloproteinase 7 (MMP7), which modulates ECM by breaking down for example fibronectin and proteoglycans, is seen in extrahepatic cholangiocytes in BA patients and serves as a biomarker for the disease (see below) [94,95]. Much however remains to be learned about the ECM status in the vicinity of extra- and intrahepatic bile ducts and how ECM may function as a niche for cholangiocytes. This can be addressed by proteomics approaches, but as discussed above, the increasing sophistication of cholangiocyte organoid systems may also allow various ECM combinations to be directly tested for their effects on cholangiocytes.

4.6. Destabilization of the cholangiocyte cell fate in BA

As discussed above, intrahepatic cholangiocytes and hepatocytes emerge from a common hepatoblast progenitor, and it is therefore of interest to explore whether cell fate decisions or stability are affected in BA. Analysis of organoids from BA patients revealed a transcriptomic shift from a cholangiocyte towards a hepatocyte fate, i.e., cholangiocyte markers such as SOX1, FOXA1 and the Notch ligand JAGGED1 were downregulated, while hepatocyte markers, including HNF4A and TTR, were upregulated [54]. This may be indicative of a destabilized cholangiocyte cell fate in the BA disease process, and in line with this reasoning it is interesting to note that hepatic progenitor cells accumulated in BA livers [96]. Furthermore, cells with a marked intermediary biliary-hepatocyte phenotype were increased in ALGS [96], suggesting that the cholangiocyte-hepatocyte differentiation process may be affected also in other cholangiopathies. To decode which differentiation or fate stability steps are affected in BA remains a priority for future research.

5. Emerging diagnostic opportunities for Biliary Atresia

The importance of improving early diagnosis of BA is well recognized [97], as the negative impact of late surgery has been demonstrated in previous studies [98], and novel biomarkers that can stratify patients with regard to the need for liver transplants are warranted. In an ideal world, the investigation for BA should be non-invasive and able to provide accurate and sensitive diagnosis during the early phase of the disease, and although not yet a reality, it is an important aim to work towards this goal. The examination of bile-stained stool in infants with suspected BA is completely non-invasive. Some countries adopted the policy of universal screening of infant stool colour and reported promising results [97], but the cost-effectiveness remains controversial due to its low specificity. Except the minor discomfort associated with venipuncture, blood tests are nearly non-invasive. MMP7 was recently identified as a biomarker with promising potential, in particular as MMP7 levels can be established from a blood test [95]. A recently published meta-analysis has reported high sensitivity and specificity for the use of MMP7 in differentiating BA from other cholestatic diseases in newborns [99]. Genetic mutations can also be identified from DNA acquired through blood tests, but while genes with specific mutations (PKD1L1) [5] or carrying common variants in BA patients, such as ADD3, CRIPTO, NODAL, LEFTY, GPC, EFEMP1, ARF6, PCNT, KIF3B and TTC17 [6–8,11], continue to be identified, genetic screening will likely only capture a small percentage of BA patients, as most cases are nonsyndromic. Traditionally, histological examination of liver biopsy sample by an experienced pathologist is considered as a relatively accurate diagnostic test [100], but acquisition of a liver biopsy is an invasive procedure, and a provisional diagnosis can only be established 6 weeks after birth [16]. The recent discovery of beta-amyloid deposits in the liver of BA patients [54] may become an adjuvant test to overcome some of the limitations in human histological analysis, but currently also requires a liver biopsy. It may however be hoped that in the near future, blood test for beta-amyloid can replace existing investigations to provide an early and the most precise diagnosis for infants with BA, similar to blood test-based analysis of beta-amyloid in Alzheimer patients.

6. Towards therapy development for Biliary Atresia

While KPE offers relief of jaundice in some BA patients, residual problems ranging from minor quality of life impairment to severe life-threatening events are common among the survivors [17].

Table 2
Registered clinical trials of therapeutics for Biliary Atresia.

