



Published in final edited form as:

*Nat Rev Dis Primers*. ; 10(1): 47. doi:10.1038/s41572-024-00533-x.

## Biliary atresia

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## Abstract

Biliary atresia (BA) is a progressive inflammatory fibrosclerosing disease of the biliary system and a major cause of neonatal cholestasis. It affects 1:5,000–20,000 live births, with the highest incidence in Asia. The pathogenesis is still unknown, but emerging research suggests a role for ciliary dysfunction, redox stress and hypoxia. The study of the underlying mechanisms can be conceptualized along the likely prenatal timing of an initial insult and the distinction between the injury and prenatal and postnatal responses to injury. Although still speculative, these emerging concepts, new diagnostic tools and early diagnosis might enable neoadjuvant therapy (possibly aimed at oxidative stress) before a Kasai portoenterostomy (KPE). This is particularly important,

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Author contributions

Introduction (P.K.H.T.); Epidemiology (P.K.H.T. and M.H.); Mechanisms/pathophysiology (P.K.H.T., C.S.M.T., V.C.H.L., C.L.M. and R.G.W.); Diagnosis/screening/prevention (P.K.H.T., M.D., C.D.L. and M.P.); Management (P.K.H.T., M.D., P.D.C. and M.P.); Quality of life (P.K.H.T. and M.P.); Outlook (P.K.H.T. and P.D.C.); Overview of the Primer (P.K.H.T.).

Competing interests

The authors declare no competing interests.

as timely KPE restores bile flow in only 50–75% of patients of whom many subsequently develop cholangitis, portal hypertension and progressive fibrosis; 60–75% of patients require liver transplantation by the age of 18 years. Early diagnosis, multidisciplinary management, centralization of surgery and optimized interventions for complications after KPE lead to better survival. Postoperative corticosteroid use has shown benefits, whereas the role of other adjuvant therapies remains to be evaluated. Continued research to better understand disease mechanisms is necessary to develop innovative treatments, including adjuvant therapies targeting the immune response, regenerative medicine approaches, and new clinical tests to improve patient outcomes.

## Introduction

Biliary atresia (BA) is a devastating inflammatory obliterative disease of the bile ducts. BA affects 1 in 5,000–20,000 newborns and has its highest incidence in Asia<sup>1,2</sup>. It is a heterogeneous, complex, multifactorial disorder with unknown pathogenesis<sup>3</sup>. Genetic and/or developmental susceptibility, injury by an environmental toxin or virus infection and immune–inflammatory dysregulation have been implicated. Irrespective of the initiating events, and often despite treatment, liver fibrosis and ultimately cirrhosis become the dominant pathologies<sup>4–6</sup>.

BA diagnosis is challenging, sometimes missed or made late, and can only be established by intraoperative cholangiography. Timely surgery by Kasai portoenterostomy (KPE), in which the obliterated extrahepatic bile duct (EHBD) is replaced with an intestinal conduit, restores bile flow in some patients. Failure to restore bile drainage is typically managed by liver transplantation, often within the first 2 years of life. However, even patients who have achieved clearance of jaundice (CoJ) frequently develop complications, including portal hypertension, cholangitis, hepatopulmonary syndrome and even malignancy due to progression of liver injury<sup>7–10</sup>.

Early diagnosis, centralization of resources and multidisciplinary management have led to improved rates of survival with the native liver<sup>11</sup>. The value of current adjuvant therapies remains debatable, highlighting the need to improve understanding of disease pathogenesis and to develop new treatment strategies<sup>12</sup>.

This Primer provides a comprehensive summary of up-to-date research on BA, covering its epidemiology, mechanisms and pathophysiology, diagnosis, management, quality of life, progression of liver injury and future directions for research.

## Epidemiology

### Incidence

BA is a rare disease that occurs in most, if not all, ethnic groups, but incidence rates differ considerably (Fig. 1). Incidence differences between ethnic groups within the same country or region suggest a genetic predisposition<sup>2,13</sup>. Studies in Western countries including Netherlands<sup>14</sup>, France<sup>15</sup>, Croatia<sup>16</sup>, Canada<sup>17</sup>, USA<sup>18</sup>, England and Wales<sup>19</sup>, Switzerland<sup>20</sup>, Finland<sup>21</sup> and Sweden<sup>22</sup> have found incidences between 1:14,000 and 1:22,000 per live births. BA is more common in East Asia, with reported incidences between 1:5000 and

1:9000 in Taiwan<sup>23</sup>, Japan<sup>24</sup>, Korea<sup>25</sup>, and Shanghai and Hong Kong, China<sup>2</sup>. In New Zealand, a striking difference in BA incidence was observed between the Māori population (1:5,000) and children of European origin (1:16,000)<sup>26</sup>. No published national data exist from Africa, South America, India or Australia.

## Demographic associations

**Environmental factors.**—Environmental factors are believed to be important in BA pathogenesis. However, results of studies analysing the effect of environmental exposures on BA incidence are inconclusive. The incidence of BA has been variably reported to be unchanged<sup>27</sup> or higher in urban<sup>28</sup> and rural settings<sup>14</sup>. Some studies report seasonal variations in the incidence of BA, with different seasonal peaks<sup>25,28,29</sup>, but most larger studies do not support seasonality<sup>18,19,30,31</sup>. Epidemiological data supporting a viral aetiology are also scarce. In Taiwan, decreasing BA incidence over time showed a statistically insignificant correlation with rotavirus vaccine coverage rates<sup>32</sup>. A European study showed that stricter COVID lockdowns were associated with larger decreases in BA incidence<sup>33</sup>.

**Sex distribution.**—The incidence of BA overall is higher in females, with a risk ratio of approximately 1.4 in most Western<sup>14,17–19,21</sup> and Asian countries<sup>25</sup>, rising to 1.8 in Japan<sup>23,24</sup>. Inexplicably, female to male ratios of 1.1:1 and 0.7:1 have been reported in China<sup>34</sup> and Sweden<sup>22</sup>, respectively. The sex distribution may also vary between different BA phenotypes, but evidence is scarce. An increased (2:1) female-to-male ratio was observed in patients with biliary atresia splenic malformation (BASM) compared with patients without BASM in a study in England and Wales<sup>35</sup>, whereas the ratios were similar in a study in Canada<sup>36</sup>.

**Maternal factors.**—Maternal diabetes mellitus during pregnancy was found to be ten times more likely to be associated with an infant with BASM than with an infant with BA without splenic malformation (isolated BA)<sup>35</sup>. Pregestational diabetes was associated with an increase in BA risk of more than twofold in two population-based studies<sup>37,38</sup>. In Sweden, no association with diabetes was seen, but maternal age >35 years and parity of four or more were associated with increases in BA risk of twofold to threefold<sup>22</sup>. Prenatal (third trimester) exposures to maternal intestinal and genitourinary infections were associated with increases in BA risk of sixfold and 1.6-fold, respectively<sup>39</sup>. First trimester anti-inflammatory asthma medication<sup>40</sup> and maternal drug abuse<sup>37</sup> were associated with increases in BA risk of more than threefold. Although not confirmed as a risk factor in population-based studies, in one cohort, 26% of 39 children with BA had been conceived through in vitro fertilization<sup>41</sup>.

**Ethnicity.**—The risk of BA is twofold to threefold higher in Asian countries and in children of Asian descent than in Caucasian populations<sup>18,26</sup> (Fig. 1). Higher incidence rates in non-white than in white children have also been reported in England and Wales<sup>3</sup>, Atlanta and New York<sup>28,29</sup>. In addition, the relative proportions of different BA phenotypes differ between ethnicities, with BASM being much more common in Western

countries<sup>3,14,15,24,25,34,36</sup>, whereas cytomegalovirus (CMV)-associated BA seems to be more frequent in Asia<sup>42–45</sup>.

**Prematurity.**—Twofold and fivefold higher BA incidence rates have been reported in children born prematurely at <37 weeks and at <32 weeks of gestation, respectively<sup>22,38,46,47</sup>. Premature children with BA present more often with associated malformations than children with BA born at term (19%<sup>47</sup>, 30%<sup>27</sup> and 40%<sup>46</sup>). In a UK study among preterm babies with BA, a higher than expected proportion of twins was observed (48%)<sup>27</sup>. Preterm children with BA tend to be older at time of KPE<sup>38,47</sup>; however, they may reach similar CoJ and native liver survival rates to those in children with BA born at term<sup>27</sup>. Small for gestational age was found to be associated with an increase in BA risk of almost fivefold in a Swedish national study<sup>22</sup>.

**CMV infection.**—Human data implicate a relationship between CMV infection and BA<sup>42–45</sup>. Patients with BA and CMV infection are considered a special subgroup owing to their clinically distinguishable characteristics, including older age at time of KPE, lower CoJ rates, shorter native liver survival, and higher pretransplant mortality<sup>42–44,48–50</sup>. Infants with BA and higher anti-CMV IgM antibody levels have a worse prognosis following KPE<sup>44,51,52</sup>.

**Syndromic biliary atresia and associated anomalies.**—Syndromic BA usually refers to the BASM syndrome. In addition to asplenia, polysplenia or double spleen, patients with BASM may also present with other anomalies, including situs inversus (50% of patients), malrotation, a preduodenal portal vein or an absent intrahepatic vena cava (Fig. 2a,b). Some studies included patients with these laterality defects but without splenic malformations<sup>36</sup>, in contrast to the original definition of BASM<sup>35</sup>. BASM accounts for 12–16% of BA cases in Western countries<sup>3,7,14,15,36</sup>, but only 0–3% in Asia<sup>24,25,34,47</sup>. However, as the incidence of isolated BA is about threefold higher in Asia, the occurrence of BASM may be constant across continents. Patients with BASM typically have EHBDs that are more atrophic, a higher risk of hepatopulmonary syndrome<sup>31,53</sup>, and inferior native liver survival rates than those with isolated BA<sup>31,35</sup>. BA has also been reported in patients with other defined syndromes, such as cat-eye syndrome, Kabuki syndrome and Kartagener syndrome<sup>3,54,55</sup>.

The incidence of congenital heart disease in BA varies between cohorts without consistent geographical differences. Cardiac malformations are observed in 6–8% of patients with BA in the UK, Nordic countries, Korea and Canada<sup>7,25,36,56</sup>, in 3% in China and India<sup>34,57</sup>, and in 15–16% in Sweden and North America<sup>22,58</sup>. BASM has been reported in 50% and cat-eye syndrome in 11% of patients with cardiac malformations, and the co-existence of other congenital anomalies is also more common than in patients with isolated BA<sup>56</sup>. Patients requiring correction of heart defects should probably undergo cardiac surgery before KPE as this approach has been related to improved CoJ rates<sup>56</sup>.

Other congenital malformations observed in patients with BA include intestinal atresia (0.5–5%)<sup>3,57</sup>, anorectal malformations, Hirschsprung disease, abdominal wall defects, hypospadias (<1%)<sup>3,34</sup>, oesophageal atresia (1–3%)<sup>3,25,57</sup> and duodenal atresia (1.5% in

a UK series, with a higher incidence in patients with BASM than in those with isolated BA<sup>3</sup>, but none in cohorts from Korea, India and China, where BASM is uncommon<sup>25,34,57</sup>).

## Mechanisms/pathophysiology

### Injury and the response to injury in BA

We suggest that BA should be viewed as the combination of an injury and a response to injury, and we consider the pathophysiology of BA from this perspective, while noting that key mechanisms may contribute to both phases (Fig. 3).

Understanding the nature of the initial insult in BA requires understanding *when* it occurs. Although BA is diagnosed exclusively in neonates, most of whom are asymptomatic at birth, two studies in the USA including a combined 400,000 newborns demonstrated that elevated levels of conjugated bilirubin within the first few days of life were 100% sensitive for later development of BA<sup>59–61</sup>. This led to a paradigm shift in the concept of BA pathophysiology, suggesting that the injury leading to BA occurs in utero or (less likely) around birth, and that the postnatal period is a time of *response* to injury marked by rapid injury progression and maladaptive healing. Recovery of injured bile ducts in the neonatal period has never been observed, although animal studies as well as a small percentage of babies with elevated conjugated bilirubin levels who never develop evidence of any kind of liver disease raise this speculative possibility<sup>60–63</sup>.

In this section, we first outline the development and anatomy of the biliary tree, with a particular reference to differences between humans and rodents; then, because experimental models (particularly rodents) are critical to research into BA pathophysiology, we describe the major current models. We then discuss key aspects of the injury phase of BA, followed by a discussion of the response to injury (Fig. 3). Of note, many aspects (for example, genetic factors and immune dysregulation) undoubtedly contribute to both the initial injury and the progressive response to that injury; they are discussed according to their likely primary role.

