Genetic Contributors and Modifiers of Biliary Atresia

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Key Words

Exome · Polymorphisms · Laterality

Abstract

To date, the etiology and pathogenic underpinning of the progression of the most prevalent serious neonatal liver disease, biliary atresia, remains elusive. This disease presents as an aggressive form of neonatal cholestasis characterized by the destruction and obliteration of the extrahepatic bile ducts within the first few weeks of life and a rapid progression of biliary fibrosis, likely due to unremitting cholestasis and retention of biliary constituents including bile acids. In ~5% of patients, biliary atresia is associated with laterality features, suggesting a genetic underpinning to a disease that begins soon after birth. However, biliary atresia does not occur within families and twins are discordant, indicating an absence of strict mendelian inheritance. Despite this, genes related to bile duct dysmorphogenesis/ciliopathies overlapping with features of biliary atresia in both humans and non-human model systems have been proposed. Taken together, strict genetic etiologies leading to a common pathway of a neonatal cholangiopathy resulting in biliary atresia remain elusive. Contributions from fibrogenesis- and inflammation-based studies suggest that early engagement of these pathways contributes to disease progression, but a recent dou-

ble-blind study did not suggest any benefit from early use of corticosteroids. However, there are genetic contributions to the adaptation and response to cholangiopathies and cholestasis that may be present in certain populations that likely impact upon the response to hepatoportoenterostomy and subsequent biliary tract function. Studies utilizing next generation sequencing technologies (e.g., exome analysis) are ongoing in several laboratories around the world; they are expected to provide insights into genetic contributions to biliary atresia outcomes. Altogether, combinations of exome sequencing and large population studies are expected to reveal causative and modifying genes relevant to patients with biliary atresia as a means to provide therapeutic targets and potential opportunities for genetic screening.

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Introduction

Biliary atresia was first comprehensively described in an extraordinary compilation of cases in 1892 by the Scottish surgeon John Thomson [1]. Dr. Thomson reported varying appearances of the extrahepatic biliary tree in these infants: some patients' biliary tracts had obstructions along with cystic components, while other patients' extrahepatic biliary tracts were completely atretic without

a discernibly patent lumen of the extrahepatic biliary tree through to the duodenum. Overall, there was clearly a variable presentation of this disease between patients, with a common feature being complete obstruction of bile flow.

Advances in treatment for this uniformly fatal neonatal liver disease were limited until the groundbreaking development of the hepatoportoenterostomy (HPE) by Dr. Morio Kasai, a Japanese pediatric surgeon, in 1959 [2]. Although it took time for surgeons outside of Japan to adopt this technique [3–5], it soon evolved to become the standard of care for biliary atresia and improved mortality among infants who received an early diagnosis prior to the development of irreversible cirrhosis or the life-threatening sequelae of cholestatic liver disease. Despite this significant surgical intervention, only ~50% of infants who undergo Kasai HPE prior to the development of cirrhosis are able to avoid liver transplantation by the age of 2, with approximately 70% of all patients eventually requiring liver transplant by adulthood (see below) [1, 6].

A critical area of investigation into biliary atresia concerns the cause, or more likely, the multiplicity of causes responsible for the aberrant development of the non-patent biliary tree, which may inform the utility of Kasai HPE and explain the marked distinction in outcomes of infants undergoing the procedure. This paper is focused upon highlighting the current state of discovery and knowledge regarding genes that may confer susceptibility to biliary atresia or modulate outcomes among infants after HPE. It is likely that a deeper understanding of the genetic profile of infants with biliary atresia will provide clinically substantive insights into the etiology and severity of the disease, and enable the development of more precise diagnostic methods and rational therapeutic targets to improve outcomes [2, 7, 8].

Biliary Atresia, Epidemiology and Salient Clinical Concepts

Among all causes of neonatal cholestasis, biliary atresia is the most prevalent, amounting to approximately 40% of all cases of neonatal cholestasis [3–5, 9]. The overall incidence of neonatal cholestasis is ~1:2,500 births [10], with varying incidences of biliary atresia reported around the world. Biliary atresia appears to be more common in Southeast Asia, with an incidence around 1:5,000 live births, compared to North America and Western Europe, which have an incidence of 1:10,000–15,000 births

[2, 11]. The reasons for the global discrepancy in the incidence of biliary atresia are unknown.

