Review

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Biliary atresia and other cholestatic childhood diseases: Advances and future challenges

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Summary

Biliary Atresia and other cholestatic childhood diseases are rare conditions affecting the function and/or anatomy along the canalicular-bile duct continuum, characterised by onset of persistent cholestatic jaundice during the neonatal period. Biliary atresia (BA) is the most common among these, but still has an incidence of only 1 in 10–19,000 in Europe and North America. Other diseases such as the genetic conditions, Alagille syndrome (ALGS) and Progressive Familial Intrahepatic Cholestasis (PFIC), are less common. Choledochal malformations are amenable to surgical correction and require a high index of suspicion. The low incidence of such diseases hinder patient-based studies that include large cohorts, while the limited numbers of animal models of disease that recapitulate the spectrum of disease phenotypes hinders both basic research and the development of new treatments. Despite their individual rarity, collectively BA and other cholestatic childhood diseases are the commonest indications for liver transplantation during childhood. Here, we review the recent advances in basic research and clinical progress in these diseases, as well as the research needs. For the various diseases, we formulate current key questions and controversies and identify top priorities to guide future research.
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Diagnostic developments for neonatal cholestasis

Key questions

- Which strategies can enhance earlier recognition of neonatal cholestasis, including biliary atresia (BA)?
- What is the value of abdominal ultrasound, nuclear isotope excretion scan, liver biopsy and endoscopic retrograde cholangiopancreatography (ERCP) in the diagnostic work up?

BA prognosis relates to timely surgical correction and therefore early diagnosis is mandatory (ideally before 30 days of age). Various diagnostic algorithms have been proposed [1–3]. A prompt diagnosis relies on the basic recognition that the infant has conjugated hyperbilirubinemia. Benign physiological or breast-milk associated unconjugated hyperbilirubinemia with normal color stools and urine is frequent, so the presence of acholic stools and pigmented urine should raise suspicion of liver disease in all jaundiced babies. Unfortunately, however, these symptoms can appear relatively late in biliary atresia (BA) and may go unrecognised. Thus, it has been recommended by the American Academy of Pediatris that all infants with jaundice persisting beyond 2–3 weeks of age should have conjugated/direct bilirubin measured to identify those infants with cholestasis who require further evaluation in a referral unit [4], which should have the capacity to perform radiological imaging, liver biopsy interpretation and exclusion of genetic conditions mimicking BA within a few

Key point

Novel mechanisms have been identified in the pathophysiology of these diseases, including the roles of viral infections, immune dysregulation, gene polymorphisms and toxins.

days. Despite these recommendations, the average age at diagnosis and treatment of BA (~60 days) has not changed over the past 20 years in the United States and many other countries [5,6], and only decreased to some extent in the United Kingdom [7].

New strategies to screen for neonatal cholestasis are clearly needed to enhance early diagnosis of BA. One such method is the provision of stool color cards to parents of newborns for identification of acholic stools. Routine screening for BA with stool color cards started in Japan in the 1990s [8], and was later introduced nationwide in Taiwan [9] and in Switzerland [10]. In Taiwan, five years after starting the stool color card screening, the rate of Kasai hepatoportoenterostomy (KPE) at <60 days increased from 49% to 66%; the jaundice free rate at 3 months after surgery from 35% to 61%, and the 5-year survival with native liver from 27% to 64% [9]. As reported by Matsui, a program involving 313,230 infants in Japan's Tochigi Prefecture between 1994 and 2011 – with an 84% card return rate – demonstrated a sensitivity, specificity, positive predictive value, and negative predictive value for BA of 77%, 99.9%, 13%, and 99.9% respectively, with a native liver survival of BA children at 5, 10 and 15 years of 88%, 77% and 49% [11]. Recently, a large-scale prospective study demonstrated the practicality and cost effectiveness of the stool color card [12]. However, the success of a stool color card program may be more limited in countries without routine 30-day old well-child visits for review of the stool color card. Finally, the possibility of using direct/conjugated serum bilirubin measurements in newborns to screen for BA has recently been proposed [13] and is being pursued in different centers.

The diagnostic role of endoscopic retrograde cholangiopancreatography (ERCP) remains controversial. In some centers with particular expertise, ERCP is used as a first-line diagnostic tool [14,15], while in others it is limited to cases where the diagnosis remains doubtful after standard diagnostic tests (typically liver biopsy) [16]. Some centers rely heavily on ultrasound findings for the diagnosis of BA, including the "triangular cord" sign [17]. Most centers, however, consider ultrasound a complementary investigation, relying mostly on histological findings (liver biopsy) and exclusion of genetic disorders mimicking BA [18] in decision making for exploratory surgery. Nuclear isotope excretion scans are no longer frequently used: the absence of excretion into the intestines does not confirm BA. After the identification of cholestasis in an infant, diagnostic evaluation that would yield the diagnosis of BA should be completed within one week in order to expedite early surgery (before ~35 days). Recognizing that genetic tests for syndromes of inherited cholestasis may take several weeks, they are not included in the typical diagnostic algorithm for BA.