### Hepatobiliary development and anatomy

The biliary tree is a continuous and arborizing network consisting of two highly heterogeneous regions with different developmental origins: hepatocytes and intrahepatic bile ducts (IHBDs) are derived from the cranial liver bud and EHBDs and the gallbladder are derived from the caudal liver bud (pars cystica)<sup>64,65</sup>. Early BA primarily affects the extrahepatic and large intrahepatic/hilar bile ducts, although progressive disease affects the entire biliary tree and the effects on intrahepatic disease of the primary injury and the post-obstructive complications are not well understood. The EHBDs and large (distal) IHBDs are exposed to bile that may have been modified during passage through the intrahepatic ductal system, and have anatomical and biochemical features that may make them particularly susceptible to injury. Additionally, repair mechanisms in these structures are different: proximal IHBDs are surrounded by hepatocytes that function as a source of bipotential (hepatocyte and cholangiocyte) cells, whereas EHBDs (and distal IHBDs<sup>66</sup>) are surrounded

by peribiliary glands that contribute to duct homeostasis and are mobilized to repair injured cholangiocyte monolayers<sup>67,68</sup>.

Human and rodent hepatobiliary trees develop with different relative time courses. Examples include the emergence of the ductal plate at weeks 7–8 of gestation in humans but after the midpoint of gestation in mice, and the apparent equivalence of the EHBD matrix of fetal humans and postnatal rodents<sup>64,69</sup>. Although the overall differences are poorly understood, they are essential to consider in using animal models.

## Disease models

Various technology platforms for disease modelling have advanced our understanding of BA, with several new or improved in vivo and in vitro disease models introduced in recent years.

**Rodent models.**—The rhesus rotavirus (RRV) mouse model of BA, in which BALB/c mice are injected intraperitoneally with RRV in the first 24 h of life, mimics key features of human BA (including hyperbilirubinaemia and EHBD obstruction)<sup>70</sup> and is the most commonly used animal model. It has yielded important insights into potential mechanisms of BA but is limited by a fatality rate >90% before the development of advanced fibrosis and other pathologies. Use of a rotavirus reassortant strain (RRV-TUCH) improves early survival to 63%<sup>71</sup>. Other models include common bile duct ligation in neonatal rats<sup>72</sup>, and BA-like disease in neonatal mice induced by the biliary toxin bilitresone<sup>63</sup>. No experimental animal models have yet generated BA in neonates after treatment of pregnant mothers, although a recent study showed abnormal bile acid synthesis in the pups of bilitresone-treated pregnant mice<sup>73</sup>.

**Zebrafish.**—Zebrafish (*Danio rerio*) and humans share substantial similarities in the development, anatomy and function of the biliary tree. The use of zebrafish in cholestasis research — in particular employing fluorescent dyes to identify biliary excretion in transparent larvae — has contributed to large-scale screening and characterization of potential biliary toxins<sup>74,75</sup>, validation of candidate genes from genetic studies<sup>76–79</sup>, and identification of the role of DNA hypomethylation in BA<sup>80,81</sup>.

**In vitro models.**—Liver derived organoids, which consist of cholangiocytes or hepatocytes in a spherical monolayer epithelium, are increasingly used in liver research<sup>82,83</sup>. Pluripotent stem cells, such as embryonic stem cells and induced pluripotent stem cells, are capable of generating hepatic progenitor cells and cholangiocyte organoids<sup>84–86</sup>, which have been used extensively in BA research to study mechanisms of cholangiocyte damage in BA. For example, breakdown in apical–basal polarity with perturbed cholangiocyte development occurred in organoids generated from the epithelial cell adhesion marker-expressing cells of patients with BA but not in organoids from cells of patients without BA<sup>87,88</sup>. In another study, CRISPR–Cas9-mediated knockout of the BA susceptibility genes *ADD3* and *GPC1* led to reductions in ductal structure formation in organoids<sup>89</sup>. Neonatal mouse EHBD explants in culture are also extensively used in BA research; following injury, these explants demonstrate BA-like cholangiocyte monolayer injury and collagen deposition<sup>90</sup>. Organs-on-



a-chip have similarly been used, sometimes with human organoid-derived cells, as in vitro models of the neonatal EHBD<sup>91,92</sup>.

Critical to BA research in the future will be improved animal models (including large-animal and maternal-exposure models) as well as multicellular, human cell-based organoids and on-chip devices paired with specialized (and potentially patient-derived) extracellular matrix (ECM) scaffolds to study cell–cell and cell–ECM interactions.

## Injury

**Genetic susceptibility.**—Despite low heritability of BA, recent large-scale genetic studies have unequivocally demonstrated that BA is a complex disorder with considerable contributions from common and rare genetic variants (Table 1). Genome-wide association studies (GWAS) identified a group of genes involved in hepatobiliary development and structure, including *ADD3*, *EFEMP1*, *ARF6*, *GPC1*, *MAN1A2*, *AFAP1* and *TUSC3* (refs. 13,77,93–97). Dysregulation of these genes may perturb embryogenesis of the biliary tree or disrupt ECM organization and integrity, thereby increasing susceptibility to extrahepatic biliary injury.

Genetic data also suggest that polygenic predisposition to ciliary dysfunction underlies BA irrespective of the clinical subtype. Cholangiocyte cilia abnormalities, including misorientation and reduction in length and quantity, have been described in both syndromic and non-syndromic forms of BA<sup>98–100</sup>. Rare biallelic variants in a ciliary gene, *PKDIL1*, were first identified in five of 67 Caucasian patients with BASM<sup>101</sup> and bile duct ligation in liver-specific *Pkd111*-deficient mice recapitulated features seen in patients, including reduced primary cilia, progressive cholangiocyte proliferation and peribiliary fibroinflammation<sup>102,103</sup>. A whole-exome sequencing study on 89 patients with non-syndromic BA and their parents showed a 2.6-fold increase in mutational burden of rare (de novo or biallelic) damaging mutations in ciliary genes in 31% of the patients<sup>78</sup>. Functional analyses showed the absence of cholangiocyte cilia in the patients with deleterious ciliary mutations. Non-motile primary cilia are at the hub of many signalling pathways, including Hedgehog, NOTCH, WNT and transforming growth factor- $\beta$  (TGF $\beta$ )–bone morphogenetic protein pathways, which are essential for bile duct development and for detecting the flow, composition, osmolality and pH of the bile<sup>104–106</sup>. Defects in these signalling pathways could cause abnormal biliary development and contribute to the pathogenesis of BA<sup>4,64</sup>. Intriguingly, abnormally low nasal nitric oxide levels, a clinical screening test for primary ciliary dyskinesia, were detected in eight of ten patients with BA and ciliary mutations<sup>78</sup>. This phenotypic overlap supports an intrinsic ciliary defect in BA and is also consistent with the pleiotropic disease manifestations affecting multiple systems in some patients with non-syndromic BA.

A GWAS in 811 patients with BA requiring liver transplantation further showed that common variants associated with the ciliogenesis and planar polarity effector (CPLANE) complex may predispose to an increased risk of BA via ciliary dysgenesis<sup>97</sup>. The CPLANE regulatory network comprises a broad spectrum of proteins (~2,000 genes) essential for ciliogenesis and cilia-mediated patterning<sup>107</sup>. A polygenic risk score aggregating the effects of ~6,000 common variants in 102 CPLANE genes showed a strong correlation with BA

( $P = 5.5 \times 10^{-15}$ ). Knockdown of the CPLANE genes *AFAP1* and *TUSC3* impeded the formation of motile cilia in mouse tracheal cells. Additionally, whole-genome sequencing of 100 patients with BA identified enrichment of rare variants of CPLANE genes<sup>97</sup>.

In line with the variable incidence of BA worldwide, locus heterogeneity is notable across ethnically diverse populations. Except for *ADD3*, BA candidate loci have been reported in either Caucasian patients or Asian patients but not both<sup>13,77,93–97,108</sup>. This may result from differences in underlying genetic architecture or reflect different levels of exposure (and, therefore, different susceptibilities) to injurious environmental agents. Nevertheless, the convergence on a limited number of functional pathways, particularly those related to ciliogenesis, supports a shared genetic predisposition across populations. Although BA-associated rare variants have reduced penetrance and common variants have modest effects individually (odds ratio (OR) 1.4–2.7; Table 1), they collectively contribute considerably to the overall risk of abnormal biliary development in BA.

Interestingly, animal models of BA demonstrate acquired ciliary defects — cholangiocyte cilia are abnormal in the RRV mouse model as well as in primary cholangiocytes treated with biliatresone<sup>74,99</sup>. Human organoids treated with biliatresone also demonstrate abnormal cilia, akin to those seen in organoids derived from patients<sup>109</sup>. Ciliary abnormalities may represent a common mechanism of disease between genetic and environmental injuries or, alternatively, defects in ciliary genes may predispose to cholangiocyte injury.

**Environmental injury.**—The injury agent in BA is not known. Viruses have long been suggested as possible aetiological factors for BA. Virus infection could cause defective bile duct development, direct injury to the bile duct epithelium, or a secondary autoimmune reaction<sup>1,110,111</sup>. CMV, Epstein–Barr virus, rotavirus and human papillomavirus have all been implicated<sup>112,113</sup> but data from humans exist mostly for CMV<sup>44,48,114</sup>. CMV-associated BA has been reported in 10–30%, 60% and 78% of patients with BA in Europe<sup>42–44</sup>, China<sup>45</sup> and South Africa<sup>115</sup>, respectively. However, studies vary in their definition of CMV positivity and diagnostic methods<sup>43–45,49,50</sup>. Proof of a causal relationship remains elusive.

Rotavirus is a double-stranded RNA virus of the Reoviridae family that can infect hepatocytes and cholangiocytes<sup>116,117</sup>. As noted above, RRV infection of neonatal mice is an established model of BA<sup>118</sup>. In one study, group C rotavirus RNA was detected in 50% of liver specimens from infants with BA but not in specimens from controls with other cholestatic diseases<sup>119</sup>. By contrast, other studies did not detect rotavirus RNA in liver specimens from infants with BA<sup>120</sup> or increased rotavirus IgM in infants with BA compared with controls<sup>121</sup>.

In proof-of-concept studies, several toxins have been shown to have biliary toxicity potentially relevant to BA. Biliatresone was isolated from Australian plants ingested by pregnant livestock after several outbreaks of a BA-like disease in their newborns<sup>74,122</sup>. Biliatresone causes selective EHBD injury in larval zebrafish and newborn mice<sup>62,63,74</sup>. Chemically, it contains a reactive  $\alpha$ -methylene ketone group and binds to reduced glutathione (GSH)<sup>123–125</sup>, the most abundant endogenous small-molecule antioxidant.



Levels of GSH are particularly low in neonatal EHBD, which may partially explain the biliatresone mechanism of action<sup>75,90</sup>. Owing to its limited distribution, biliatresone is probably not relevant to human BA but provides evidence that prenatal exposure to a toxin could lead to BA in neonates while sparing their mother. Preliminary data suggest that microcystin-RR, found in harmful algal blooms, is a biliary toxin, and like biliatresone, is specific in causing redox stress in neonatal (as opposed to adult) cholangiocytes<sup>126</sup>.

An Egyptian study in infants with BA and their mothers found that BA can result from maternal exposure to the hepatocarcinogen aflatoxin B1 and its toxic metabolite aflatoxin B1-8,9-epoxide, which is normally detoxified by glutathione *S*-transferase Mu 1 (GSTM1)-mediated conjugation to GSH<sup>127,128</sup>. These unconfirmed data are intriguing because of the link to GSH.

The role of the maternal microbiome in BA is an emerging area of research. Treatment of pregnant mice with antibiotics during pregnancy and lactation led to a substantial reduction in the incidence of RRV-induced experimental BA in their pups, associated with colonic enrichment of the butyrate-producing bacteria *Anaerococcus lactolyticus*<sup>129</sup>. Butyrate administration to pregnant mice rendered their newborns resistant to experimental BA, with intestinal enrichment of Bacteroidetes and Clostridia and an increase in faecal glutamate and glutamine, which protected cholangiocytes against natural killer (NK) cell-mediated toxicity<sup>130</sup>. Notably Bacteroidetes and Clostridia were under-represented in the faecal microbiota of patients with BA with decreased glutamate and glutamine<sup>130,131</sup>. These findings may have broad therapeutic implications.

**Developmental susceptibility to injury.**—Oxidative stress is an important cause of teratogenesis and fetal injury<sup>132</sup>. Hypoxia is necessary for some stages of development, and the uterine environment during most of the first trimester is low in oxygen and energy metabolism is based on glycolysis. At the end of the first trimester, oxygen levels in the uterine environment increase and metabolism switches to oxidative phosphorylation, suggesting that this is potentially a time of redox stress if antioxidant production lags behind the increased production of reactive oxygen species (ROS)<sup>133</sup>. Birth and the immediate postnatal period are additional times of potential redox stress due to both ischaemia–reperfusion (from contractions) and the start of breathing with the switch to the postnatal circulation<sup>134</sup>. A systems biology study combining RNA sequencing (RNA-seq), GWAS and whole-exome sequencing of human BA tissue identified hypoxia signalling as a potential mediator of BA-like bile duct abnormalities; this study validated the potential role of hypoxia in bile duct abnormalities in zebrafish<sup>135</sup>. Immunostaining of human BA tissue showed expression of hypoxia-inducible factor 1 $\alpha$  (HIF1 $\alpha$ ) in hepatic and porta hepatis cholangiocytes; HIF1 $\alpha$  activation may result from hypoxia and/or oxidative stress<sup>136</sup>.