Treatment tested	Masking	No of participating centres	Locations	Study period	Phase of study	ClinicalTrials.gov Identifier
Prednisolone	Double	1	Europe	1/2000 – 9/2008	3	NCT00539565
Corticosteroid	Triple	14	North America	11/2005 – 1/2013	3	NCT00294684
Intravenous immunoglobulin	Open label	7	North America	10/2013 – 7/2016	1,2	NCT01854827
Pentoxifylline	Open label	1	North America	1/2013 – 1/2022	2	NCT01774487
Granulocyte Colony-Stimulating Factor	Open label	2	North America, Asia	1/2018 – 1/2020	1	NCT03395028
N-Acetyl cysteine	Open label	1	North America	5/2018 – 12/2024	2	NCT03499249
Odevixibat	Triple	70+	North America, Europe, Middle East, Asia Pacific	7/2020 – 6/2024	3	NCT04336722
Maralixibat	Triple	7	North America, Europe	7/2021 – 4/2023	2	NCT04524390

Despite numerous attempts to improve the operative outcomes by modifications of surgical techniques, the result of KPE remains unsatisfactory and less than 60% of BA patients could remain transplant-free after 10 years from the KPE. Moreover, the native liver survivors are not 'cured' as more than half of them would suffer from complications including portal hypertension, recurrent cholangitis and progressive liver failure [101]. Even though liver transplant is generally regarded as the salvage treatment for a failed KPE, it is a major operation with significant morbidities and necessitates life-long immunosuppression [102]. In some countries, this treatment option is furthermore handicapped by the shortage of organ donors.

Novel treatments are thus needed, and there are at least two principal avenues towards therapy development for BA: pharmacological treatments that block or ameliorate the disease, or directly repairing or replacing the ailing bile duct tissue. In the first category, there are a number of clinical trials testing repurposed therapeutics (Table 2), but the efficacy of these trials is not yet known. An additional recent example is the outcome of characterizing the immunological responses in BA patients and the RRV mouse model [67]. As discussed above, B cell lymphogenesis persisted after birth in BA patients causing autoimmune responses, and in a small-scale clinical trial, a B-cell modifying therapy using rituximab resulted in a reduction of B cells combined with restoration of macrophage function.

On the tissue repair side, the technology for engineering endodermal tissues in vitro on decellularized matrices has improved during the last few years [103], and may be applied to engineering bile ducts. Engineered neo-bile ducts were successfully transplanted into pigs and well tolerated [104]. Recently, Sampaziodis et al., (2021) showed that extrahepatic organoids could successfully be transplanted to repair intrahepatic biliary ducts in human livers ex vivo and organoid-derived cells regenerated 40-85% of the injected ducts [28]. In immunocompromised mice where biliary duct damage was induced, only cholangiocyte organoids, but not MSC, grafted and similarly led to an amelioration of the duct damage [28]. Interestingly, primary cholangiocyte were not successful as donor cells in the restoration process, indicating that cells may need to be passed through the organoid state prior to transplantation. In conclusion, although there is still some way to go before therapies for BA are in routine clinical use, data from pharmacological modulations as well as cell transplantation experiments show considerable promise.

7. Conclusions

Biliary atresia is a devastating disease affecting newborns, and there is thus a dire need for therapy development. There is considerable progress on several fronts, ranging from recent insights into the underlying pathomechanisms to direct small-scale clinical trials using immunomodulatory therapies. There is also progress in advancing BA diagnosis, which is important to better stratify patients, in particular to decide if and when liver transplants are required. Innovative research using different model systems, including organoids, is also likely to further accelerate the discovery process for new pharmacological intervention principles and cell therapy approaches. There is thus reason to be cautiously optimistic that in a not-too-distant future, we will be able to develop therapies to ameliorate, or even cure, biliary atresia, for the benefit of the patients.

Contributors

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All authors have read and approved the final version of the manuscript.

Outstanding questions

BA remains a devastating inflammatory disease, primarily affecting newborns, and some outstanding questions remain before the disease can be fully combated. Firstly, it is important to further analyze the underlying pathomechanisms, and in detail learn at which steps during intra- and extra-hepatic differentiation steps the various disease-causing agents act. Secondly, there is room for improving regarding BA diagnostics, striving towards accurate and minimally invasive procedures. Finally, future research will hopefully provide pharmacological or cell-based therapies that are effective in routine clinical use.

Search strategy and selection criteria

Data for this review have been collected from Pubmed using search themes including: Biliary Atresia, liver disease, cholangiocyte, hepatoblast, therapy, diagnosis, mouse, rotavirus, zebrafish and organoid. Articles published in the timeframe 1990-2021 have been used.