Top priorities for enhancing early diagnosis of neonatal cholestasis

- We recommend a broader implementation of screening strategies, in particular the stool color card, with implementation that is tailored to country-specific infant care models. Educating parents of newborns is essential to the success of the stool color card program. To minimise diagnostic delay, patients with neonatal cholestasis should be evaluated in (or under guidance of) an experienced center that can complete the evaluation rapidly and, if indicated, can perform an intraoperative cholangiogram and KPE without delay.

Advancing the prognosis of biliary atresia

Key questions

- To what extent has the prognosis of BA been characterised among different centers and countries? How has this evolved over time?
- What are the major causes and predictors of morbidity and mortality of patients with BA?
- Is centralized care essential for improving prognosis?

Paediatric registries have proven useful for detailing epidemiology of BA and for benchmarking both their short and long-term outcomes [7,19–23]. The UK registry was the basis for the first report of a significantly higher post-KPE jaundice free survival rate in high (>5 cases/year) *vs.* low surgical volume centers resulting in centralization of KPE surgery to only 3 high volume centers [20,24]. In contrast, France has a decentralised policy for the care of BA [6]. The national institutes of health sponsored childhood liver disease research network (ChiLDReN) is a consortium of 16 specialised centers in North America (www.childrennetwork.org), using similar clinical care protocols (without centralizing care) within prospective longitudinal study of 8 rare liver diseases, evaluating and tracking BA outcomes and their predictors [25]. Table 1 describes outcomes in several other disease registries, national or otherwise.

The French registry reported a BA incidence of 1:19,400 live births [26], similar to other reports from western Europe and Canada, and somewhat lower than the incidence in USA [19,21,22,25,27].

Abbreviations: ALGS, Alagille syndrome; BARD, Biliary Atresia and Related Diseases; PFIC, Progressive Familial Intrahepatic Cholestasis; KPE, Kasai hepatoportoenterostomy; NeSBAR, Netherlands Study group for Biliary Atresia Registry; ChiLDReN, Childhood Liver Disease Research Network; ERCP, endoscopic retrograde cholangiopancreatography; START, Steroids in Biliary Atresia Randomised Trial; BASM, Biliary Atresia Splenic Malformation syndrome; ASBT, apical sodium dependent bile acid transporter; CM, choledochal malformation; GGT, gamma glutamyltransferase.

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Table 1. Overview of national or multicenter registries on biliary atresia, based on published reports. Biliary atresia registries, national or otherwise.

Country, period, and No. of centers Follow-up (median, range)	No. of patients	No. of KPE	Age at KPE days (in average)	Primary LTx	Survival overall	Survival with native liver	Jaundice free after KPE*	Reference
Canada, 1992-2002, 3 centers Follow-up 5.5 years (0.4-14.2 years)	230	207	64	10%	83% (1)	39% (1)	n.a.	[23]
France, 1986-2009, 45 centers Follow-up 9.5 years (0.3-24.6 years)	1107	1044	59	4%	79% (2)	40% (3)	38% (4)	[6]
Germany, 2001-2015, 29 centers Follow-up 3.3 years (2.1-7.1)	183	159	57	11%	83% (5)	20% (5)	18% (5)	[111]
Japan, 1989-1999, 93 centers Follow-up 5 resp. 10 years[#]	1381	1181	n.a.	0.1%	75% (3)	60% (3)	57%** (4)	[28]
Netherlands, 1987-2008, 6 centers Follow-up 6.9 years (0.1-21.9 years)	231	214	59	3%	73% (1)	46% (1)	36% (1)	[21]
Switzerland, 1994-2004, 7 centers Follow-up 4 years[#*]	48	43	68	10%	92% (3)	37% (3)	37% (3)	[112]
UK[&], 1999-2009, 3 centers Follow-up 4 years	443	424	54	3%	89% (6)	46% (1)	55% (7)	[7]
USA/Biliary Atresia Research Consortium, 1997-2000; 9 centers (not a national registry) Follow-up 2 years[#]	104	104	61	n.a.[##]	87% (5)	56% (5)	38% (7)	[25]

[&]United Kingdom (England and Wales); *jaundice free defined as bilirubin <20 μmol/l; **jaundice free defined as bilirubin <34.2 μmol/l; [#]length of total follow-up not available; [##]patients not undergoing KPE had been excluded; [(1)]4 years; [(2)]at last follow-up; [(3)]5 years; [(4)]undefined period; [(5)]2 years; [(6)]10 years; [(7)]6 months.
KPE, Kasai hepatic portoenterostomy; LTx, liver transplantation; n.a., not available.