The redox state of the human EHBD during development is not known. The EHBDs of rodents and zebrafish have low levels of GSH in the neonatal and larval stages compared with the adult stage and with the IHBDs, suggesting susceptibility to redox stress<sup>75,90,126</sup>. Evidence of redox stress in liver tissue is a negative prognostic factor in patients with BA, whereas upregulation of genes involved in GSH metabolism is correlated with increased transplant-free survival<sup>137,138</sup>. Consistent with fetal and neonatal EHBD susceptibility to

redox stress as a risk factor for BA, toxins and viral infections (including CMV infection) cause redox stress<sup>139,140</sup>. Gestational diabetes and preterm birth, both linked to BA, are similarly associated with redox stress<sup>132,141</sup>. In support of a causal association between redox stress and BA, the antioxidant *N*-acetyl cysteine (NAC) reduced RRV-associated EHBD damage in mouse pups<sup>138</sup>, and a small pilot study of NAC treatment in babies with BA showed improved bile flow, decreased ROS production and decreased inflammation<sup>142</sup>.

In addition to low levels of GSH, the neonatal mouse EHBD has immature tight junctions that predispose to bile leakage, a collagen-poor submucosa that facilitates spread of leaked bile, and a sparse, immature epithelial apical glycocalyx that predisposes to injury from hydrophobic bile acids<sup>143,144</sup>. Neonatal EHBDs with these developmental immaturities seem to be particularly sensitive to bile-acid-mediated injury<sup>145</sup>. The bile acid pool changes considerably between prenatal and perinatal periods<sup>146,147</sup>, but the relative toxicity of prenatal, perinatal and postnatal bile has not been established.

**Susceptibility factors with unknown mechanisms.**—Sex, ethnicity, maternal diabetes and prematurity are susceptibility factors with undetermined mechanisms.

**$\beta$ -Amyloid deposition.**— $\beta$ -Amyloid (A $\beta$ ) deposition has been identified in the liver of patients with BA, the RRV mouse model and BA organoids<sup>87</sup>. Importantly, addition of exogenous A $\beta$  to normal control liver organoids was sufficient to cause BA-like morphological changes. Thus, BA could be grouped under diseases with A $\beta$  deposition, such as Alzheimer disease and cerebral amyloid angiopathy; however, in contrast to the extracellular deposits in other amyloid diseases, A $\beta$  deposition in BA seems to be intracellular. A $\beta$  deposition is a novel pathobiological feature of BA and may provide new diagnostic biomarkers (A $\beta$ 42/A $\beta$ 40) and adjuvant therapies (anti-A $\beta$ ).

Taken together, multiple factors — genetic, environmental, developmental and others, as-yet undefined — may contribute, alone or in combination, to the initial injury that probably occurs in the prenatal period.

## Response to injury

**Dysregulated and aberrant immune responses.**—A leading hypothesis in the pathogenesis of BA is that, in the genetically predisposed infant with immature or altered biliary development, cholangiocyte injury leads to exaggerated autoimmune and autoinflammatory responses targeting cholangiocytes, resulting in progressive bile duct damage, fibrosis and obliteration<sup>5</sup>. In BA, there is abundant evidence that adaptive immune cells (T cells<sup>148–152</sup> and B cells<sup>152–155</sup>) and innate immune cells (NK cells<sup>130,148,156</sup>, macrophages<sup>157–160</sup>, dendritic cells<sup>157,161</sup> and neutrophils<sup>142,162–164</sup>) contribute to biliary injury and disease pathogenesis (Fig. 4).

In addition, various inflammatory pathways contribute to biliary injury in BA. Extensive investigations on the role of T cells showed that early in biliary injury a T helper 1 (T<sub>H</sub>1) (that is, IFN $\gamma$  and TNF)-predominant response occurs<sup>165–167</sup> followed by a subsequent T<sub>H</sub>2 (that is, IL-33) response that is associated with bile duct proliferation and fibrosis<sup>150,168,169</sup>. IL-33 activates a type 2 innate lymphoid cell (ILC2) that shows plasticity and includes the

subsets of natural ILC2s (nILC2) and inflammatory ILC2s (iILC2). IL-33-activated nILC2s produce IL-13, leading to cholangiocyte hyperplasia and epithelial repair<sup>150,170</sup>. When the nILC2 IL-13–IL-4R $\alpha$ –STAT6 pathway (regulated by amphiregulin) is inhibited, iILC2s predominate and the full experimental BA phenotype ensues<sup>170</sup>.

The role of B cells in BA pathogenesis has also been investigated. Liver immune profiling in infants with BA by single-cell RNA-seq (scRNA-seq)<sup>152</sup> confirmed previous findings of cytotoxic CD8<sup>+</sup> T cell expansion and aberrant NK cell and macrophage responses, and revealed aberrant hepatic B cell lymphopoiesis. In the RRV BA mouse model, administration of anti-CD20 B cell-depleting antibodies<sup>152</sup> or use of B cell receptor-deficient mice<sup>155</sup> resulted in decreased liver CD4<sup>+</sup> and CD8<sup>+</sup> T cell levels and increased survival. In addition, B cell activation was associated with accumulation of non-specific IgG autoantibodies<sup>152</sup> and cholangiocyte-targeted IgM autoantibodies that correlated with worse outcomes<sup>153</sup>.

Cholangiocyte proteins may be identified as foreign by immune system components owing to alterations by the disease agent or exposure of sequestered proteins. Alternatively, there may be molecular mimicry, whereby viral proteins or toxic moieties have a high sequence homology with cholangiocyte proteins, with cross-reactivity resulting in adaptive ‘autoimmune’ responses<sup>171</sup>. Deficits in the CD4<sup>+</sup>FOXP3<sup>+</sup> regulatory T (T<sub>reg</sub>) cells that are responsible for inhibiting autoreactive T cells<sup>172</sup> in both human BA and animal models resulted in autoreactive T cells targeting cholangiocytes and contributing to disease pathogenesis<sup>173–177</sup>. Epigenetic modifications of FOXP3 (ref. 178) could cause functional T<sub>reg</sub> cell deficiencies in BA, and decreased T<sub>reg</sub> cell frequency was associated with higher bilirubin levels in infants after KPE<sup>179</sup>. Another potential trigger of an ‘autoimmune’ response in BA is maternal microchimerism, whereby maternal cells entering the fetus may alter its response to self-antigens and trigger autoimmunity<sup>180</sup>. Many investigators have identified increased numbers of maternal cells in the liver of patients with BA compared with the numbers in the liver of control patients without BA<sup>181–184</sup>. Whether or not these maternal cells are pathogenic or simply bystanders related to inflammation is unclear. A working theory is that the maternal microchimeric cells initially function as effector T cells causing biliary injury in utero (similar to a graft-versus-host response). Postnatally, the retained maternal cellular proteins are seen as foreign targets, eliciting an autoimmune-like response (host-versus-graft response)<sup>185</sup>.

BA mimics autoinflammatory diseases, which are defined by dysregulation or disturbance of the innate immune system, with macrophages and neutrophils as the main effector cells<sup>186</sup>. Macrophages were found to be recruited to the liver via a dendritic cell–T<sub>H</sub>17 cell–macrophage axis and contributed to cholangiocyte injury in a mouse model of BA<sup>157</sup>. An effector mechanism of liver macrophages within murine BA includes the NLRP3 inflammasome, a potent producer of pro-inflammatory cytokines and cytotoxic molecules<sup>187</sup>. scRNA-seq demonstrated that the livers of infants with BA have deficits within a regulatory (anti-inflammatory) macrophage subset, which would promote chronic macrophage activation<sup>160</sup>.

In murine BA, CD177<sup>+</sup> neutrophil extracellular trap (NET)-forming neutrophils resulted in cholangiocyte injury, whereas CD177<sup>-/-</sup> mice did not develop BA<sup>163</sup>, and patients with BA demonstrated a predominance of CD177<sup>+</sup> neutrophils in peripheral blood that led to increased NET production and resulted in cholangiocyte apoptosis in in vitro studies<sup>162</sup>. Similarly in patients, increasing levels of plasma IL-8 (neutrophil activator), neutrophil elastase and NETs positively correlated with bilirubin levels 60 days after KPE, whereas rising IL-8 levels were associated with an increased risk of transplantation or death 1 year after KPE<sup>179</sup>, suggesting that persistent neutrophil activation promotes biliary injury and fibrosis.

**Inflammation and bile acid metabolism.**—The relationship between bile acids and inflammation is dichotomous: on the one hand, bile acid-mediated cellular injury can lead to subsequent activation of inflammation and, on the other hand, inflammation directly altering bile acid metabolism could result in bile acid retention<sup>188</sup>. In the RRV mouse model of BA, early inflammatory responses resulted in persistent downregulation of multiple bile acid transporters and nuclear receptors, despite high bile acid concentrations in the liver<sup>189</sup>. A potential cause of the inflammation-induced downregulation of bile acid transport may be an epigenetic phenomenon, with production of microRNAs that specifically target bile acid transporters and/or nuclear receptors<sup>190</sup>. By contrast, low levels of PPAR $\alpha$  and FXR (regulators of bile acid formation and transport) were associated with upregulation of inflammatory and fibrotic pathways in BA, suggesting bile-acid-induced inflammation<sup>191</sup>.

Taken together, these studies suggest that a sustained induction of adaptive and innate immune responses in the setting of dysregulated immunity results in chronic biliary injury, bile acid retention and fibrosis, contributing to the pathogenesis of BA.

### The ECM in disease progression

The ECM is a complex and dynamic cellular environment that provides biochemical and biomechanical cues as part of wound healing and fibrosis. The ECM of the fetal EHBD in humans and other species has minimal collagen but large amounts of hyaluronic acid (HA). Interestingly, wound healing in fetal sheep and neonatal mice features considerable HA, rather than collagen, deposition<sup>69,143</sup>. As fetal wound healing<sup>192</sup> is scarless (regenerative rather than reparative), this raises questions about the effects of the switch to an adult wound healing programme in late gestation and the perinatal appearance of actively collagen-secreting fibroblasts in the EHBD submucosa<sup>143</sup>. Fibrotic remnants excised during KPE from patients with BA show that type I collagen is outside the thickened HA ring that surrounds epithelial remnants (obliterated bile duct lumen), raising the possibility that swelling of the highly hygroscopic HA could contribute to duct obstruction<sup>143,193</sup>.

Multiple studies in patients with BA also support a key role for collagen degradation in disease progression. Expression of matrix metalloproteinase 7 (MMP7), which breaks down ECM proteins, is elevated in cholangiocytes of patients with BA<sup>194</sup>, and MMP7 is a biomarker for the disease<sup>195</sup>. In the liver from patients with BA, MMP7 was mainly produced by hepatocytes, bile duct epithelial cells and some Kupffer cells, and elevated

MMP7 expression was detected not only in these cells but also in interstitial fibrous tissue<sup>196</sup>, suggesting that alterations in the ECM niche are linked to disease progression.

## Outcome

The response phase after the initial injury involves dysregulation of immunity, inflammation and the ECM. These factors underpin BA pathogenesis and disease progression, and are critical in determining CoJ and native liver and overall survival. Identifying suitable therapeutic targets and optimal timing for new interventions is the next challenge in improving patient outcomes.

## Diagnosis, screening and prevention

### Diagnosis

Up to 50% of newborns experience transient jaundice after birth, but only around one in 2,500 full-term neonates develops cholestatic jaundice, characterized by increased conjugated bilirubin ( $>17 \mu\text{mol/l}$ ;  $1 \text{ mg/dl}$ )<sup>197</sup>. Although some asymptomatic infants in whom conjugated hyperbilirubinaemia is detected on neonatal screening may not develop BA, persistent neonatal cholestasis should be considered pathological, and BA is the most common cause (25–40%)<sup>198</sup>. Prompt diagnostic evaluation is necessary in all infants who remain jaundiced beyond 2 weeks of age if formula-fed or 3 weeks if breastfed<sup>197</sup>. Differential diagnosis includes monogenic aetiologies (25%), such as Alagille syndrome, other congenital causes, such as  $\alpha 1$ -antitrypsin deficiency, cystic fibrosis, tyrosinaemia, choledochal cyst and others, total parenteral nutrition-associated cholestasis and so-called idiopathic neonatal hepatitis. The antenatal history is rarely specific for BA, but cystic BA may be detected during maternal ultrasonography, with a differential of a choledochal cyst, together with some of the associated anomalies (particularly cardiac anomalies) typically seen in BASM (Fig. 2).

Serum liver biochemistry changes, imaging results and liver biopsy findings may suggest BA, but these are not specific for the disease. Features on ultrasonographic scans that suggest BA include the presence of a hypoplastic or atrophic gallbladder and fibrosis of the bile ducts in the porta hepatis (triangular cord sign). Sometimes the cystic element of cystic BA may be visible<sup>2,197,199,200</sup>. Radioisotope hepatobiliary scans may show absence of hepatic isotope excretion but this finding may not be discriminatory<sup>197,201</sup>. Percutaneous liver biopsy has been the mainstay of preoperative diagnosis of BA for some time, particularly in North America and the UK. Histology may show portal inflammation, bile plugs within the duct lumen, bile duct proliferation, ductular reaction (DR) and fibrosis. The estimated diagnostic accuracy of a needle liver biopsy is ~90% (95% CI 85–95%)<sup>202,203</sup>. The risk of a false-negative biopsy is increased in infants  $<30$  days old, due to immature liver histopathology<sup>202,204</sup>. The need to wait for 4–7 days for the histological diagnosis of BA has led some centres in Asia to advocate for diagnostic laparoscopy followed immediately by KPE if the operative diagnosis is BA.