From a clinical standpoint, it is often challenging to differentiate a child with biliary atresia from one that has physiological jaundice or another form of cholestasis, thereby hampering prompt diagnosis. One of the few hallmarks that can be utilized for diagnosis or even for public health intervention includes the lack of pigment in the stool, arising from a frank impairment of bile flow. The clinical presence of acholic stools is sometimes inapparent to the (often new) parents of these children as well as to caregivers. However, once caregivers are appropriately educated, reporting of stool color can serve as a tremendously useful screening tool for the identification of biliary atresia in areas of high prevalence. The successful use of a stool color card provided to parents of all infants born in Taiwan led to the capture of nearly every child with biliary atresia within the second year of its implementation [3–5, 12]. This simple population-wide screening tool is an exemplary intervention with demonstrable efficacy in promoting earlier detection and referral for Kasai HPE. At present, the analysis of dried blood spots derived from the standard newborn screening card has not proven useful for biliary atresia, primarily because of the lack of a diagnostic metabolite. However, recent data suggests that measurement of direct bilirubin levels within the first 2 days of life could be used as a screening tool [6, 7, 13].

Clinically, infants with biliary atresia develop firm hepatomegaly and splenomegaly as young as 4–8 weeks of age, and laboratory tests usually reveal elevations of ALT, AST, and total and direct bilirubin, while GGT and alkaline phosphatase levels often rise to levels >1,000 U/dl [7, 8, 14]. Ultrasonographic imaging may reveal an absent or atretic gallbladder, as well as fibrotic regions in the porta hepatis (“triangular cord”) and, intriguingly, increased subcapsular flow [9, 14–17]. The finding of increased subcapsular flow may perhaps provide a readily utilized screening technique to help differentiate biliary atresia from other forms of neonatal cholestasis [14]. Notably, infants with biliary atresia exhibit a spectrum of presentations, including some with the presence of a gallbladder, a normal serum GGT, and pigmented stools early in life, which may obscure the diagnosis. To confirm the presence of biliary tract obstruction, the utility of a percutaneous liver biopsy has substantial value, which can lead to prompt histologic recognition of peribiliary fibrosis and bile duct plugs, or conversely, a disorder unrelated to biliary atresia, thus abrogating the need for a cholangiogram or Kasai HPE. Once the possibility of biliary atresia is entertained, a surgical referral is warranted to perform an

intraoperative cholangiogram, and if patency of the biliary tree is not visualized, to proceed to Kasai HPE.

It is well-established that delay in diagnosis of biliary atresia negatively affects the outcome of Kasai HPE, with worse outcomes reported by some studies if the infant is >45 days of age [11, 18, 19]. Often, children present after a routine pediatric visit at ~60 days of age, at which point some patients have already progressed to cirrhosis and end-stage liver disease, precluding any benefit from the intervention. Children with cystic presentations of biliary atresia may have a better response to Kasai HPE; conversely, children with associated congenital malformations and laterality features typically have a worse prognosis (see below) [20–23]. The lack of a uniform screening tool, coupled with the generally normal appearance of the persistently jaundiced infant, often leads to these marked delays in diagnosis, a central gap in optimizing the care of these infants.

In general, outcomes after Kasai HPE are divided among patients who achieve restoration of bile flow and those who do not. Among those with restored biliary drainage, as evidenced by a total serum bilirubin 3 months after Kasai HPE <2 mg/dl (or <34 micromolar), the majority of patients are able to postpone or avoid liver transplantation, while children whose jaundice does not resolve typically develop stigmata of cirrhosis and require evaluation for liver transplantation in the first year of life [6, 11, 19, 24–27]. Unfortunately, there is no medical therapy that has been shown to alter the outcome of the Kasai HPE, including a recent double-blind study evaluating a potential role for corticosteroids [28]. Overall, the stratification of patients into one group or the other is poorly understood.

Data from a large, population-wide cohort study suggests that if a child does not require liver transplantation by the age of 5, over 70% are likely to survive with their native liver into adulthood [6]. Despite recognition of important prognostic factors, such as the anatomy of the biliary remnant, presence of laterality features, age at HPE, and experience of the surgical center [6, 21], the outcome for an individual patient is largely unpredictable, which suggests that other factors may be responsible for determining disease progression.