However, these rates are much lower than those reported from Asia (e.g., Japan, 1:9,640) [28] and in the smaller populations of French Polynesia (1:3,401) [29] and the Maoris of New Zealand (1:3,124) [30]. The reasons for this remain obscure.

Top priorities for advancing the prognosis of biliary atresia

- To study the relationships between clinical and therapeutic interventions and outcomes through expanding the reach and depth of data in databases. Expanded databases in BA will also facilitate collaborative studies to "enable better assessment of disease risk, understanding of disease mechanisms, and prediction of optimal therapy" – as proposed by the National Institute of Health of the USA [31]. The recently launched online registry "bard-online" (www.bard-online.com) might aid in the collection of multinational data, including from countries without registries.

Treatment of biliary atresia after Kasai portoenterostomy

Key questions

- Which strategies following the Kasai portoenterostomy (KPE) may delay or prevent the need for liver transplantation?
- What are accepted prognostic parameters for long-term success of KPE?
- What can be learned from variance in outcome of BA and KPE among different centers and countries?

The most important advances for the long-term prognosis of BA have been the development of KPE [32] and of paediatric liver transplantation. The development of effective medical treatments following KPE to delay need for liver transplantation has been limited. Davenport *et al.* reported a randomised, double-blind placebo-controlled trial of oral prednisolone treatment *vs.* placebo in BA patients post-KPE [33]. The steroid regime did not reduce the need for liver transplantation within 1 year, but subgroup analysis suggested beneficial effects on serum bilirubin in infants aged less than 70 days at KPE. In a follow-up open-labeled study in young BA patients (<70 days at KPE), this observation was confirmed together with a statistically significant increase in the proportion able to clear their jaundice. Despite this, there was no statistical difference in either 4-year patient survival or native liver survival [34].

In the largest randomised controlled double-blind clinical trial in BA thus far, Bezerra *et al.* studied the effects of a 13 week course of steroids on clearance of jaundice with native liver at 6 months after KPE. This began with 4 weeks of high dose intravenous or oral methylprednisolone *vs.* placebo [35]. The clearance of jaundice was not statistically different between the two groups, but a small clinical benefit could not be excluded. Survival with native liver at 2 years was virtually identical between the treatment and control groups. Earlier onset of serious adverse events occurred during treatment in the steroid group compared to the placebo group,

raising concerns for high dose steroid therapy in the face of no demonstrable benefit.

Some other drugs have been hypothesised to modify the prognosis of BA including antibiotics (administered in the immediate post-operative period after KPE or as prolonged prophylaxis against cholangitis) and choleretic agents, such as ursodeoxycholic acid. However, there are no published randomised controlled or pragmatic clinical trials post-KPE that are statistically powered to firmly support either antibiotic treatment or choleretic agents. Current use of these agents is therefore opinion- and center-based rather than evidence-based.

The available trials indicate that age at surgery may influence the responsiveness to treatment. It has recently been proposed that the presence of cytomegalovirus IgM-positivity defines a phenotype of BA with inferior outcomes [36]. Thus, age at KPE and evidence of cytomegalovirus infection should be considered when establishing inclusion and exclusion criteria of future study designs. Another factor that may influence the (lack of) therapeutic response to steroids in BA is the stage of disease, such as advanced fibrosis in patients older than 70 days of age at KPE. Recently, an unprecedented high success rate has been reported with respect to long-term native liver survival in Japan after portoenterostomy: 88%, 77% and 49% at 5, 10 and 15 years, respectively [11]. It is still unclear whether existing practices and techniques in Japan could be helpful in improving global outcomes of patients with BA. It cannot be excluded that differences in genetic background or environmental factors account for the improved prognosis.

Top priorities for improving the treatment success of biliary atresia after Kasai portoenterostomy

The high success rates recently reported in Japan suggest that the prognosis of BA can be further improved. Top priorities for improving the treatment success are:

- Identification of predictors of the responsiveness to medical treatments after KPE, through analysis of clinical, laboratory, genetic and radiological characteristics.
- Studies of environmental, medical, and surgical approaches that may be linked to the variance in outcomes in different centers and countries.
- Exploration of new therapeutic strategies (e.g., anti-fibrotic drugs or farnesoid X-receptor agonists) that presently are in development, particularly in adult cholestatic diseases, to assess their ability to improve native liver survival.