Definitive diagnosis is achieved on laparotomy or laparoscopy with possible intraoperative cholangiography. BA is usually classified according to the level of proximal obstruction:

types I, II and III are characterized by atresia at the levels of the common bile duct, common hepatic duct and porta hepatis, respectively<sup>1</sup>. Absence of bile in the gallbladder is highly suggestive of BA, whereas presence of bile in the gallbladder almost always excludes a diagnosis of BA, with the possible exception of type I BA. Nevertheless, results of intraoperative cholangiography are not always conclusive. Contrast flowing from the gallbladder to the intestine does not rule out a more proximal obstruction, and visualization of the intrahepatic ducts may require pressure on the common bile duct. A hypoplastic but patent biliary tree may be a feature of Alagille syndrome<sup>6</sup>.

New, less-invasive diagnostic methods have been developed with the aim of improving accuracy. Serum MMP7 seems to have high discriminatory potential with an area under the curve reaching 0.90–0.99 (refs. 194,205,206). The optimal cut-off concentration of MMP7 depends on the analytical method, and further research is warranted before routine clinical application<sup>207,208</sup>. Plasma A $\beta$  levels have been reported to enhance diagnostic accuracy in combination with liver biochemistry<sup>209</sup>, and increased liver stiffness (measurements of >7.7 kPa in infants up to the age of 90 days and >8.8 kPa in infants older than 90 days) has shown promising accuracy in differentiating between BA and non-BA cholestasis<sup>210</sup>. However, large multicentre studies are needed to confirm these observations<sup>211</sup>.

## Screening

BA fulfils most of the criteria of a condition suitable for newborn screening<sup>212</sup>. The original screening method used in Japan, Taiwan, and some parts of Canada, Germany and Switzerland involved distribution of stool colour cards (SCC) to parents, with subsequent evaluation in those shown to be pale. Digital versions are now available<sup>212</sup>. SCC screening resulted in earlier age at time of KPE and improved native liver survival rates<sup>213</sup>. Although universal use and long-term evidence of the benefit of SCC screening have not been established<sup>214–216</sup>, in a study in China, this method led to earlier diagnosis and better prognosis<sup>217</sup>.

The recognition that BA is present at birth in most infants<sup>59</sup> led to an increase in studies involving measurement of fractionated bilirubin in whole-blood samples, and in dried blood spots in a two-stage screening programme as a marker of cholestasis<sup>60,218–220</sup>. Such screening programmes have decreased the age at KPE and improved surgical outcomes<sup>60,213</sup>. Elevated conjugated bilirubin in early whole-blood samples has been shown to be a sensitive screening tool for BA, but technical difficulties remain in developing consistent conjugated bilirubin screening using dried blood samples for large-scale screening in different laboratories.

## Prevention

Prevention of BA remains elusive. Some epidemiological data link maternal intestinal and genitourinary infections<sup>39</sup>, first-trimester anti-inflammatory asthma medication<sup>40</sup> and maternal drug abuse with an increased risk of BA<sup>37</sup>. Experimental studies have suggested benefits of altering the maternal gut microbiome in BA prevention<sup>129–131</sup>. However, no clinical data exist that show whether intervention on these risk factors can prevent the development of BA in susceptible infants.



## Management

### Kasai portoenterostomy

**Open Kasai portoenterostomy.**—Modern surgery for BA dates from the 1950s and 1960s. Kasai transformed BA from a mostly untreatable and lethal condition into a treatable and chronic disease with the introduction of portoenterostomy. In a KPE, the obliterated bile duct remnant is sectioned in the porta hepatis and reconstructed using a jejunal Roux loop (Fig. 5). Microscopic examination shows patent biliary ductules at the site of anastomosis retaining a connection with the intrahepatic biliary system<sup>8,221</sup>. The nature of the IHBDs can sometimes be directly appreciated in patients with cystic BA when the cholangiogram shows a myriad of intercommunicating etiolated ductules (Fig. 2c). Since the 1970s, KPE has become the global standard treatment for BA. Ohi extended the level of hilar dissection with the upper limit being flush with the liver capsule but not breaching the liver parenchyma<sup>222</sup>. This radical dissection achieves complete excision of the extrahepatic biliary system exposing the widest possible portal plate for reconstruction. Typically, the Roux loop is 30–40 cm long and passes behind the colon (Fig. 5). Subsequent modifications of this procedure, including the creation of a ‘valve’ from partial intussusception of the mucosa, have not shown additional benefit<sup>223</sup>.

**Laparoscopic Kasai portoenterostomy.**—Early attempts to replicate the open procedure in a laparoscopic approach had little success. In the past 10 years, surgeons in high-volume centres, particularly in China, have reinvigorated laparoscopic KPE<sup>224,225</sup>. However, compared with open KPE, the extent of the dissection is fairly restrained and similar to Kasai’s original description without the subsequent extended dissection proposed by Ohi. This is predominantly because a safe extended dissection is much more difficult to accomplish with current laparoscopic technology.

KPE can also be accomplished using robotic technology (three arms, four ports). However, in a report from China<sup>226</sup>, hospital stay was prolonged to ~10 days, compared with 6–7 days for open KPE typically seen in the West<sup>227</sup>. This is obviously contradictory to one of the purported benefits of laparoscopy. In one of the largest published experiences of laparoscopic KPE<sup>225</sup>, patients remained in hospital for 30 days, largely for intravenous corticosteroid therapy. These techniques seem to have limited real advantages beyond cosmesis. Real outcomes data from prospective studies vary considerably and suggest rates of CoJ ranging from 41%<sup>228</sup> to >80%<sup>229</sup>. The only long-term study involving laparoscopic KPE showed a dramatic difference in 10-year native liver survival: 45% (5/11) after laparoscopic KPE and 85% (17/20) after open KPE ( $P = 0.03$ )<sup>230</sup>.

**Outcomes of Kasai portoenterostomy.**—Multiple national series and large consortia reports on the outcomes of KPE have been published<sup>7,9,10,15,20,23,213,231–238</sup> (Table 2). These are predominantly from Japan, Taiwan and Europe with few from the Americas, South Asia or Africa. The key outcome measures are time to KPE, CoJ to normal levels, native liver survival and true survival. Reports with the lowest time to KPE either come from regions with some form of screening programme (for example, Taiwan<sup>23,213</sup>) or from regions with centralized care (for example, England and Wales<sup>238</sup>). Similarly, the highest

CoJ rates have been reported from Asia (for example, Taiwan and Japan<sup>9,213</sup>) or in a centralized European series (England and Wales, the Nordic consortium<sup>7,238</sup>), suggesting that high patient volumes positively influence outcomes. By contrast, the lowest CoJ rate (~24%) has been reported in high-income countries where care is not centralized (for example, Germany<sup>232</sup>).

## Liver transplantation

Liver transplantation is the treatment for real or expected KPE failures. In a retrospective cohort study in 3,438 patients with BA listed for liver transplantation, 15% were listed for primary transplantation (no prior KPE), 17% for salvage transplantation after early failure (at <1 year of age) and 67% after late failure (at >1 year of age)<sup>239,240</sup>. For many, particularly in the USA, liver transplantation as a primary procedure has become increasingly attractive<sup>239</sup>, owing to poor immediate and long-term KPE outcomes. In a review from 2024, the inevitability of KPE failure in the natural history of the disease was noted as an argument for primary liver transplantation, whereas an argument against the procedure is that some patients do have excellent long-term outcomes following KPE, and that the real problem is the inability to predict the outcome at the time of presentation<sup>241</sup>. Primary transplantation clearly has value in some patients. In the UK, <5% of all infants presenting with BA undergo the procedure<sup>238</sup>. Typically, these are patients who present late (for example, >100 days old) and have obvious liver cirrhosis and associated ascites or portal hypertension<sup>238</sup>.

## Prognosis at time of KPE

Important prognostic factors for BA can be divided into intrinsic factors related to the natural history and nature of the disease and external factors related to its management<sup>242</sup>.

**Intrinsic factors.**—The effect of age in isolated BA is undeniable: those <30 days old at KPE have CoJ rates of >70%, whereas those >100 days old at KPE have CoJ rates of <40%<sup>238</sup>. The type of extrahepatic anatomy is also crucial. Effective surgery for cystic BA (5–10%), which is usually type I (with bile in the cyst), should still involve a radical resection and is associated with CoJ of >80%; however, some evidence suggests that late failure of this surgery is particularly evident during adolescence<sup>238</sup>. BASM-associated and CMV-infection-associated BAs are associated with inferior outcomes compared with isolated BA<sup>243</sup>.

Liver biomarkers at time of KPE can predict outcome, but their precision and clinical usefulness are limited. The best known biomarker is the APRI, a composite of aspartate aminotransferase and platelet levels, and a surrogate for liver fibrosis. Large series have shown significantly different long-term native liver survival depending on the initial APRI quartile<sup>50</sup>. A large Chinese series also showed a negative effect of having a low  $\gamma$ -glutamyl transferase (<300 IU/l) at the time of KPE<sup>244</sup>. Serum MMP7 has only recently been evaluated, not only as a diagnostic biomarker but also for its prognostic value<sup>245</sup>.

Evaluation of the degree of histological liver fibrosis at KPE has been studied but its prognostic value has been disappointing. The Childhood Liver Disease Research Network

(ChiLDReN)<sup>202</sup> reviewed 316 liver biopsy samples using an array of histological features (for example, fibrosis, portal oedema and others) and found none to have any relationship with CoJ, although some relatively small differences emerged in terms of native liver survival. For example, the hazard ratio for the need for transplantation was 1.73 (95% CI 1.21–2.48) times greater in patients with stage 3 or 4 fibrosis than in those with stage 0–2 fibrosis (Scheuer system).

New liver or serum biomarkers have been suggested to have prognostic value, although most have not yet entered routine clinical practice. For example, secretin receptor mRNA expression in the liver correlated strongly with both CoJ and native liver survival<sup>246</sup>. Serum levels and hepatic expression of IL-8 is high in infants with BA and low levels have been associated with improved native liver survival<sup>247</sup>. Similarly, serum FGF19 levels were associated with ductular proliferation but not liver fibrosis, and predicted KPE outcomes, with those with levels >109 pg/ml having markedly diminished native liver survival<sup>248</sup>. These high levels are believed to originate from the liver, with aberrant hepatic expression induced by the high levels of bile acids in cholestasis seen in BA. Finally, Ki67, a marker for cellular proliferation, may be more actively expressed in the liver from patients with BA, and higher expression has been associated with worse native liver survival<sup>249</sup>.

**Extrinsic factors.**—The obvious extrinsic factor is the expertise of the surgical team and the technique adopted. Most studies investigating centre experience and case-mix have found a good correlation between larger centre size and improvements in outcome, although some found satisfactory results for smaller centres<sup>234</sup>. This finding led to a policy of centralization and concentration of KPE and transplant resources for BA, initially implemented in England and Wales in 1999 (ref. 11). Subsequent outcome studies showed a dramatic improvement in national KPE outcomes with a reduction in the time to KPE to <50 days and improvement in CoJ rates<sup>238</sup>. This policy has now been adopted by several other (predominantly Northern European) countries<sup>7,250</sup>.

Clearly, there are major geographical obstacles to centralization, such as in North America and Australia<sup>236,251</sup>. The nature of the health-care system may preclude centralization where there is competition between potential providers (for example, in Germany and the USA)<sup>232</sup>.

## Adjuvant therapies

The use of adjuvant treatments following KPE is controversial with an arguably marginal evidence base and many institutions sticking with outdated protocols in the absence of certainty. Nevertheless, the field may be entering a period of increased optimism. As BA is a rare disease, incontrovertible trial-based evidence of the benefit of these therapies remains slim. The merits of each pharmacological agent should be assessed individually.

**Antibiotics and ursodeoxycholic acid.**—The use of both agents is widespread and uncontentious but with virtually no actual evidence. Cholangitis is a common postoperative problem — it occurs in up to 80% of patients after KPE in some recent series<sup>252</sup> — and its treatment usually involves intravenous antibiotics for 1–2 weeks. Bacterial cultures are rarely positive, but one study showed a prevalence of the Gram-positive *Enterococcus faecium* and the Gram-negative organisms *Escherichia coli*, *Enterobacter cloacae* and

*Klebsiella pneumoniae*<sup>252</sup>. In one Chinese multicentre ( $n = 14$ ) open-label study involving 211 children, the severity of cholangitis was graded as ‘mild’, ‘moderate’ and ‘severe’, and each grade was randomized to a different regimen<sup>253</sup>. The duration of fever was the primary outcome. Meropenem alone was found to be equivalent to cefoperazone in mild and moderate cholangitis and the addition of intravenous immunoglobulin improved the efficacy of meropenem in severe cholangitis. Prophylactic antibiotic regimens of variable duration (1–12 months) have been designed; however, little scientific evidence favours one over another according to one systematic analysis<sup>254</sup>. Ursodeoxycholic acid use is prevalent but only one study showed biochemical improvement in a withdrawal–reintroduction format in 16 children with stable disease<sup>255</sup>.