Declaration of Competing Interest

The authors declare that they have no competing interests.

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Supplementary materials

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References

[1] Spada M, Riva S, Maggiore G, Cintorino D, Gridelli B. Pediatric liver transplantation. World Journal of Gastroenterology 2009;15:648–74.
[2] Tiao MM, Tsai SS, Kuo HW, Chen CL, Yang CY. Epidemiological features of biliary atresia in Taiwan, a national study 1996-2003. Journal of Gastroenterology and Hepatology (Australia) 2008;23:62–6.
[3] Davenport M, de Ville de Goyet J, Stringer M, Mieli-Vergani G, Kelly D, McClean P, et al. Seamless management of biliary atresia in England and Wales (1999–2002). The Lancet 2004;363:1354–7.
[4] Chung PHY, Zheng S, Tam PKH. Biliary atresia: East versus west. Seminars in Pediatric Surgery 2020;29:150950.
[5] Berauer JP, Mezina AI, Okou DT, Sabo A, Muzny DM, Gibbs RA, et al. Identification of Polycystic Kidney Disease 1 Like 1 Gene Variants in Children With Biliary Atresia Splenic Malformation Syndrome. Hepatology 2019;70:899–910.
[6] Cui S, Leyva-Vega M, Tsai EA, Eauclaire SF, Glessner JT, Hakonarson H, et al. Evidence from human and zebrafish That GPC1 is a biliary atresia susceptibility gene. Gastroenterology 2013;144:1107–15 e3.
[7] Chen Y, Gilbert MA, Grochowski CM, McEldrew D, Llewellyn J, Waisbourd-Zinman O, et al. A genome-wide association study identifies a susceptibility locus for biliary atresia on 2p16.1 within the gene EFEMP1. PLoS Genetics 2018;14:e1007532.
[8] Ningappa M, So J, Glessner J, Ashokkumar C, Ranganathan S, Min J, et al. The role of ARF6 in biliary atresia. PLoS ONE 2015;10:e0138381.
[9] Mezina A, Karpen SJ. Genetic contributors and modifiers of biliary atresia. Digestive Diseases 2015;33:408–14.
[10] Girard M, Panasyuk G. Genetics in biliary atresia. Current Opinion in Gastroenterology 2019;35:73–81.
[11] Lam W, Tang C, So M, Hus J, Chung P, Nicholls J, et al. Identification of a wide spectrum of ciliary gene mutations in nonsyndromic biliary atresia patients implicates ciliary dysfunction as a novel disease mechanism. EBioMedicine 2021;71:103530.
[12] Harpavat S, Garcia-Prats JA, Shneider BL. Newborn Bilirubin Screening for Biliary Atresia. New England Journal of Medicine 2016;375:605–6.
[13] Averbukh LD, Wu GY. Evidence for viral induction of biliary atresia: A review. Journal of Clinical and Translational Hepatology 2018;6:410–9.
[14] Lorent K, Gong W, Koo KA, Waisbourd-Zinman O, Karjoo S, Zhao X, et al. Identification of a plant isoflavonoid that causes biliary atresia. Science Translational Medicine 2015;7:286ra67.
[15] Scottoni F, Davenport M. Biliary atresia: Potential for a new decade. Seminars in Pediatric Surgery 2020;29:150940.
[16] Bezerra JA, Wells RG, Mack CL, Karpen SJ, Hoofnagle JH, Doo E, et al. Biliary Atresia: Clinical and Research Challenges for the Twenty-First Century. Hepatology 2018;68:1163–73.
[17] Tam PKH, Chung PHY, St Peter SD, Gayer CP, Ford HR, Tam GCH, et al. Advances in paediatric gastroenterology. The Lancet 2017;390:1072–82.
[18] Fiorotto R, Raizner A, Morell CM, Torsello B, Scirpo R, Fabris L, et al. Notch signaling regulates tubular morphogenesis during repair from biliary damage in mice. Journal of Hepatology 2013;59:124–30.
[19] Hankeova S, Salplachta J, Zikmund T, Kavkova M, Hul V, Brinek A, et al. DUCT reveals architectural mechanisms contributing to bile duct recovery in a mouse model for Alagille syndrome. ELife 2021;10:e60916.