Developments in understanding of the pathogenesis of biliary atresia

Key questions/controversies

- What are the genetic susceptibility factors for BA?
- Which viruses may trigger BA and does viral infection occur prenatally?
- Does immune dysregulation or autoimmunity play a role in the progressive bile duct injury after KPE?
- Do the intestinal microbiome and innate immunity play a role in BA pathogenesis and the rapid progression of fibrosis, even after successful KPE?
- Is there evidence that a toxin or vascular insult causes human BA?

There are several phenotypes of BA, each likely with its own etiology and mechanistic underpinning. Genetic expression studies combined with histologic examination of hepatobiliary tissues at diagnosis suggest that there may be inflammatory and fibrosing subtypes of BA, each with its own pattern of progression. There are subtypes of BA associated with congenital malformations (fetal or embryonic BA) suggesting abnormal bile duct morphogenesis in the etiology of BA. Biliary atresia splenic malformation syndrome (BASM; about 4–14% of cases) is associated with laterality defects, which suggest a genetic or epigenetic etiology [37,38]. Other subtypes include the presence of major congenital malformations without laterality defects (<5% of cases), or those with laterality defects but without splenic malformation (<5% of cases) [39,40]. Finally, a so called cystic biliary atresia has recently been described in up to 8% of BA [41], which includes the presence of a cyst within an otherwise obliterated biliary tree.

The majority of BA infants do not have congenital malformations (“isolated BA”, formerly known as perinatal or acquired BA). Almost all BA infants in fact have elevated serum direct bilirubin within the first 5 days of life [13], calling into question if perinatal cases are not indeed all prenatal in onset. Several susceptibility genes have been described based on GWAS and targeting sequencing approaches [42–46]. There are at least two theories regarding pathogenesis of isolated BA, based on human observations and mouse models. A viral-induced, immune or autoimmune mediated inflammatory obstruction of the biliary tree is the most commonly accepted theory based on strong experimental evidence from the rhesus rotavirus (RRV) Balb/C newborn mouse model [47–49]. Both T-cell [50,51] and B-cell-mediated autoimmunity [49,52] have been implicated as well as dysfunction of regulatory

T-cells [53,54], activation of innate immunity and NK cells [55] and dendritic cells [56], activation of a pro-inflammatory gene footprint in liver tissue [57] and the loss of cholangiocyte primary cilia [58]. Recently, the contribution of interleukin-17 to the inflammation and destruction of the biliary system has been demonstrated, both in infants with BA and in the RRV newborn mouse model [59,60].

A more recent provocative toxin theory for BA pathogenesis is centered on a plant toxin (biliatresone). It is proposed to be responsible for both the BA that occurs naturally in Australian livestock, and for a BA-like lesion in zebrafish [61,62]. Biliatresone appears to act by interfering with cholangiocyte polarity involving both Sox and Notch pathways. Ongoing investigations will need to determine the mechanisms of bile duct injury and obstruction by biliatresone and whether it is involved in human BA.

Finally, a vascular hypothesis for biliary atresia is based on the findings of anatomic variants of hepatic artery and arterial hyperplasia in liver of some cases of human BA [63]. It is currently unclear however, whether vascular changes are causative, the result of injury or part of the remodeling process [64].

In summary, the available data point to roles of single nucleotide polymorphisms (e.g., *CFC1* and *ADD3* genes) and extrinsic factors (e.g., viruses and toxins) as susceptibility and/or triggering factors that target bile ducts. An initial injury may be accompanied by a dysregulated or immature immune response that produces the fibrosing and obstructing phenotype of BA (Fig. 1).

Top priorities to understand the pathogenesis of biliary atresia

Top priorities to increase the understanding of the pathogenesis are:

- Identification of genetic variants that are more relevant to pathogenesis of syndromic forms of BA, and if found, characterise their functional significance.
- Assessment of possible influences of genetic variants on severity of disease and response to surgical treatment.
- Systematic biological approach to identify if common immunological factors can be identified in the pathogenesis of BA and BA-related liver fibrosis and if they are amenable to therapeutic interventions.
- Assessment of the role of specific toxins that target bile duct epithelia (e.g., biliatresone) in the pathogenesis of BA in humans.

Choledochal malformation

Key questions

- Does prenatally discovered choledochal malformation (CM) require a different treatment strategy than postnatally diagnosed CM?
- What is the right timing of surgery in patients with asymptomatic CM?
- What is the life-long risk of bile duct malignancy in CM patients?

The diagnosis and treatment of CM has been rather controversial, probably due to the very low incidence and the large variability in clinical and anatomical presentation. CM can be diagnosed pre- or postnatally. The optimal mode of diagnostic imaging and time of surgical resection remain unclear. Preliminary data from a recent Dutch survey highlight that magnetic resonance cholangiopancreatography (MRCP) was routinely used in 71% and the more invasive ERCP in 29% of departments [65].