**Anti-inflammatory therapy: corticosteroids and immunoglobulin.**—Inflammation has a key role in the early pathophysiology of some infants with BA, after which fibrosis takes over. Over the past 20 years, corticosteroids have been explored as postoperative adjuvant therapy to modulate this inflammatory response. There have been two prospective randomized controlled trials (RCTs), and a number of large prospective open-label trials<sup>256,257</sup> (Table 3). For example, a RCT trial in the UK using a low-dose regimen (2 mg/kg/day oral prednisolone) showed little clinical difference but significantly reduced bilirubin levels in the corticosteroid arm<sup>256,258</sup>. A multicentre RCT (START trial by ChiLDReN) using intravenous or oral 4 mg/kg/day prednisolone was powered to detect a difference of 25% in CoJ, and showed a difference of 15% in CoJ (compared with placebo) in patients <70 days old at KPE, but without achieving statistical significance<sup>259</sup>. A follow-on prospective study from the original UK study using an increased dose (5 mg/kg/day oral prednisolone) in infants <70 days old at KPE showed the same difference in CoJ but because of a larger number of participants the results were statistically significant<sup>256</sup>. A larger, Chinese single-surgeon trial showed significant effects on CoJ and native liver survival with a 10-week regimen of intravenous or oral 4 mg/kg/day prednisolone<sup>257</sup>.

Most large centres in Europe, China and Japan use high-dose corticosteroids in a variety of regimens and some American centres, despite the negative results of the START trial, also use corticosteroid-based regimens<sup>260,261</sup>. In these centres, CoJ rates of >60% may be achieved. It is also important to recognize that age at KPE is an important factor in the efficacy of corticosteroids<sup>262</sup>. An interesting question is whether corticosteroid therapy should be repeated for disease relapses. This use is common practice in Japanese centres, which have the best overall results worldwide. Of note, corticosteroids may have adverse effects. A post hoc analysis of child growth following the START trial showed impaired length, weight and head circumference growth trajectories for at least 6 months after KPE<sup>263</sup>.

The anti-inflammatory potential of intravenous immunoglobulin administration has been proven in several autoimmune and inflammatory disorders. However, a ChiLDReN phase Ib/II clinical trial<sup>264</sup> of intravenous immunoglobulin in BA failed to show a beneficial effect compared with the placebo arm of the START trial.

**Antiviral therapy.**—Specific antiviral therapy against CMV has been used in small, single-centre studies<sup>265,266</sup>. At least one study showed remarkably improved outcomes for

CoJ and reduced need for transplantation<sup>267</sup> in patients with BA who were CMV-positive. Currently, oral valganciclovir is favoured based on ease of use and efficacy<sup>42,267</sup>.

**Targeting bile acid metabolism.**—Three major randomized trials investigating targeting bile acid metabolism are currently in progress. Maralixibat (NCT04524390)<sup>268</sup> and odevixibat (NCT04336722)<sup>269</sup> are ileal bile acid transporter (IBAT) inhibitors that reduce the re-uptake of bile acids in the terminal ileum, and could diminish bile acid accumulation after KPE<sup>270</sup>. Obeticholic acid (NCT05321524)<sup>271</sup> is a modified primary bile acid and a potent agonist of nuclear farnesoid X receptor (FXR). FXR activation may reduce endogenous bile acid production, promote bile acid export from hepatocytes and inhibit a variety of NF-κB mediated inflammatory precursors (such as TNF) and may limit the severity of fibrosis<sup>272</sup>.

The two IBAT inhibitors have shown promising results with reductions in serum bile acids and pruritus in other allied neonatal cholestatic conditions, such as Alagille syndrome<sup>273,274</sup>. However, early unpublished results from the phase II maralixibat trial suggest no significant effect on the primary and secondary outcomes according to a [press release](#).

### Potential for regeneration

Regenerative medicine aims to restore or replace damaged tissues and organs using various methods, including stem cells, gene therapy, tissue engineering and organoids, and is emerging as a potential solution for various liver diseases including BA<sup>275</sup> (Fig. 6).

**Stem cell therapy.**—Stem cells generated from induced pluripotent stem cells, embryonic stem cells or directly from tissue-derived cells can be injected into the liver or the bile ducts to promote regeneration and repair of damaged tissues. Liver-derived and cholangiocyte-derived organoids are most promising because they can replace both the damaged epithelium and niche<sup>276</sup>. Stem cells can also act by modulating responses to inflammation and fibrosis. In preclinical models, autologous bone-marrow-derived mononuclear cells (BMMNC), haematopoietic stem cells, mesenchymal stem cells and endothelial progenitors improved liver cirrhosis by reducing hepatic fibrosis and inducing recovery of liver function, but limited clinical data are currently available. An early study from New Delhi using BMMNC injected via the hepatic artery and portal vein showed improvements in postoperative biochemistry but patient survival at 1 year was low, limiting the relevance of the study<sup>277</sup>. A clinical trial from Hanoi testing a regimen of two autologous BMMNC administrations in children with BA and liver cirrhosis showed no change in fibrosis or histology on liver biopsy, or in endoscopic features, but bilirubin levels and the Paediatric End-Stage Liver Disease values decreased<sup>278</sup>. Injection of granulocyte colony stimulating factor to mobilize haematopoietic stem cells has also been studied in infants after KPE<sup>279</sup>. This study showed a reasonable safety profile and the desired haematopoietic response, but with very limited differences in outcomes apart from less frequent cholangitis compared with controls.

**Tissue engineering.**—Tissue engineering using scaffolds, bioreactors or 3D printing can create artificial or bioengineered bile ducts that mimic the natural ones. This methodology can be very effective in replacing EHBDs, but there are limitations to the engineering

of intrahepatic ducts even though preliminary data showed their ability to repair human biliary epithelium<sup>12,280</sup>. Future treatments of liver failure may also benefit from using tissue-engineered liver which takes advantage of cells, such as organoids, ECM and signalling molecules<sup>82,83,275</sup>. Functional engineered livers using both synthetic and natural biomaterials such as decellularized livers have been transplanted in large-animal models, but 3D bioprinting may offer a more tailored regenerative option for patients with BA in the future<sup>281</sup>. These regenerative therapies are still at the experimental stage and face considerable challenges, including safety, efficacy, scalability and ethical issues, before they may become available in practice.

## Quality of life

The most important clinical manifestations of progressive liver disease and indications for liver transplantation include portal hypertension, cholangitis and advanced cirrhosis associated with synthetic liver failure, recurring cholestasis and ascites, which greatly affect patients' quality of life<sup>282,283</sup>. Few long-term studies in patients with BA are available. KPE outcomes seem to be better in highly specialized centres in East Asia, but the only study comparing an Asian and a UK centre, published in 2001, showed no difference in objective scoring<sup>284</sup>.

Although successful KPE resolves histological cholestasis, DR and liver fibrosis persist, reflected by expansion of cholangiocytes and activated portal myofibroblasts with increased expression of cytokeratin 7 (K7)/*KRT7*, a cholangiocyte marker, and  $\alpha$ -smooth muscle actin/*ACTA*, a marker of activated hepatic stellate cells<sup>21,285,286</sup>. DR is a central pathological feature in cholangiopathies in which expansion of reactive neocholangiocytes is thought to promote liver fibrogenesis by activation of myofibroblasts<sup>287</sup>. DR initially decreases in patients in whom bilirubin levels normalize after KPE<sup>285,286,288</sup>, but it increases after unsuccessful KPE. Progression of DR is also associated with the extent of liver fibrosis and transplant-free survival both at and after KPE<sup>288</sup>. The key biliary elements of DR, bile duct expansion and K7<sup>+</sup> hepatocytes, are closely correlated with serum bile acid levels, which predict transplant-free survival following KPE<sup>288,289</sup>. The underlying cholangiopathy advances variably, and nearly 50% of patients surviving with their native liver at age 2 years require liver transplantation by adulthood<sup>290,291</sup>.

By school age, 43% of children undergoing protocol follow-up liver biopsies following successful KPE have cirrhosis; 79–97% adult native liver survivors also show histological, clinical and/or ultrasonographic signs of cirrhosis<sup>292–294</sup> (Table 4). Chronic advanced liver fibrosis predisposes to development of both benign hepatic nodules (6%) and hepatocellular carcinoma (HCC) (1%) in native liver survivors<sup>295,296</sup>. Surveillance with biannual  $\alpha$ -fetoprotein measurement and abdominal ultrasonography is recommended, and tumour biopsy should be obtained on suspicion of malignancy<sup>295–297</sup>. HCC diagnosis warrants prompt evaluation for liver transplantation.

Portal hypertension affects most patients, typically developing at a young age paralleling the progression of liver fibrosis, and is frequently complicated by oesophageal varices<sup>292</sup>. By a median age of 8.5 years, 62% of 348 paediatric native liver survivors had developed



splenomegaly, thrombocytopenia or both<sup>298</sup>. Oesophageal varices were verified at a median age of 8.6 years in 60% of native liver survivors who underwent endoscopy<sup>299</sup>. Ascites is a less common manifestation of portal hypertension, detected in <10% of paediatric native liver survivors<sup>300</sup>. At a mean age of 19 years, 69% and 64% of native liver survivors had portal hypertension and oesophageal varices, respectively<sup>301</sup>, and 46–70% of adult native liver survivors demonstrated clinical, ultrasonographic or endoscopic signs of portal hypertension<sup>293,294</sup>. In paediatric native liver survivors presenting with splenomegaly and thrombocytopenia at a median age of 8.5 years, the 5-year cumulative incidence of variceal bleeding exceeded 10%<sup>298</sup>, and a systematic review reported gastrointestinal bleeding in 21% of adults<sup>302</sup>. Although primary endoscopic prophylaxis of oesophageal varices remains controversial in children with BA, variceal bleeding is preventable by endoscopic surveillance and eradication, particularly in patients with a functioning KPE<sup>299,303</sup>. Infrequent pulmonary complications of portal hypertension include hepatopulmonary syndrome and portopulmonary hypertension, both of which are indications for listing for liver transplantation to avoid the development of irreversible cardiopulmonary complications that would be a contraindication for transplantation<sup>282,304</sup>. Hepatopulmonary syndrome affects 5–7% of children surviving with their native liver and is characterized by hypoxia and intrapulmonary vasodilatation<sup>282,300</sup>. Portopulmonary hypertension is diagnosed in <1% of children with end-stage liver disease, predominantly female adolescents<sup>305</sup>. It is asymptomatic until increased pulmonary artery pressures cause hypoxia, dyspnoea and, eventually, right-sided heart failure<sup>305</sup>.

Cholangitis episodes negatively affect native liver survival<sup>282,306</sup>. Two-thirds of 219 paediatric native liver survivors aged 5–18 years had experienced cholangitis, 17% during the preceding year, while 31% of adult native liver survivors faced cholangitis aged 17–30 years<sup>293,300</sup>. Although widely used, oral antibiotic prophylaxis fails to reduce the incidence of cholangitis episodes according to a meta-analysis<sup>254</sup>. Approximately 10–15% of patients with BA develop intrahepatic bile lakes, predisposing to recurrent cholangitis by bile stagnation<sup>307</sup>. The presence of bile lakes should be assessed with MRI so that they can be effectively drained to reduce cholangitis episodes and maintain native liver survival<sup>307</sup>. Future research should focus on development of accurate diagnostic tools and efficient prevention and treatment of cholangitis<sup>308</sup>.

Although most older patients experience stable growth, undergo normal pubertal development, and reach normal adult height and weight, growth and biometrics of nutritional status should be regularly assessed to avoid malnutrition<sup>300,304</sup>. Vitamin D deficiency is common even after successful KPE despite enteral supplementation, and predisposes to rickets and osteoporosis<sup>309</sup>. Although the risk of metabolic bone disease is highest in patients in whom jaundice does not clear, fractures have been reported in 12–15% after successful KPE<sup>301,309</sup>. Infants with BA are at risk of neurodevelopmental delays, particularly after unsuccessful KPE<sup>310</sup>. Most North American native liver survivors aged 3–12 years showed normal neurodevelopment, whereas two smaller European national studies, which also included liver-transplanted patients, found impaired motor skills and decreased intelligence quotient, which were associated with advanced liver disease<sup>311–313</sup>. Compared with healthy controls, all domains of patient and parent-proxy reported health-related quality of life were decreased at a mean age of 9.7 years in 221 children surviving with their native

liver, with the greatest differences occurring in school functioning<sup>314</sup>. Adult native liver survivors, particularly women, have reported decreased general health perception, which is correlated with complications of liver disease<sup>315,316</sup>. Based on limited data, most adult native liver survivors demonstrate educational levels and employment rates comparable to those in the general population<sup>293,301,316</sup>.

Patients with BA require lifelong structured multidisciplinary follow-up in specialist centres to optimize safe transition of care to adult practice and timing of liver transplantation, as adult liver disease scores are not appropriate for these patients<sup>282,297,304,317</sup> (Fig. 7). For successful transition, health professionals caring for the adult population should be familiarized with the long-term liver disease complications and the challenging timing of liver transplantation, and young adult patients with BA deserve a holistic management approach that does not overlook psychosocial aspects and comprehensive patient education about their condition<sup>282,297,304</sup>.