[20] Lemaigre FP. Development of the Intrahepatic and Extrahepatic Biliary Tract: A Framework for Understanding Congenital Diseases. Annual Review of Pathology: Mechanisms of Disease 2020;15:1–22.
[21] Prior N, Hindley CJ, Rost F, Meléndez E, Lau WWY, Göttgens B, et al. Lgr5+ stem/progenitor cells reside at the apex of a heterogeneous embryonic hepatoblast pool. Development 2019;146:dev174557.
[22] Schaub JR, Huppert KA, Kurial SNT, Hsu BY, Cast AE, Donnelly B, et al. De novo formation of the biliary system by TGFβ-Mediated Hepatocyte Transdifferentiation. Nature 2018;557:247–51.
[23] Spence JR, Lange AW, Lin SCJ, Kaestner KH, Lowy AM, Kim I, et al. Sox17 Regulates Organ Lineage Segregation of Ventral Foregut Progenitor Cells. Developmental Cell 2009;17:62–74.
[24] Clotman F, Lannoy VJ, Reber M, Cereghini S, Cassiman D, Jacquemin P, et al. The onecut transcription factor HNF6 is required for normal development of the biliary tract. Development 2002;129:1819–27.
[25] Coffinier C, Gresh L, Fiette L, Tronche F, Schütz G, Babinet C, et al. Bile system morphogenesis defects and liver dysfunction upon targeted deletion of HNF1β. Development 2002;129:1829–38.
[26] Dong PDS, Munson CA, Norton W, Crosnier C, Pan X, Gong Z, et al. Fgf10 regulates hepatopancreatic ductal system patterning and differentiation. Nature Genetics 2007;39:397–402.
[27] Aizarani N, Saviano A, Sagar Mailly L, Durand S, Herman JS, et al. A human liver cell atlas reveals heterogeneity and epithelial progenitors. Nature 2019;572:199–204.
[28] Sampaziotis F, Muraro D, Tysoe OC, Sawiak S, Beach TE, Godfrey EM, et al. Cholangiocyte organoids can repair bile ducts after transplantation in the human liver. Science 2021;371:839–46.
[29] Halpern KB, Shenhav R, Matcovitch-Natan O, Tóth B, Lemze D, Golan M, et al. Single-cell spatial reconstruction reveals global division of labour in the mammalian liver. Nature 2017;542:352–6.
[30] Halpern KB, Shenhav R, Massalha H, Toth B, Egozi A, Massasa EE, et al. Paired-cell sequencing enables spatial gene expression mapping of liver endothelial cells. Nature Biotechnology 2018;36:962–70.
[31] Dobie R, Wilson-Kanamori JR, Henderson BEP, Smith JR, Matchett KP, Portman JR, et al. Single-Cell Transcriptomics Uncovers Zonation of Function in the Mesenchyme during Liver Fibrosis. Cell Reports 2019;29:1832–47.
[32] Riepenhoff-Talty M, Schaekel K, Clark FH, Mueller W, Uhnoo I, Rossi T, et al. Group A Rotaviruses Produce Extrahepatic Biliary Obstruction in Orally Inoculated Newborn Mice. Pediatric Research 1993;33:394–9.
[33] Hertel PM, Estes MK. Rotavirus and biliary atresia: can causality be proven? Current Opinion in Gastroenterology 2012;28:10–7.
[34] Danial E, Fleck-Derderian S, Rosenthal P. Has Rotavirus Vaccination Decreased the Prevalence of Biliary Atresia? Journal of Clinical Gastroenterology 2019;53:e348–51.
[35] Mohanty SK, Lobeck I, Donnelly B, Dupree P, Walther A, Mowery S, et al. Rotavirus Reassortant–Induced Murine Model of Liver Fibrosis Parallels Human Biliary Atresia. Hepatology 2020;71:1316–30.
[36] Zhang R, Lin Z, Fu M, Guan X, Yu J, Zhong W, et al. The Role of Neonatal Gr-1+ Myeloid Cells in a Murine Model of Rhesus-Rotavirus–Induced Biliary Atresia. The American Journal of Pathology 2018;188:2617–28.
[37] Garrido M, Escobar C, Zamora C, Rejas C, Varas J, Párraga M, et al. Bile duct ligature in young rats: A revisited animal model for biliary atresia. European Journal of Histochemistry 2017;61:2803.