Nongenetic Etiologic Theories of Biliary Atresia

The putative causes of biliary atresia can be separated into a variety of etiologic categories, including toxin-related, viral, immunological, maternal fetal factors, and ge-

netic [29–33]. From Australia, there are intriguing data related to an episodic form of newborn lamb cholangiopathy associated with a change in diet due to harsh weather conditions, whereby ingestion of a plant-derived toxin from the species of *Dysphania* damages the developing biliary tree [34]. However, an exogenous toxin has yet to be associated with the disease in humans. Investigations into a viral etiology have yielded mixed results, implicating various strains of rotavirus, reovirus, and cytomegalovirus, among others, but there have been inconsistencies in the detection of viruses in patient samples. Preclinical and animal models have identified intriguing contributions to disease progression from various immunological mediators, viruses, and immune cell types, several of which have been demonstrated in human studies [11, 21, 30, 31, 33, 35–37]. Currently, there is a paucity of support for a single viral or immunological insult in the etiopathogenesis of biliary atresia, though these factors certainly may play modifying roles for disease progression.

Epidemiological data further diminishes support for a viral etiology for this disease since biliary atresia does not occur outside of the immediate newborn period and the incidence is not epidemic among humans, whereas an immune-mediated or autoimmune process is less plausible due to the lack of recurrence after transplantation. These observations establish the need to consider alternative modalities of disease causation.

Proposed Genetic Etiologies of Biliary Atresia

Among the more salient aspects of biliary atresia is that approximately 10% of subjects present with anatomical features known as the biliary atresia splenic malformation syndrome [18, 38, 39]. These patients have anomalies that are usually associated with laterality malformations either above or below the diaphragm in the context of biliary atresia. Typical anomalies above the diaphragm include tetralogy of Fallot, dextrocardia, anomalies of the pulmonary or cardiac vasculature, and atrial and ventricular septal defects. Below the diaphragm, in addition to biliary tract anomalies, patients may present with midline or left-sided liver, polysplenia or asplenia, and interrupted inferior vena cava [40]. The laterality defects exhibited in biliary atresia splenic malformation syndrome are phenotypically similar to those observed in the ciliopathies, a heterogeneous group of diseases caused by functional and structural defects in genes that encode cilia proteins [41–48]. Cilia are evolutionarily conserved, aid in establishing the left-right axis in vertebrates, and are present on many

cell types, including the apical surface of cholangiocytes, where cilium function is integral to bile flow and bile ductular formation [49]. Moreover, diseases such as autosomal recessive polycystic kidney disease (*PKHD1* gene) are associated with biliary cystic malformations that are grouped together as Caroli's disease, and expression of the *PKHD1* gene product, polycystin, was found to be drastically reduced in the livers of biliary atresia patients with and without attendant renal cysts, in contrast to normal liver [25, 46]. Moreover, investigations into rhesus rotavirus murine models of biliary atresia have found substantial loss of primary cilia from extrahepatic cholangiocytes, which was corroborated by the finding of decreased ciliation in extrahepatic ducts in children with nonsyndromic biliary atresia, although cilia appeared normal in neighboring peribiliary glands [50]. Ultimately, determining whether abnormalities in the development or function of cholangiocyte cilia are the basis for biliary atresia requires more extensive genetic and immunohistochemical analyses. Finally, the high incidence of biliary atresia in areas of Southeast Asia as well as Polynesia is suggestive of an inherited predisposition, although a local dietary or viral factor cannot be excluded [51].

Observations that argue against a Mendelian paradigm of inheritance of biliary atresia include the lack of familial penetrance, although there are rare cases of familial biliary atresia [52, 53], and discordant presentation of biliary atresia among twins, including monozygotic twins [54–57]. However, a growing canon of developmental diseases display unconventional patterns of inheritance, including Bardet-Biedl syndrome, a clinically heterogeneous ciliopathy marked by oligogenic inheritance, whereby mutations in multiple genes interact to generate the phenotype. A similar phenomenon related to gene-gene interactions, or epistasis, may account for the diversity of presentations in biliary atresia. Nonmendelian inheritance is also observed in Alagille syndrome, which is the result of a haploid insufficiency of *JAG1* or its receptor *NOTCH2*, which has a varied clinical presentation within families as well as between patients [58, 59]. Considerations for epigenetic modification (e.g. methylation) as a cause of biliary atresia requires further support and may necessitate direct studies of hepatocyte or cholangiocyte DNA in patients.