## Outlook

The global burden of BA remains substantial. CoJ with timely KPE is achievable in 50–70% of patients with BA in centres with relevant expertise, and this approach should, therefore, become the benchmark for less-developed communities to target in the short term. However, in the medium and long term, further improvements in patient outcomes are likely to require multiple innovative measures inspired by a comprehensive understanding of the complex mechanisms of disease causation and progression (Fig. 8).

Early diagnosis will be an important first step in the new management paradigm. New biomarkers, probably in combination, informed by big data and analysed with artificial intelligence, may enable improved newborn screening and a diagnosis to be made in the first few weeks of life. Expediting the diagnosis will provide a larger window for neoadjuvant therapy before KPE. The distinction between prenatal and perinatal insult to the extrahepatic biliary tree and postnatal hepatic response to biliary obstruction will enable more targeted medical interventions in different phases of disease progression. For example, neonatal susceptibility to redox stress and hypoxia suggests that antioxidants, such as NAC, may be more appropriate for neoadjuvant therapy. Likewise, testing of butyrates (that modulate glutamate and glutamine) and ciliary dysfunction remedies (repurposing drug targets developed for other primary ciliopathies) may be warranted during the early injury phase of BA.

Surgery will continue to be the cornerstone of BA management. Centralization of surgery and optimizing medical interventions for complications after KPE, such as cholangitis, will improve patient outcomes and provide a sufficiently large patient pool to better understand early (at <1 year of age) and late (at >1 year of age) KPE failures and their underlying mechanisms.

Emerging adjuvant therapies targeting immune response to obstruction, bile acid homeostasis and fetal wound healing are promising and should be evaluated with robust multicentre prospective randomized trials. It is possible that some emerging therapies may

work better in either the injury or the response phase, whereas others may be beneficial in both phases, which exist as a continuum. An additional question is whether the emerging therapies and existing therapies (for example, corticosteroids) would work better alone or in combination.

Regenerative medicine approaches are a speculative strategy for BA. Human stem cell and organoid research provides a preclinical platform for disease modelling and drug screening, capable of diagnostic and therapeutic breakthroughs<sup>275</sup>. Stem cells modulating inflammatory or immune dysregulation or liver fibrosis progression may be tested as adjuvants to KPE and tissue engineering may ultimately replace liver transplantation. With focused academia–industry collaboration, some of these emerging research directions might be successful and transform this devastating disease into a curable condition.

## Acknowledgements

P.K.H.T., C.S.M.T. and V.C.H.L. disclose funding support for the research for this work from Theme-based Research Scheme (T12-712/21-R), CERG (17105119), HMRP (09201836) and Commissioned HMRP (PR-HKU-1) from Hong Kong SAR Government; P.K.H.T. was also supported by Macau FDCT0011/2023/AKP. R.G.W. was supported by NIH R01 DK119290 and Children's Hospital of Philadelphia-Fred and Suzanne Biesecker Paediatric Liver Center.

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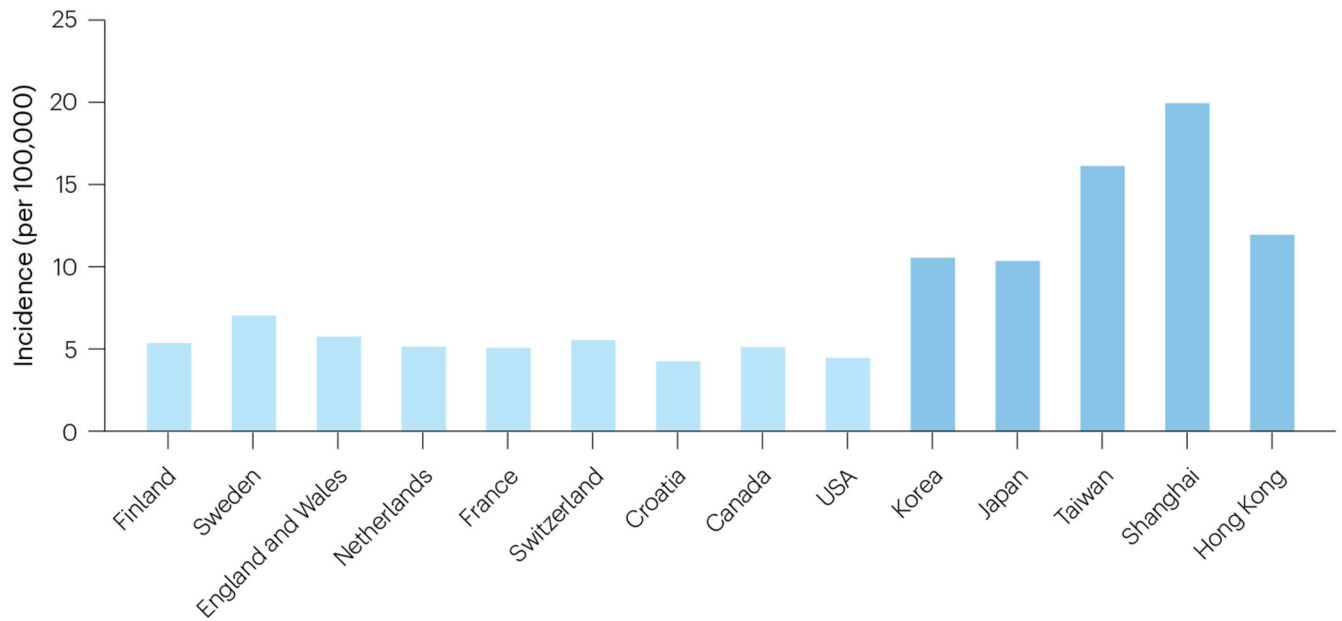
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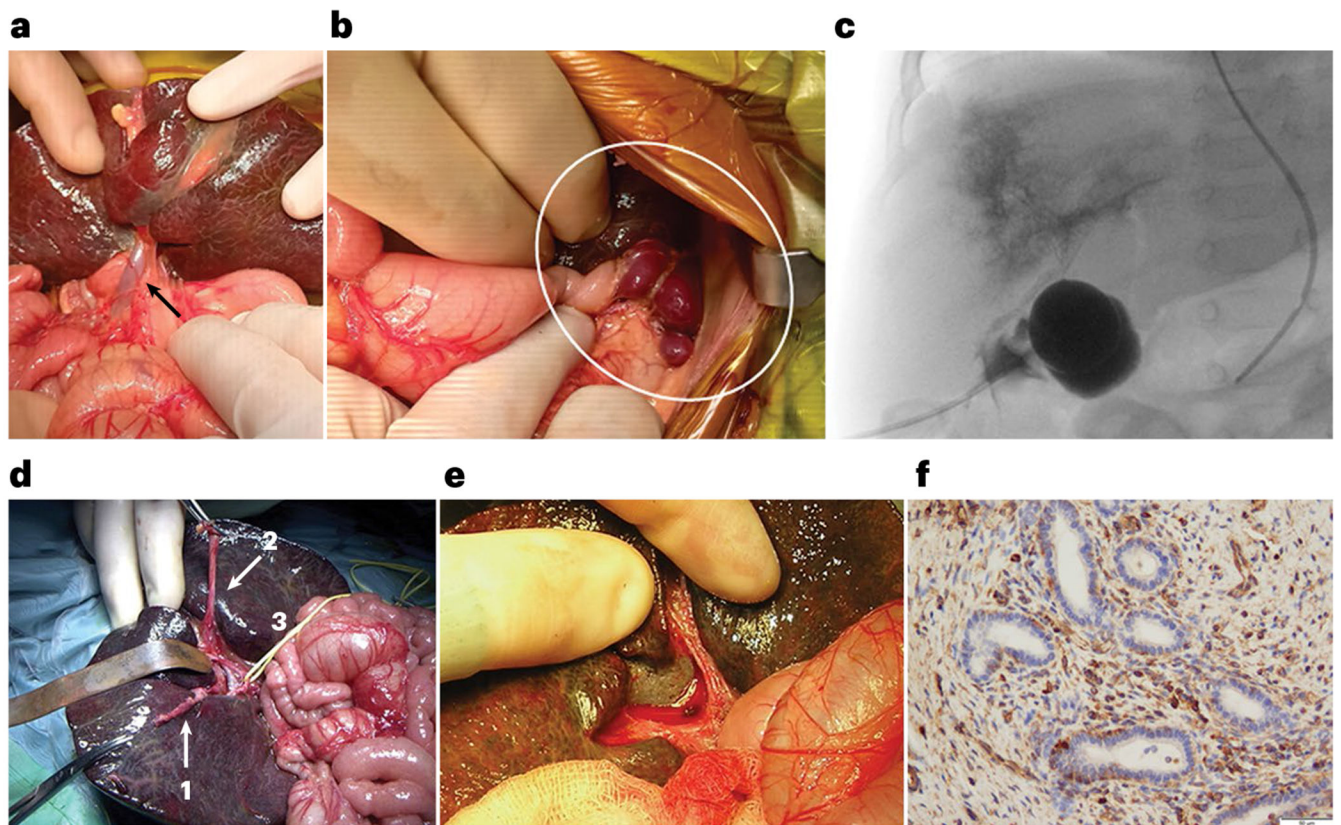
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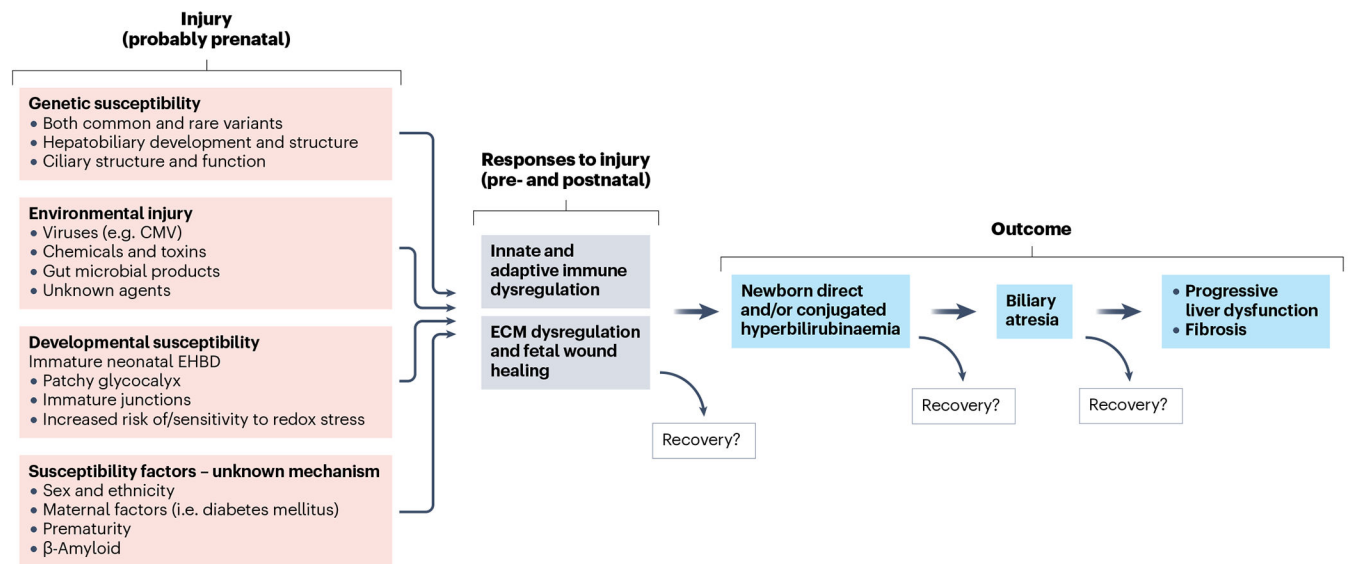
**Fig. 1 |. Global incidence of BA.**

Incidences of BA reported in nationwide or multicentre studies. Overall, higher incidences were reported for East Asian than for Western countries/regions.