[38] Yang Y, Wang J, Zhan Y, Chen G, Shen Z, Zheng S, et al. The synthetic toxin biliatresone causes biliary atresia in mice. Laboratory Investigation 2020;100:1425–35.
[39] Pham D-H, Zhang C, Yin C. Using Zebrafish to Model Liver Diseases-Where Do We Stand? Current Pathobiology Reports 2017;5:207–21.
[40] Lorent K, Yeo SY, Oda T, Chandrasekharappa S, Chitnis A, Matthews RP, et al. Inhibition of Jagged-mediated notch signaling disrupts zebrafish biliary development and generates multi-organ defects compatible with an Alagille syndrome phenocopy. Development 2004;131:5753–66.
[41] Novoa B, Figueras A. Zebrafish: Model for the Study of Inflammation and the Innate Immune Response to Infectious Diseases. Adv Exp Med Biol 2012;946:253–75.
[42] Zhao X, Lorent K, Wilkins BJ, Marchione DM, Gillespie K, Waisbourd-Zinman O, et al. Glutathione antioxidant pathway activity and reserve determine toxicity and specificity of the biliary toxin biliatresone in zebrafish. Hepatology 2016;64:894–907.
[43] Tang V, Cofer ZC, Cui S, Sapp V, Loomes KM, Matthews RP. Loss of a candidate biliary atresia susceptibility gene, add3a, causes biliary developmental defects in zebrafish. Journal of Pediatric Gastroenterology and Nutrition 2016;63:524–30.
[44] Cheung Y, Wu Z, Garcia-Barcelo M-M, Tam PKH, Ma ACH, Lui VCH. Deletion of interleukin enhancer binding factor 2 (ILF2) resulted in defective biliary development and bile flow blockage. Journal of Pediatric Surgery 2021;56:352–9.
[45] Cofer ZC, Cui S, Eauclaire SF, Kim C, Tobias JW, Hakonarson H, et al. Methylation microarray studies highlight PDGFA expression as a factor in Biliary atresia. PLoS ONE 2016;11:e0151521.
[46] Matthews RP, Eauclaire SF, Mugnier M, Lorent K, Cui S, Ross MM, et al. DNA hypomethylation causes bile duct defects in zebrafish and is a distinguishing feature of infantile biliary atresia. Hepatology 2011;53:905–14.
[47] Guan Y, Xu D, Garfin PM, Ehmer U, Hurwitz M, Enns G, et al. Human hepatic organoids for the analysis of human genetic diseases. JCI Insight 2017;2:e94954.
[48] Marsee A, Roos FJM, Verstegen MMA, Gehart H, de Koning E, Lemaigre F, et al. Building consensus on definition and nomenclature of hepatic, pancreatic, and biliary organoids. Cell Stem Cell 2021;28:816–32.
[49] Huch M, Gehart H, van Boxtel R, Hamer K, Blokzijl F, a Verstegen MM, et al. Long-Term Culture of Genome-Stable Bipotent Stem Cells from Adult Human Liver. Cell 2015;160:299–312.
[50] Andersson ER, Chivukula IV, Hankeova S, Sjöqvist M, Tsoi YL, Ramsköld D, et al. Mouse Model of Alagille Syndrome and Mechanisms of Jagged1 Missense Mutations. Gastroenterology 2018;154:1080–95.
[51] Sato K, Zhang W, Safarikia S, Isidan A, Chen AM, Li P, et al. Organoids and Spheroids as Models for Studying Cholestatic Liver Injury and Cholangiocarcinoma. Hepatology 2021;74:491–502.
[52] Rimland CA, Tilson SG, Morell CM, Tomaz RA, Lu WY, Adams SE, et al. Regional Differences in Human Biliary Tissues and Corresponding In Vitro–Derived Organoids. Hepatology 2021;73:247–67.
[53] Sampaziotis F, Justin AW, Tysoe OC, Sawiak S, Godfrey EM, Upponi SS, et al. Reconstruction of the mouse extrahepatic biliary tree using primary human extrahepatic cholangiocyte organoids. Nature Medicine 2017;23:954–63.
[54] Babu RO, Lui VCH, Chen Y, Yiu RSW, Ye Y, Niu B, et al. Beta-amyloid deposition around hepatic bile ducts is a novel pathobiological and diagnostic feature of biliary atresia. Journal of Hepatology 2020;73:1391–403.