The introduction of minimally invasive surgery has certainly changed the approach to CM. Liem *et al.* confirmed the potential for excellent results in 400 Vietnamese children using a laparoscopic approach [66]. In a meta-analysis on 679 patients, Narayanan *et al.* reported no differences in the rates of bile leak, cholangitis, operative time, hospital stay and reoperation after laparoscopic hepaticoduodenostomy *vs.*

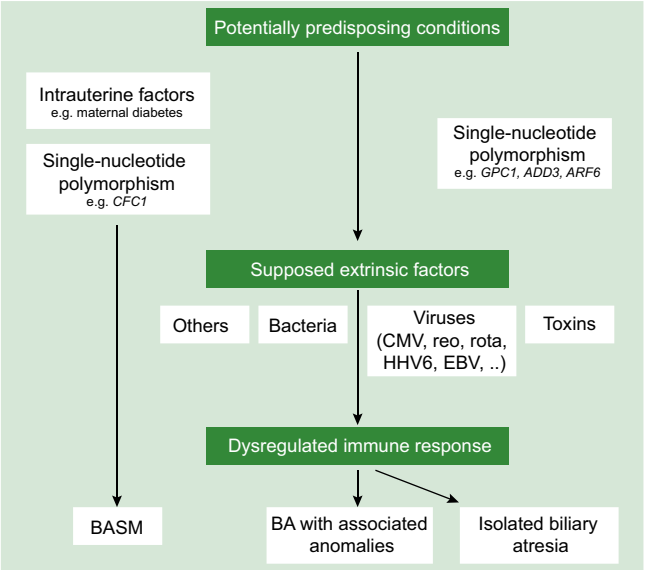

Fig. 1. The two favored hypotheses for biliary atresia share the assumption of genetic predisposition. In patients with BASM, a single nucleotide polymorphism in *CFC1* has been described [42]. In patients with isolated BA, single nucleotide polymorphisms in other genes have been implied, such as *GPC1*, *ADD3* and *ARF6* [43–46]. In patients with a so called acquired or isolated form of BA, the two-hit theory holds that extrinsic factors (e.g., an infectious agent or biliary toxin) might induce a dysregulated immune response, which may develop into a potentially self-perpetuating autoimmune process [38].

Review

Key point

Novel genetic causes of childhood cholestatic liver diseases are increasingly being identified, which also has helped to better understand the normal physiology.

traditional hepaticojejunostomy [67]. However, the incidence of reflux/gastritis was much higher after hepaticoduodenostomy. At this moment there is a virtual absence of defined registries and of impartial assessment of the available diagnostic and therapeutic approaches to CM. Similarly, there is an absence of prospectively collected data on both the natural history of CM and its post-surgical course, which limits our ability to predict the long-term prognosis and cancer risk in individual patients.

Top priorities to improve diagnosis and treatment of choledochal malformation

- Development of multicenter/multicountry patient registries, such as "bard-online", to allow (sub)classification of CM and assessment of treatment results and long-term course of disease. Over time, the data can provide greater insights into variation in disease presentation and clinical course.
- Defining the optimal surgical procedure, short- and long-term outcome, morbidities, optimal prevention and treatment of cholelithiasis, and the life-long risk of bile duct malignancy.

Alagille syndrome: diagnosis and treatment

Key questions/controversies

- Are there effective medical treatments for intractable pruritus?
- What is the spectrum of non-hepatic morbidities in affected patients?
- Which patients benefit from liver transplantation and what is the outcome?

Alagille syndrome (ALGS) is an autosomal dominant multisystem condition [68,69] that is caused by mutations in *JAG1* or *NOTCH2* in the Notch signaling pathway. These mutations cause defective bile duct morphogenesis and angiogenesis, and abnormalities in skeletal, ocular, cardiovascular and kidney development [70].

ALGS is characterised by bile duct paucity and at least 3 out of 5 clinical features: cholestasis, cardiac defects, skeletal abnormalities, ocular abnormalities and characteristic facies [68]. The majority of patients with cholestasis have growth failure with fat malabsorption, metabolic bone disease, pruritus and hypercholesterolemia with xanthomas [71]. Management is based on intensive nutrition, fat soluble vitamin supplementation, choleretic agents and/or bile resins to reduce cholesterol. Management of pruritus is troublesome and may involve the addition of rifampicin or naltrexone. When medical treatment fails external partial biliary diversion may be required. Current clinical trials are investigating whether LUM001, which inhibits the apical sodium dependent bile acid transporter (ASBT) and prevents the reabsorption of bile acids in the terminal ileum, may improve quality of life, liver function, and reduce itching (Clinicaltrial.gov identifiers: NCT02047318, NCT01903460, NCT02057692, NCT02160782) [72,73]. Cirrhosis and portal hypertension are rare early in childhood and 50% of children regain normal liver function without significant cholestasis by adolescence. However, only approximately 50% of ALGS patients presenting with neonatal cholestasis survive into adult life with their native liver. Management should include monitoring for the development of abdominal and intracranial vascular anomalies and for hepatocellular carcinoma, and multidisciplinary care of potential cardiac and renal failure [74–76].