An intriguing body of work suggests that there may be a component of the maternal environment or microchimerism that could contribute to the phenotypic heterogeneity and nonclassical genetic inheritance of biliary atresia [60, 61].

More recent inquiries into the genetic underpinning of biliary atresia have taken the form of genome-wide asso-

ciation studies that investigate the association of individual, common genetic variants with disease, known as single nucleotide polymorphisms (SNPs). A genome-wide association study conducted in the Han Chinese population identified a potential susceptibility region for biliary atresia between the genes *ADD3* and *XPNPEP1*, chromosome10q25.1, and the association of one SNP with disease was replicated in independent Chinese and Thai samples [62, 63]. More in-depth sequencing of a Han Chinese sample identified that a 5-SNP risk haplotype was associated with biliary atresia and the genotype correlated with reduced levels of *ADD3* expression [64]. An attempt to replicate the association in a Caucasian cohort discovered a stronger signal in the first intron of *ADD3* following SNP imputation, although the exact genotype at this SNP was not predictive of the degree of *ADD3* expression. The *ADD3* gene encodes adducin- γ , a membrane skeletal protein that functions in regulating cell-cell adhesions in various epithelia and is more highly expressed in fetal liver relative to adult liver [65].

Another potential causative gene, glypican 1 (*GPC1*), has garnered attention through genome-wide association studies of DNA copy number variation that identified a potential region of susceptibility to biliary atresia on chromosome 2q37.3, which was replicated in an independent cohort of patients demonstrating heterozygous deletion of *GPC1* as the sole gene in this region [66]. Glypican family members are involved in various signaling and developmental pathways in hepatocytes and cholangiocytes, though *GPC1* had not been previously associated with liver development or function. In addition to reporting abnormal expression of *GPC1* by cholangiocytes in patients with biliary atresia, the authors knocked down the *GPC1* analog in zebrafish, which led to a markedly disordered biliary ductular network [66].

Other genes involved in bile duct dysmorphogenesis and cholestasis, the immune response, vasculogenesis and left-right patterning have been proposed to contribute to biliary atresia on the basis of clinical investigations and nonhuman model systems, including *CFTR*, *JAG1*, *ZIC3*, *CFC1*, *INV*, *MIF*, *VEGF*, *IFN- γ* , and *SOX17* [46, 66–73].

At this point in time, the application of genome-wide studies has led to the discoveries of two credible candidate genes that may underlie the development of biliary atresia: *ADD3* and *GPC1*. We anticipate that larger genetic studies across a variety of ethnic and racial strata, especially with the advent of next-generation sequencing technologies, will uncover additional genes that may play a role in the etiology of biliary atresia.

The genetic profile of an individual can greatly influence the clinical severity of a disease, and despite the phenotypic heterogeneity of biliary atresia, to date, there are no known genetic modifiers of outcomes after the HPE procedure. Several authors have suggested that genes with biological relevance to biliary function may be involved, including A1AT, JAG1, and CFTR, but these remain to be explored in detail [33, 46, 66, 74, 75].

A substantive body of evidence has accrued over the last 10–15 years detailing the remarkable capacity of the liver to handle cholestatic loads, engaging pathways from the membrane to the cytoplasm to alter nuclear transcriptional programs, particularly through regulation of the members of the nuclear receptor family, centered upon the bile acid receptor FXR [76, 77]. The liver can adapt remarkably well to bile acid retention, but this response is inadequate in patients who develop cholestatic liver disease, suggesting that compensatory mechanisms may fail in predisposed individuals when exposed to nonphysiologic stress, such as biliary atresia. Given the patient-to-patient variability in response to cholestatic and fibrotic liver diseases, it is imperative to consider the role of genetic variance in shaping the adaptive response to cholestasis.