[55] Amarachintha SP, Mourya R, Ayabe H, Yang L, Luo Z, Li X, et al. Biliary organoids uncover delayed epithelial development and barrier function in biliary atresia. Hepatology 2021 epub ahead of print.

[56] Waisbourd-Zinman O, Koh H, Tsai S, Lavrut PM, Dang C, Zhao X, et al. The toxin biliatresone causes mouse extrahepatic cholangiocyte damage and fibrosis through decreased glutathione and SOX17. Hepatology 2016;64:880–93.

[57] Fried S, Gilboa D, Har-Zahav A, Lavrut PM, Du Y, Karjoo S, et al. Extrahepatic cholangiocyte obstruction is mediated by decreased glutathione, Wnt and Notch signaling pathways in a toxic model of biliary atresia. Scientific Reports 2020;10:7599.

[58] Tian L, Ye Z, Kafka K, Stewart D, Anders R, Schwarz KB, et al. Biliary Atresia Relevant Human Induced Pluripotent Stem Cells Recapitulate Key Disease Features in a Dish. Journal of Pediatric Gastroenterology and Nutrition 2019;68:56–63.

[59] Chen S, Li P, Wang Y, Yin Y, de Ruiter PE, Verstegen MMA, et al. Rotavirus Infection and Cytopathogenesis in Human Biliary Organoids Potentially Recapitulate Biliary Atresia Development. MBio 2020;11 e301968-20.

[60] Du Y, Khandekar G, Llewellyn J, Polacheck W, Chen CS, Wells RG. A Bile Duct-on-a-Chip With Organ-Level Functions. Hepatology 2020;71:1350–63.

[61] Xu Y, Yu J, Zhang R, Yin Y, Ye J, Tan L, et al. The perinatal infection of cytomegalovirus is an important etiology for biliary atresia in China. Clinical Pediatrics 2012;51:109–13.

[62] Davenport M. Biliary atresia: Clinical aspects. Seminars in Pediatric Surgery 2012;21:175–84.

[63] Wen J, Xiao Y, Wang J, Pan W, Zhou Y, Zhang X, et al. Low doses of CMV induce autoimmune-mediated and inflammatory responses in bile duct epithelia of regulatory T cell-depleted neonatal mice. Laboratory Investigation 2015;95:180–92.

[64] Wang W, Donnelly B, Bondoc A, Mohanty SK, McNeal M, Ward R, et al. The Rhesus Rotavirus Gene Encoding VP4 Is a Major Determinant in the Pathogenesis of Biliary Atresia in Newborn Mice. Journal of Virology 2011;85:9069–77.

[65] Pang S-Y, Dai Y-M, Zhang R-Z, Chen Y-H, Peng X-F, Fu J, et al. Autoimmune liver disease-related autoantibodies in patients with biliary atresia. World Journal of Gastroenterology 2018;24:387–96.

[66] Luo Y, Brigham D, Bednarek J, Torres R, Wang D, Ahmad S, et al. Unique Cholangiocyte-Targeted IgM Autoantibodies Correlate With Poor Outcome in Biliary Atresia. Hepatology 2021;73:1855–67.

[67] Wang J, Xu Y, Chen Z, Liang J, Lin Z, Liang H, et al. Liver Immune Profiling Reveals Pathogenesis and Therapeutics for Biliary Atresia. Cell 2020;183:1867–83 e26.

[68] Tucker RM, Feldman AG, Fenner EK, Mack CL. Regulatory T cells inhibit Th1 cell-mediated bile duct injury in murine biliary atresia. Journal of Hepatology 2013;59:790–6.

[69] Miethke AG, Saxena V, Shivakumar P, Sabla GE, Simmons J, Chougnet CA. Postnatal paucity of regulatory T cells and control of NK cell activation in experimental biliary atresia. Journal of Hepatology 2010;52:718–26.

[70] Ortiz-Perez A, Donnelly B, Temple H, Tiao G, Bansal R, Mohanty SK. Innate Immunity and Pathogenesis of Biliary Atresia. Frontiers in Immunology 2020;11:1–14.