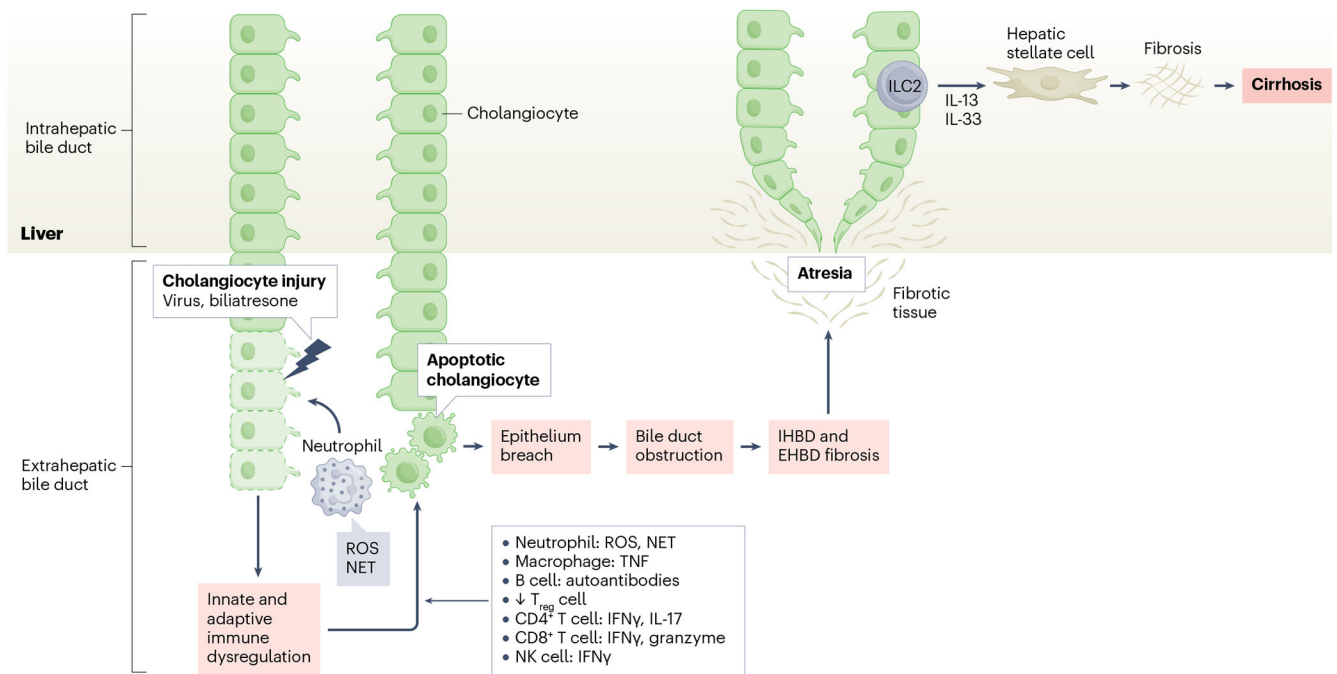
**Fig. 2 |. Aetiological heterogeneity of BA.**

Biliary atresia splenic malformation syndrome showing a pre-duodenal portal vein (part **a**, arrow) and left-sided polysplenia (part **b**, circled). Cystic biliary atresia with cholangiogram (part **c**) showing contrast retention in the cyst, absence of flow into the intestine, and abnormally fine, interconnecting etiolated intrahepatic bile ducts. Isolated biliary atresia (part **d**) showing (1) mobilized atrophic gallbladder, (2) umbilical vein and (3) sling around right hepatic artery. Cut surface of the porta hepatis before Roux loop reconstruction (part **e**). Photomicrograph of cut surface showing scattered biliary ductules within a fibroinflammatory stroma (vimentin-positive) (part **f**). Part **f** reproduced with permission from La Pergola E et al. (2021)<sup>321</sup>.

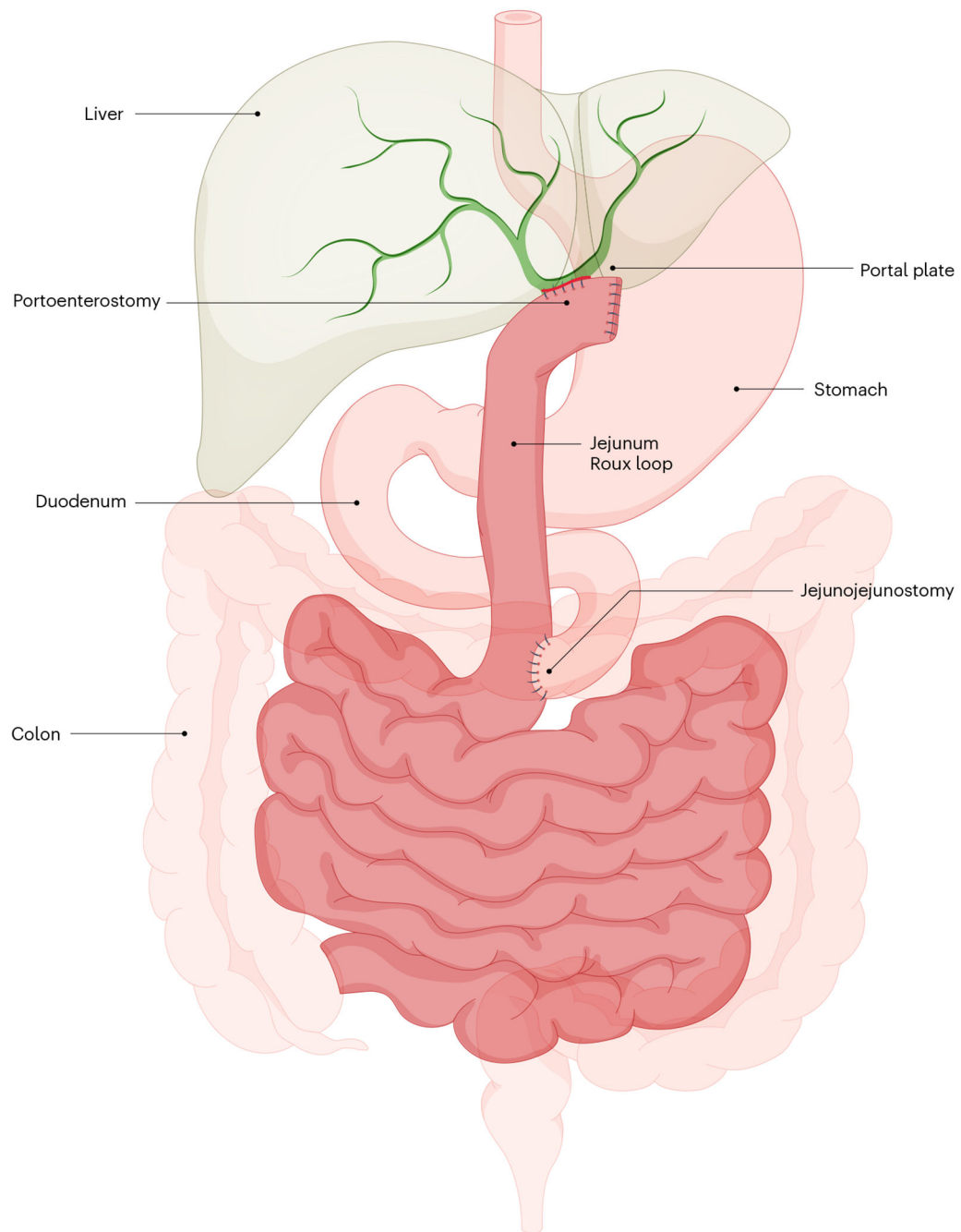


**Fig. 3 |. BA as a combination of injury and maladaptive response.**

Biliary atresia (BA) is viewed as the result of an injury, probably prenatal, and the response to that injury, probably occurring in both the prenatal and postnatal periods. Agents that may contribute to injury include genetic causes (most notably genetic alterations that lead to abnormal primary cilia), environmental agents and developmental susceptibilities of the immature extrahepatic bile duct (EHBD). Other factors, such as sex and ethnicity, can predispose to BA. Dysregulated immune and wound healing responses comprise the response to injury and facilitate progression of the disease. Clinically detectable outcomes of the disease progress to BA and, subsequently, liver dysfunction and liver fibrosis. The potential for recovery after injury by regenerative or reparative healing is unknown and remains speculative. CMV, cytomegalovirus; ECM, extracellular matrix.

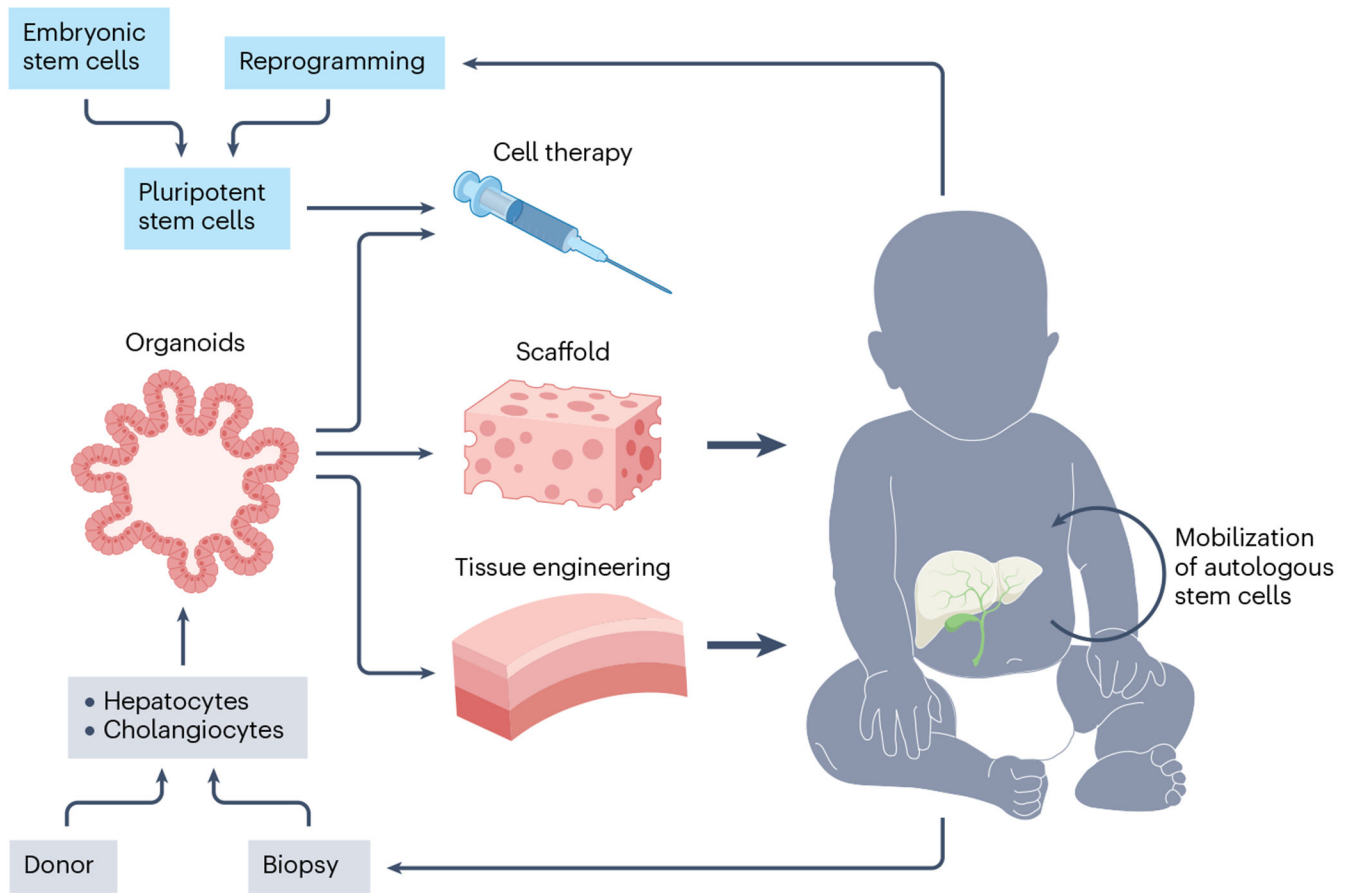


**Fig. 4 |. Hypothetical contribution of immune dysregulation to the pathogenesis of biliary atresia.** Injury by virus infection or toxins of altered cholangiocytes triggers a cascade of early innate (autoinflammatory) and subsequent adaptive (autoimmune) responses that proceed unchecked in the setting of regulatory T (T<sub>reg</sub>) cell deficits. The chronic inflammation further damages cholangiocytes and activates stellate cells, resulting in progressive biliary fibrosis. EHBD, extrahepatic bile duct; IHBD, intrahepatic bile duct; ILC2, type 2 innate lymphoid cell; NET, neutrophil extracellular traps; NK, natural killer; ROS, reactive oxygen species.