Indications for liver transplantation include liver failure and complications of portal hypertension, intractable pruritus or deforming xanthomata, repeated bone fractures due to intractable metabolic bone disease, growth impairment and poor quality of life. The assessment is complex because of multisystem involvement, particularly cardiac or renal disease, and the need to exclude vascular anomalies. One and five-year graft and patient survival are lower in ALGS than in BA with death <30 days after transplant higher in ALGS due to graft failure, neurological, and cardiac complications [77]. Ocular disorders may include optic atrophy due to intracranial hypertension, retinal demyelination and chorioretinal atrophy [78]. Renal involvement occurs in 40% of *JAG1* positive individuals. Renal dysplasia and renal tubular acidosis are common and renal insufficiency may require renal transplantation [70].

Top priorities to improve diagnosis and treatment of Alagille Syndrome

- Clinical practice studies and patient registries to define the long-term natural history of disease (both hepatic and extra-hepatic manifestations) and the effects of interventions. Long-term monitoring of sequelae of ALGS is warranted (renal disease, cardiovascular disease, and in case of cirrhosis, development of hepatocellular carcinoma) using case-control analyses from adolescent and adult ALGS patients enrolled into registries. This will require the involvement of hepatologists treating adult patients.
- Trials targeting either the pathogenesis of end-stage liver disease or of pruritus (including antifibrotic drugs and inhibitors of ASBT) are indicated. Given the

low incidence of ALGS, collaboration across many centers will be mandatory in order to conduct properly powered clinical trials.
- Develop a better understanding of the mechanisms by which partial biliary diversion may be beneficial, and prediction of which patients can benefit from these surgical interventions.

Progressive familial intrahepatic cholestasis

Key questions

- What are the genetic and molecular underpinnings of progressive familial intrahepatic cholestasis (PFIC) or PFIC-like diseases?
- What is the mechanism underlying the success of partial biliary diversion strategies and do they prevent the development of end-stage liver disease and hepatocellular carcinoma?
- Do patients respond to strategies to medically reduce the bile acid pool size through inhibition of ASBT?
- What are the mechanisms of increased risk of hepatocellular carcinoma in PFIC type 2?

PFIC encompasses a group of autosomal recessive disorders of bile formation. Their pathogenesis can be divided into two groups based on the high or low level of serum gamma glutamyltransferase (GGT) [79–81]. In cholestasis, serum GGT is low when bile acids are not secreted into the bile and high when bile acids are secreted into the bile, but either concomitantly biliary phospholipid secretion is absent or bile outflow is obstructed. Low-GGT PFIC is associated with bile acid synthesis defects, and with mutations in *ATP8B1* (PFIC type 1), *ABCB11* (PFIC type 2), or *TJP2* (TJP2 deficiency) [82–85]. The mutant TJP2 protein is associated with defective cellular localization and disruption of tight-junction structure. Up to 40% of phenotypic low-GGT PFIC cases do not have mutations in these genes. High-GGT PFIC is associated with several diseases, among which are mutations in *ABCB4* (PFIC type 3) [86].

Low-GGT PFIC is relentlessly progressive if not treated, although more rapid in PFIC type 2 compared to PFIC type 1. The current first-line therapy is partial external bile diversion (PEBD) in patients with severe pruritus [87,88]. This therapy appears to work by creating a relatively hydrophilic bile acid composition, thus improving bile formation [89]. Patients with no canalicular expression of functional bile salt export pump (BSEP) (i.e., severe *ABCB11* mutations) and patients who already have cirrhosis prior to PEBD can be expected to fail PEBD [90].

PEBD was first reported to be effective treatment for pruritus in PFIC in 1988 [87,88] and has gained wide acceptance as the first-line therapy for low-GGT PFIC [87,90–93] as well as in some cases of severe ALGS [90,94]. It is effective in relieving pruritus in both conditions and improves growth, at the "cost" of an external stoma. Two variant approaches for biliary diversion recently have been reported, namely laparoscopic PEBD [95] and open button cholecystostomy [96]. Ileal exclusion has been used to treat pruritus in PFIC patients with some success [88,97,98], although in general it is considered less effective than PEBD. There has been a recent interest in internal surgical bile diversion from gallbladder to colon [99,100], but the safety and efficacy of the procedure are as yet unproven.