[71] Mohanty S, Donnelly B, Temple H, Ortiz-Perez A, Mowery S, Lobeck I, et al. HMGB1 release by cholangiocytes governs biliary atresia pathogenesis and correlates with increases in afflicted infants. Hepatology 2021;74:864–78.

[72] Klemann C, Schröder A, Dreier A, Möhn N, Dippel S, Winterberg T, et al. Interleukin 17, Produced by $\gamma\delta$ T Cells, Contributes to Hepatic Inflammation in a Mouse Model of Biliary Atresia and Is Increased in Livers of Patients. Gastroenterology 2016;150:229–41 e5.

[73] Möhn N, Bruni E, Schröder A, Frömmel S, Gueler F, Vieten G, et al. Synthetic retinoid AM80 inhibits IL-17 production of gamma delta T cells and ameliorates biliary atresia in mice. Liver International 2020;40:3031–41.

[74] Ishii M, Vroman B, LaRusso NF. Morphologic demonstration of receptor-mediated endocytosis of epidermal growth factor by isolated bile duct epithelial cells. Gastroenterology 1990;98:1284–91.

[75] Vroman B, LaRusso NF. Development and characterization of polarized primary cultures of rat intrahepatic bile duct epithelial cells. Laboratory Investigation 1996;74:303–13.

[76] Fabris L, Fiorotto R, Spirli C, Cadamuro M, Mariotti V, Perugorria MJ, et al. Pathobiology of inherited biliary diseases: a roadmap to understand acquired liver diseases. Nature Reviews Gastroenterology and Hepatology 2019;16:497–511.

[77] Masyuk AI, Huang BQ, Ward CJ, Gradilone SA, Banales JM, Masyuk T v, et al. Biliary exosomes influence cholangiocyte regulatory mechanisms and proliferation through interaction with primary cilia. American Journal of Physiology - Gastrointestinal and Liver Physiology 2010;299:990–9.

[78] Zhou Y, Ji H, Xu Q, Zhang X, Cao X, Chen Y, et al. Congenital biliary atresia is correlated with disrupted cell junctions and polarity caused by Cdc42 insufficiency in the liver. Theranostics 2021;11:7262–75.

[79] Raynaud P, Carpentier R, Antoniou A, Lemaigre FP. Biliary differentiation and bile duct morphogenesis in development and disease. International Journal of Biochemistry and Cell Biology 2011;43:245–56.

[80] Huang BQ, Masyuk T v, Muff MA, Tietz PS, Masyuk AI, LaRusso NF. Isolation and characterization of cholangiocyte primary cilia. American Journal of Physiology - Gastrointestinal and Liver Physiology 2006;291:500–9.

[81] Masyuk AI, Masyuk T v, Splinter PL, Huang BQ, Stroope AJ, LaRusso NF. Cholangiocyte Cilia Detect Changes in Luminal Fluid Flow and Transmit Them Into Intracellular Ca2+ and cAMP Signaling. Gastroenterology 2006;131:911–20.

[82] Masyuk AI, Gradilone SA, Banales JM, Huang BQ, Masyuk T v, Lee SO, et al. Cholangiocyte primary cilia are chemosensory organelles that detect biliary nucleotides via P2Y12 purinergic receptors. American Journal of Physiology - Gastrointestinal and Liver Physiology 2008;295:725–34.

[83] Frassetto R, Parolini F, Marceddu S, Satta G, Papacciuoli V, Pinna MA, et al. Intrahepatic bile duct primary cilia in biliary atresia. Hepatology Research 2018;48:664–74.

[84] Hartley JL, O'Callaghan C, Rossetti S, Consugar M, Ward CJ, Kelly DA, et al. Investigation of primary cilia in the pathogenesis of biliary atresia. Journal of Pediatric Gastroenterology and Nutrition 2011;52:485–8.

[85] Karjoo S, Hand NJ, Loarca L, Russo PA, Friedman JR, Wells RG. Extrahepatic cholangiocyte cilia are abnormal in biliary atresia. Journal of Pediatric Gastroenterology and Nutrition 2013;57:96–101.