Since its first descriptions, the underlying cause(s) and outcome contributor(s) to biliary atresia, the most common serious pediatric liver disease of infancy, remain largely unknown. Recent work indicates that this knowledge gap is likely to be shortened due to large-scale clinical consortia, application of modern whole exome and whole genome genetic screenings, and novel immunophenotyping. As these unknowns become conscribed to historical rather than active clinical issues, we expect to be able to understand bile duct morphogenesis, developmental cholangiopathies, roles for ciliary function, adaptations to bile acid retention, and means to effect cholestasis. Along with identification of genes and small molecules that play central roles in pathogenesis and adaptation to cholestasis in biliary atresia and other cholestatic disorders, an integrated systems biology approach may generate novel screening tools for disease identification and therapeutic targets. The dim past of our understanding of biliary atresia is beginning to appear brighter.

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References

- Thomson J: On Congenital Obliteration of the Bile-Ducts. *Edinburgh Medical Journal* 1892;38:565.
- Kasai M, Suzuki S: A new operation for 'non-correctable' biliary atresia: hepatic portoenterostomy. *Shujutsu* 1959;13:733–739.
- Lilly J: The Japanese operation for biliary atresia: remedy or mischief? *Pediatrics* 1975;55:12–19.
- Koop C: Biliary atresia and the Kasai operation. *Pediatrics* 1975;55:9–11.
- Gellis S: Biliary atresia. *Pediatrics* 1975;55:8–9.
- Chardot C, Buet C, Serinet MO, Golmard JL, Lachaux A, Roquelaure B, et al: Improving outcomes of biliary atresia: French national series 1986–2009. *J Hepatol* 2013;58:1209–1217.
- Harpavat S, Finegold MJ, Karpen SJ: Patients with biliary atresia have elevated direct/conjugated bilirubin levels shortly after birth. *Pediatrics* 2011;128:e1428–e1433.
- Neimark E, Leleiko NS: Early detection of biliary atresia raises questions about etiology and screening. *Pediatrics* 2011;128:e1598–e1599.
- Hoerning A, Raub S, Dechêne A, Brosch MN, Kathemann S, Hoyer PF, et al: Diversity of disorders causing neonatal cholestasis – the experience of a tertiary pediatric center in Germany. *Front Pediatr* 2014;2:65.
- Arya G, Balistreri W: Pediatric liver disease in the United States: epidemiology and impact. *J Gastroenterol Hepatol* 2002 1;17:521–525.
- Wildhaber BE: Biliary atresia: 50 years after the first kasai. *ISRN Surg* 2012;2012:132089.
- Hsiao C, Chang M, Chen H, Lee H, Wu T, Lin C, et al: Universal screening for biliary atresia using an infant stool color card in Taiwan. *Hepatology* 2008;47:1233–1240.
- Powell JE, Keffler S, Kelly DA, Green A: Population screening for neonatal liver disease: potential for a community-based programme. *J Med Screen* 2003;10:112–116.
- El-Guindi MA, Sira MM, Sira AM, Salem TA, El-Abd OL, Konsowa HA, et al: Design and validation of a diagnostic score for biliary atresia. *J Hepatol* 2014;61:116–123.
- El-Guindi MA, Sira MM, Konsowa HA, El-Abd OL, Salem TA: Value of hepatic subcapsular flow by color Doppler ultrasonography in the diagnosis of biliary atresia. *J Gastroenterol Hepatol* 2013;28:867–872.
- Lee M, Kim M, Lee M, Yoon C, Han S, Oh J, et al: Biliary atresia: color doppler US findings in neonates and infants. *Radiology* 2009;252:282–289.
- Takamizawa S, Zaima A, Muraji T, Kanegawa K, Akasaka Y, Satoh S, et al: Can biliary atresia be diagnosed by ultrasonography alone? *J Pediatr Surg* 2007;42:2093–2096.
- Davenport M, Caponcelli E, Livesey E, Hadzić N, Howard E: Surgical outcome in biliary atresia: etiology affects the influence of age at surgery. *Ann Surg* 2008;247:694–698.