We feel that surgical approaches are still needed to interrupt the enterohepatic circulation with the goal to improve pruritus and growth, but there is no clinical trial demonstrating superiority of one surgical approach over the others. This notwithstanding, we have the general impression that ileal exclusion may not be as effective as PEBD. A common approach has been to perform PEBD, and the possible conversion to ileal bypass later in life, based on outcome and patient/family preference. If a biliary diversion approach fails, or if complications arise, (e.g., development of hepatocellular carcinoma in PFIC type 2 patients [101,102]), liver transplantation is indicated.

Non–surgical opportunities may be on the horizon to replace surgery as treatment for these diseases. For patients with specific mutations in *ABCB11* (primarily miss-sense mutations), the basic transporter defect may be (partially) overcome by chaperones/small molecule strategies (e.g., 4-phenybutyrate and glycerol phenylbutyrate) that promote protein folding and enhance the functional expression of transport proteins in the liver [103]. Inhibition of intestinal bile acid absorption is being investigated as an alternative approach to treat these diseases. Miethke *et al.* recently inhibited ileal bile acid re-uptake using the competitive ASBT inhibitor SC-435 in *Abcb4*$^{-/-}$ mice, a model of PFIC type 3 [73]. SC-435 treatment dramatically reduced plasma total bilirubin and alanine transaminase (ALT) levels and improved liver histology and inflammatory gene expression compared to controls, suggesting that ASBT may be a promising pharmacological target for "toxic" bile-induced cholangiopathies such as PFIC3. Counterintuitively, ASBT inhibition may also prove to be valuable for PFIC-1 and -2 patients, despite insufficient bile acid secretion across the canalicular membrane. PFIC-2 patients with at least some functional canalicular BSEP expression can be responsive to PEBD [90], and could therefore also be responsive by pharmacological interruption of the enterohepatic circulation. Clinical trials of ASBT inhibitors for treatment of PFIC (and ALGS)

Key point

The improving prognosis of patients with these diseases has enabled their growing up to adulthood, which underlines the increasing need for close cooperation between pediatric and adult-oriented professionals.

are underway (clinicaltrials.gov identifier: NCT02057718, NCT02160782, NCT02047318).

Top priorities to improve diagnosis and treatment of PFIC

A number of genes (*ATP8B1*, *ABCB11*, *ABCB4*, and *TJP2*) have been causally linked to the etiology of PFIC. The screening for mutations in these genes in the clinic should be incorporated into diagnostic algorithms for children with chronic cholestasis. Top priorities for the field moving forward are:

- Establishing the relationships between PFIC genotype and therapeutic response (i.e., responsiveness to PEBD) is needed to better allow prognostication and personalise specific treatments in individual patients.
- Determining whether newer surgical procedures to divert bile directly into the colon are effective and safe, and could replace external diversion strategies.
- Identifying the mechanism underlying the increased risk of hepatocellular carcinoma in patients with PFIC-2. It has recently been shown that the genomic modifications in hepatocellular carcinoma of PFIC-2 patients can be distinguished from those arising in other cholestatic liver diseases [104], what could provide a target for elucidation of the mechanism.
- Assessing the therapeutic value of new pharmacologic agents to interrupt the enterohepatic circulation of bile acids or improve intracellular trafficking of mutant protein.
- Continue to identify new genetic causes of PFIC in patients negative for current genotypes.

Table 2. Top priorities in research and clinical management of biliary atresia and cholestatic childhood disease.

Fundamental research
Identification and functional characterization of genetic variants relevant for the pathogenesis of syndromic forms of BA
Systematic biological approach to identify pathogenic factors in BA and in BA-related liver fibrosis
Assessment of the role of specific toxins (e.g., biliatresone) in the pathogenesis of BA in humans
To identify the molecular mechanism underlying the increased risk of hepatocellular carcinoma in patients with PFIC-2
Clinical research and management
Broad implementation of screening strategies, in particular the stool color card
Evaluation of patients with neonatal cholestasis in an experienced center for rapid evaluation and, if indicated, Kasai hepatoportoenterostomy
To expand the databases of BA and other cholestatic childhood diseases to determine relationships between clinical and therapeutic interventions and outcomes
To analyse the variance in outcomes between different centers and countries
To assess the potential value of novel medical strategies (e.g., anti-fibrotic drugs, farnesoid X-receptor agonists, ASBT inhibition, chaperones) for extending the native liver survival and/or decreasing pruritus
To establish patient registries for Alagille syndrome patients with monitoring of sequelae into adulthood
Ability to prognosticate and personalise specific treatments in individual PFIC patients
To determine the safety and value of new surgical procedures based on internal biliary diversion

Transition from paediatric to adult care for BA and other cholestatic childhood disease patients

Key questions

- What are the most important medical risks for BA and BA-related diseases patients reaching adulthood with their native livers and what is the optimal timing of listing for liver transplantation?
- How can the transition from paediatric to adult care be facilitated through the acquisition of self-responsibility and self-management?