[86] Bangs F, Anderson K v. Primary cilia and Mammalian Hedgehog signaling. Cold Spring Harbor Perspectives in. Biology 2017;9:a028175.

[87] Rock N, McLin V. Liver involvement in children with ciliopathies. Clinics and Research in Hepatology and Gastroenterology 2014;38:407–14.

[88] van de Laarschot LFM, Drenth JPH. Genetics and mechanisms of hepatic cystogenesis. Biochimica et Biophysica Acta - Molecular Basis of Disease 2018;1864:1491–7.

[89] Mao Y, Tang S, Yang L, Li K. Inhibition of the notch signaling pathway reduces the differentiation of hepatic progenitor cells into cholangiocytes in Biliary Atresia. Cellular Physiology and Biochemistry 2018;49:1115–23.

[90] Siebel C, Lendahl U. Notch signaling in development, tissue homeostasis, and disease. Physiological Reviews 2017;97:1235–94.

[91] Hageman JH, Heinz MC, Kretzschmar K, van der Vaart J, Clevers H, Snippert HJG. Intestinal Regeneration: Regulation by the Microenvironment. Developmental Cell 2020;54:435–46.

[92] Luo Z, Shivakumar P, Mourya R, Gutta S, Bezerra JA. Gene Expression Signatures Associated With Survival Times of Pediatric Patients With Biliary Atresia Identify Potential Therapeutic Agents. Gastroenterology 2019;157:1138–52.

[93] Kyrönlahti A, Godbole N, Akinrinade O, Soini T, Nyholm I, Andersson N, et al. Evolving Up-regulation of Biliary Fibrosis–Related Extracellular Matrix Molecules After Successful Portoenterostomy. Hepatology Communications 2021;5:1036–50.

[94] Lertudomphonwanit C, Mourya R, Fei L, Zhang Y, Gutta S, Yang L, et al. Large-scale proteomics identifies MMP-7 as a sentinel of epithelial injury and of biliary atresia. Science Translational Medicine 2017;9:1–12.

[95] Jiang J, Wang J, Shen Z, Lu X, Chen G, Huang Y, et al. Serum MMP-7 in the diagnosis of biliary atresia. Pediatrics 2019;144:e20190902.

[96] Fabris L, Cadamuro M, Guido M, Spirli C, Fiorotto R, Colledan M, et al. Analysis of liver repair mechanisms in alagille syndrome and biliary atresia reveals a role for notch signaling. American Journal of Pathology 2007;171:641–53.

[97] Williams R, Alessi C, Alexander G, Allison M, Aspinall R, Batterham RL, et al. New dimensions for hospital services and early detection of disease: a Review from the Lancet Commission into liver disease in the UK. The Lancet 2021;397:1770–80.

[98] Nio M, Wada M, Sasaki H, Tanaka H. Effects of age at Kasai portoenterostomy on the surgical outcome: a review of the literature. Surgery Today 2015;45:813–8.

[99] He L, Ip DKM, Tam G, Lui VCH, Tam PKH, Chung PHY. Biomarkers for the diagnosis and post-Kasai portoenterostomy prognosis of biliary atresia: a systematic review and meta-analysis. Scientific Reports 2021;11:11692.

[100] Davenport M, Heaton N, Superina R. Surgery of the liver, bile ducts and pancreas in children editors. Boca Raton: CRC Press; 2017.

[101] Chung PHY, Chan EKW, Yeung F, Chan ACY, Mou JWC, Lee KH, et al. Life long follow up and management strategies of patients living with native livers after Kasai portoenterostomy. Scientific Reports 2021;11:11207.

[102] Sundaram SS, Mack CL, Feldman AG, Sokol RJ. Biliary atresia: Indications and timing of liver transplantation and optimization of pretransplant care. Liver Transplantation 2017;23:96–109.

[103] Giobbe GG, Crowley C, Luni C, Campinoti S, Khedr M, Kretzschmar K, et al. Extracellular matrix hydrogel derived from decellularized tissues enables endodermal organoid culture. Nature Communications 2019;10:5658.

[104] Struecker B, Hillebrandt KH, Raschzok N, Jöhrens K, Butter A, Tang P, et al. Implantation of a tissue-engineered neo-bile duct in domestic pigs. European Surgical Research 2016;56:61–75.