**Fig. 5 |. Kasai portoenterostomy.**

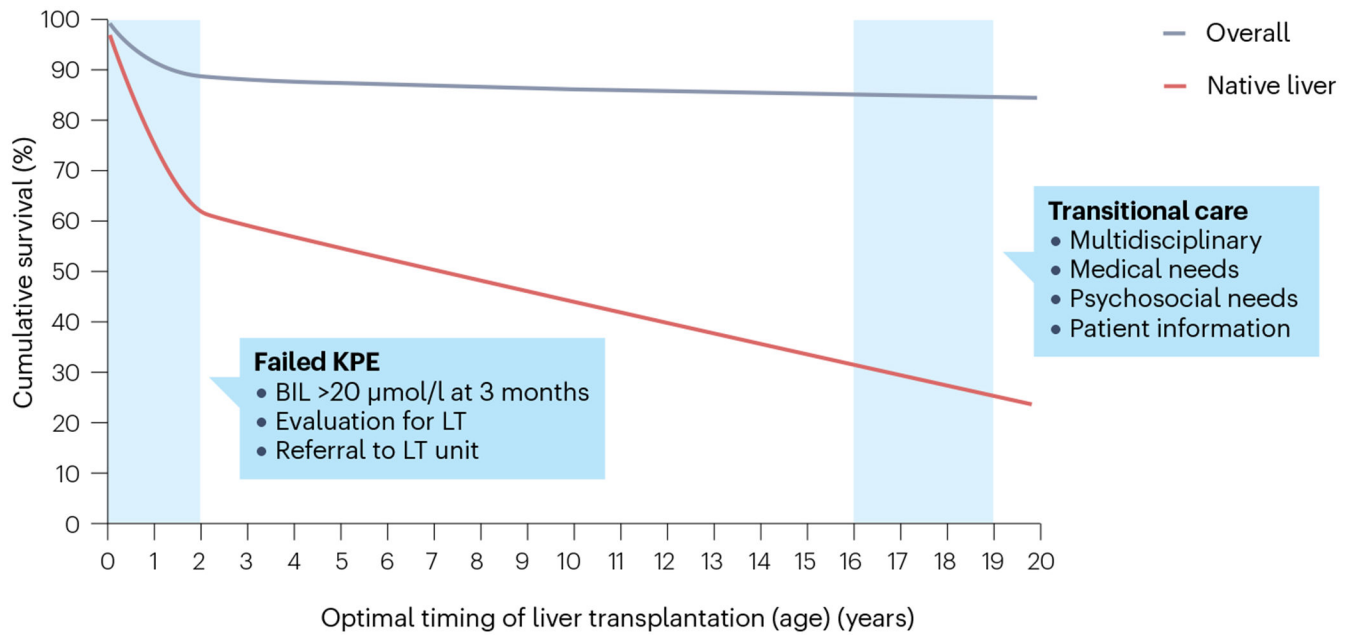
The mobilized jejunum is transected, and its proximal end is anastomosed to the transected portal plate (portoenterostomy) to enable bile drainage from the intrahepatic biliary system into the intestine via remnant patent biliary ductules. The proximal limb of the small intestine (downstream of the duodenum) is anastomosed to the 30–40 cm retrocolic Roux loop (jejunojunctionostomy).



**Fig. 6 |. Potential regenerative strategies in biliary atresia treatment.**

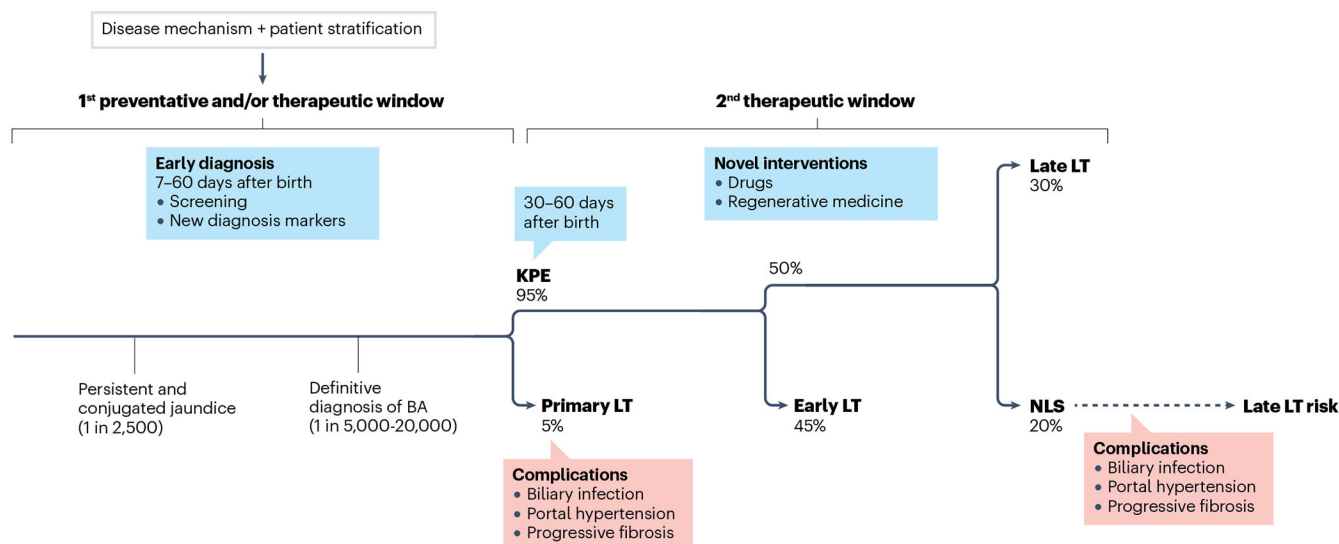
Pluripotent stem cells derived from reprogramming of primary somatic cells from patients or embryonic stem cells can be differentiated into various cell types useful for liver regeneration as cellular or tissue engineering therapies. Progenitors could also be used for mobilization of patients' cells which can help regeneration through modulation of inflammation and fibrosis.





**Fig. 7 |. Postoperative follow-up of native liver survivors.**

For optimal timing of liver transplantation, native liver survivors should be monitored and managed by multidisciplinary teams in specialist centres throughout their life. Evaluation for liver transplantation should occur promptly after failed Kasai portoenterostomy (KPE), as most patients develop rapidly progressing end-stage liver disease within 1–2 years. Despite successful KPE, the underlying cholangiopathy advances variably increasing the proportion of transplanted patients over time. Successful transition of young patients to adult health services requires special cooperative efforts between paediatric and adult specialists and comprehensive patient education. BIL, bilirubin; LT, liver transplantation.



**Fig. 8 |. Future research directions and effects on patient outlook.**

Discovery of disease mechanisms and identification of gene sets for patient stratification could lead to early diagnosis and new interventions at several important time points to improve the outcomes in patients with biliary atresia (BA). Currently, patients undergo Kasai portoenterostomy (KPE) (85–95%) at ~2 months of age, or primary liver transplantation (LT) (5–15%) because of late diagnosis. Improved screening with new biomarkers would enable diagnosis of emerging BA in early infancy (7–60 days), providing the first preventative and/or therapeutic window for earlier KPE and avoidance of primary LT. Introduction of neoadjuvant therapies targeting the injury phase of BA, such as the antioxidant *N*-acetylcysteine in the expanded window before KPE, might improve clearance of jaundice rate and reduce early (at <1 year of age) LT incidence (currently 17–40%). After KPE, patients experience complications leading to progression of liver pathology requiring late (at >1 year of age) LT (currently 30–67%). During this second therapeutic window, disease mechanism-inspired novel drugs, targeting the dysregulated immune-inflammation and ECM response phase could reduce recalcitrant cholangitis and fibrosis responsible for late LT, and increase long-term native liver survival (NLS) (currently only 40%). For the remaining patients currently requiring LT, regenerative medicine might reduce or replace the need for LT.

Table 1 |

BA-associated genetic loci

Loci	Odds ratio (95% confidence interval)	P value	Population	Mechanism	Ref.
Common variants					
<i>ADD3</i>	2.1 (1.4–3.2)	$6.9 \times 10^{-9}$	Chinese	Biliary morphogenesis	13,93
	1.7 (1.5–2.0)	$4.1 \times 10^{-11}$	Chinese		318
	1.7 (1.3–2.2)	$7.2 \times 10^{-5}$	Chinese		319
	1.7 (1.2–2.6)	$2.5 \times 10^{-3}$	Thai		320
	1.5 (1.1–1.9)	$2.0 \times 10^{-3}$	Caucasian		108
<i>EFEMP1</i>	1.5 (1.3–1.8)	$3.4 \times 10^{-8}$	Caucasian	ECM	94
<i>AFAP1</i>	1.4 (1.3–1.5)	$3.9 \times 10^{-8}$	Caucasian	Ciliogenesis	97
<i>TUSC3</i>	1.4 (1.3–1.5)	$1.3 \times 10^{-7}$	Caucasian	Ciliogenesis	97
<i>MAN1A2</i>	2.6 <sup>a</sup>	$7.0 \times 10^{-10}$	Caucasian	Ciliogenesis	95
<i>ARF6</i>	2.7 <sup>a</sup>	$4.2 \times 10^{-8}$	Caucasian	Biliary morphogenesis	96
Rare variants					
<i>GPC1</i> (deletions)	138,7 <sup>a,b</sup>	$4.4 \times 10^{-10}$	Caucasian	Biliary morphogenesis	77
<i>PKDIL1</i> (biallelic variants)	–	–	Caucasian	Ciliogenesis	101
Ciliary genes ( <i>PCNT</i> , <i>KIF3B</i> , <i>TTC17</i> and others)	2.5 (1.2–6.07)	0.034	Chinese	Ciliogenesis	78

BA, biliary atresia; ECM, extracellular matrix.

<sup>a</sup>No standard error was provided.

<sup>b</sup>Imprecise estimate due to a small number of cases.

Table 2 |

Outcomes of KPE

Country	Age at KPE (days)	Number of patients	Study period	Clearance of jaundice (%)	Native liver survival (%)	Ref.
<b>Asia</b>						
Taiwan	NA	102	2004–2010	61	57 (3 years)	213
Japan	NA	3,160	1989–2015	58	49 (10 years)	9
Taiwan	58–53 (mean)	540	1997–2011	NA	65 (no time point)	23
<b>Europe (centralized care)</b>						
England and Wales	51–48 (median)	867	1999–2019	61	47 (10 years)	238
Nordic consortium	64 (median)	158	2005–2016	64	53 (5 years)	7
<b>Europe (non-centralized care)</b>						
France	59 (median)	1,428	1997–2015	39	35 (10 years)	15
Netherlands	59 (median)	214	1987–2008	34	46 (4 years)	231
Switzerland	68 (median)	48	1994–2004	40	33 (4 years)	20
Germany	NA	173	2010–2014	24 <sup>a</sup>	29 (2 years)	232
<b>Other</b>						
Saudi Arabia	70 (median),	204 (diagnosed), 146 (KPE)	2000–2018	45 (KPE)	26 (10 years) 73 (10 years overall survival)	233
Canada	64 (median)	230	1992–2002	NA	39 (4 years)	234
New Zealand	63 (median)	95	2002–2014	NA	30 (5 years)	26
<b>North American consortia</b>						
USA (nine centres; BARC)	61 (mean)	104	1997–2000	40	52 (2 years)	10
USA (16 centres; PROBE)	58 (mean)	137	2004–2011	50	53 (2 years)	235
USA and Canada (16 centres; PROBE)	65 (median)	237 (136 underwent surgical drainage)	2004–2010	46	47 (2 years)	236
USA Western Surgical Consortium (nine centres)	64 (mean)	223	2009–2017	NA	41 (no time point)	237

KPE, Kasai portoenterostomy; NA, not available.

<sup>a</sup>Jaundice-free native liver.

**Table 3 |**

Adjuvant corticosteroid trials

Country	Number of patients	Regimen	Outcome	Ref.
<b>Randomized placebo-controlled trials</b>				
UK	71	Oral 2 mg/kg/day (low-dose) prednisolone 4 weeks	↓Bilirubin at 3 months ( $P=0.06$ ) Improved with lower age at KPE	258
North America	140	IV or oral 4 mg/kg/day prednisolone 9 weeks	CoJ 59 vs 49 at 6 months ( $P=0.43$ ) Improved with lower age at KPE No difference in transplant-free survival	259
<b>Prospective open-label trials</b>				
UK	153	Oral 2 mg/kg/day ( $n=18$ ) prednisolone Oral 5 mg/kg/day ( $n=44$ ) prednisolone	All infants <70 days of age at KPE. CoJ (no corticosteroids) 52% vs (corticosteroids) 66% ( $P=0.037$ ) at 6 months	256
China	200	IV or oral 4 mg/kg/day prednisolone 10–12 weeks	↑CoJ at 6 months 54% vs 31% ( $P<0.001$ ) ↑NLS (12 months) 66% vs 50% ( $P=0.02$ )	

CoJ, clearance of jaundice; IV, intravenous; KPE, Kasai portoenterostomy; NLS, native liver survival.

Main complications and follow-up of chronic liver disease in paediatric and adult long-term native liver survivors

Table 4 |

Complication	Prevalence in children (%)	Prevalence in adults (%)	Surveillance measures	Ref.
Cirrhosis	40–50	80–90	Serum liver biochemistry, APRI, abdominal ultrasonography, liver stiffness, liver biopsy	292–294
Portal hypertension	60–70	50–70	Spleen size, platelets, liver stiffness, echo cardiography	282,292–294,298,299,301
Oesophageal varices	50–60	20–30	Endoscopic primary/secondary prophylaxis	298,299,302,303
Bleeding	10–15	20–30		
Cholangitis	60–70	50–60	Antibiotic prophylaxis, MRCP and scintigraphy if recurrent	254,293,294,300,304,306,307
Liver malignancy	<1	>1	6-monthly ultrasonography and serum AFP	295,296,302,304
Impaired growth	<5	0–20%	Anthropometric and biometric surveillance	293,300,304
Bone fractures, osteoporosis, osteopenia	10–20	?	Serum vitamin D levels, bone mineral density surveillance	300,309
Neurodevelopmental and/or educational shortcomings	0–50	0–30	Surveillance for neurocognitive and motor skills	293,301,310–313,316
Impaired quality of life	0–30	0–30	Psychosocial assessment	293,301,314–316

AFP, α-fetoprotein; APRI, aspartate aminotransferase to platelets ratio index; MRCP, magnetic resonance cholangiopancreatography.