There is an increasing proportion of patients with BA or another cholestatic childhood disease surviving into adulthood, requiring expertise in these disorders by adult-orientated physicians and hepatologists. Up to 61% of BA patients with their native liver who reach adulthood develop severe hepatic complications, such as cholangitis, portal hypertension with variceal bleeding and, although infrequently, hepatocellular carcinoma [105]. Lind *et al.* reported that adult BA patients with native livers had a lower perceived general health and a higher score on the social domain section of the Health Status and Quality of Life questionnaire compared to the general population [106]. This is comparable to the ChiLDReN registry experience reporting that Health Related Quality of Life in BA children with native livers was poorer than in healthy children (although similar to BA patients who underwent liver transplantation), with poorer social functioning in the younger children [107].

The indication and timing of liver transplantation in young adults with BA or another cholestatic childhood disease is challenging both from a medical and psychosocial point of view. Adult listing criteria might not be relevant to childhood liver diseases. Data on course of life in young adults transplanted in childhood show delay in reaching developmental milestones, but less risky behavior with regards to substance abuse and gambling compared to the general population [108]. Non-adherence to treatment is a major concern in the post-transplant setting and the long-term graft and patient survival in patients transplanted between the ages of

12–17 years is poorer compared to the younger population.

The present key issues of transition of care are partially related to cultural differences between paediatric and adult-orientated health care, as well as to unfamiliarity of the adult care-givers with the underlying diseases. Mutual awareness of patient specific health risks, typically involving disease-related psychosocial development, is expected to result in patient-centered transition programs. Specific complication screening programs need to be developed since the diseases involved are usually accompanied by life-long increased risks. Currently unmet needs include proven strategies for adequately achieving self-responsibility and self-management by individuals who have had a severe medical condition that begins in the paediatric age [109]. King's College Hospital (London, UK) is presently expanding the adult hepatology training program with a training course aimed at the care of young adult patients with a liver disease that originated in childhood.

Top priorities to improve transition from paediatric to adult care for BA or other cholestatic childhood disease patients

The survival of BA or other cholestatic childhood disease patients into adulthood has created a rather novel patient group for adult-orientated health care professionals. Specific co-morbidities (e.g., cholangitis, portal hypertension, and short stature) and indications and timing of listing for transplantation in BA or another cholestatic childhood disease require close collaboration with paediatric hepatologists for development of clinical protocols for adult patients with BA or another cholestatic childhood disease liver diseases in order to improve the outcome of these patients.

Conclusions and perspectives

The prevalence of BA and other cholestatic childhood diseases is rare, which requires collaborative efforts to address the top priorities formulated for these disorders (summarised in Table 2). Dissemination of current research advances in the context of BA has led to a unifying hypothesis (Fig. 1). Isolation of a novel compound, biliatresone, causing natural BA in sheep (and in a zebrafish model) will open up a new research avenue. Areas of controversies that require new data include whether the centralization of BA surgery and care improves outcome. Determining the optimal screening strategies for BA and neonatal cholestasis is essential in order to ensure earlier diagnosis and better outcomes. The identification of new genetic causes of cholestatic childhood diseases continues, underlining the progress of insight as well as the value of detailed phenotypic and genetic analysis of the still unresolved causes of childhood cholestasis syndromes [110]. In related disorders, new therapies are emerging that are engineered to target the molecular cause or pathophysiology of certain types of PFIC and ALGS. These therapeutic advances will be a welcome addition to the rather limited therapeutic portfolio of treating the secondary metabolic and nutritional consequences and performing partial biliary diversion or liver transplantation. Until new therapies are shown to be effective and safe, ongoing multi-centered and multinational collaboration to study these rare diseases is critical to continue the development and testing of novel therapies. With the current success of achieving adulthood in many patients, we are challenged to define the best strategies to transfer the care of these "grown children" from paediatric to adult-orientated liver clinics.

Key point

The prevalence of many cholestatic childhood diseases is rare, but they are responsible for the majority of indications for liver transplantation at pediatric age.

Key point

The low prevalence of the diseases requires multi-center and multinational collaborations to address remaining clinical and therapeutic key questions.

Conflict of interest

The authors who have taken part in this study declared that they do not have anything to disclose regarding funding or conflict of interest with respect to this manuscript.

Authors' contributions

Study concept and design: HJV, JAB, MD, RJS, CP; Drafting of the manuscript: HJV, JAB, MD, RAS, GM-V, JBH, RJS, DAK, BU, PFW, MS, CP; Critical revision of the manuscript for important intellectual content: all authors; editing of final draft: HJV, JAB, MD, RJS, CP.

Review

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