- 19 Serinet MO, Wildhaber BE, Broué P, Lachaux A, Sarles J, Jacquemin E, et al: Impact of age at Kasai operation on its results in late childhood and adolescence: a rational basis for biliary atresia screening. *Pediatrics* 2009;123:1280–1286.
- 20 De Matos V, Erlichman J, Russo PA, Haber BA: Does 'cystic' biliary atresia represent a distinct clinical and etiological subgroup? A series of three cases. *Pediatr Dev Pathol* 2005;8:725–731.
- 21 Davenport M: Biliary atresia: clinical aspects. *Seminars in pediatric surgery* 2012;21:175–184.
- 22 Arora A, Patidar Y, Khanna R, Alam S, Rastogi A, Negi SS: Cystic biliary atresia: confounding and intriguing. *J Pediatr* 2012;161:562.
- 23 Suzuki T, Hashimoto T, Hussein MH, Hara F, Hibi M, Kato T: Biliary atresia type I cyst and choledochal cyst: can we differentiate or not? *J Hepatobiliary Pancreat Sci* 2013; DOI: 10.1007/s00534-013-0605-3
- 24 Serinet MO, Broué P, Jacquemin E, Lachaux A, Sarles J, Gottrand F, et al: Management of patients with biliary atresia in France: results of a decentralized policy 1986–2002. *Hepatology* 2006;44:75–84.
- 25 Hartley JL, Davenport M, Kelly DA: Biliary atresia. *Lancet* 2009;374:1704–1713.
- 26 de Vries W, De Langen ZJ, Aronson DC, Hulscher JBF, Peeters PMJG, Jansen-Kalma P, et al: Mortality of biliary atresia in children not undergoing liver transplantation in the Netherlands. *Pediatr Transplant* 2011;15:176–183.
- 27 de Vries W, Homan-van der Veen J, Hulscher JBF, Hoekstra-Webers JEHM, Houwen RHJ, Verkade HJ, et al: Twenty-year transplant-free survival rate among patients with biliary atresia. *Clin Gastroenterol Hepatol* 2011;9:1086–1091.
- 28 Bezerra JA, Spino C, Magee JC, Shneider BL, Rosenthal P, Wang KS, et al: Use of corticosteroids after hepatoporeostomy for bile drainage in infants with biliary atresia: the START randomized clinical trial. *JAMA* 2014;311:1750–1759.
- 29 Balistreri WF, Grand R, Hoofnagle JH, Suchy FJ, Ryckman FC, Perlmutter DH, et al: Biliary atresia: current concepts and research directions. Summary of a symposium. *Hepatology* 1996;23:1682–1692.
- 30 Mack CL, Sokol RJ: Unraveling the pathogenesis and etiology of biliary atresia. *Pediatr Res* 2005;57:87R–94R.
- 31 Petersen C: Biliary atresia: the animal models. *Semin Pediatr Surg* 2012;21:185–191.
- 32 Nakamura K, Tanoue A: Etiology of biliary atresia as a developmental anomaly: recent advances. *J Hepatobiliary Pancreat Sci* 2013;20:459–464.
- 33 Petersen C, Davenport M: Aetiology of biliary atresia: what is actually known? *Orphanet J Rare Dis* 2013;8:128.
- 34 Harper P, Plant JW, Unger DB: Congenital biliary atresia and jaundice in lambs and calves. *Aust Vet J* 1990;67:18–22.
- 35 Bezerra J: Potential etiologies of biliary atresia. *Pediatr Transplant* 2005;9:646–651.
- 36 Mack C: The pathogenesis of biliary atresia: evidence for a virus-induced autoimmune disease. *Semin Liver Dis* 2007;27:233–242.
- 37 Feldman AG, Mack CL: Biliary atresia: cellular dynamics and immune dysregulation. *Semin Pediatr Surg* 2012;21:192–200.
- 38 Davenport M, Tizzard SA, Underhill J, Mieli-Vergani G, Portmann B, Hadzić N: The biliary atresia splenic malformation syndrome: a 28-year single-center retrospective study. *J Pediatr* 2006;149:393–400.
- 39 Guttman OR, Roberts EA, Schreiber RA, Barker CC, Ng VL; Canadian Pediatric Hepatology Research Group: Biliary atresia with associated structural malformations in Canadian infants. *Liver Int* 2011;31:1485–1493.
- 40 Schwarz KB, Haber BH, Rosenthal P, Mack CL, Moore J, Bove K, et al: Extrahepatic anomalies in infants with biliary atresia: results of a large prospective North American multicenter study. *Hepatology* 2013;58:1724–1731.
- 41 Teichberg S, Markowitz J, Silverberg M, Aiges H, Schneider K, Kahn E, et al: Abnormal cilia in a child with the polysplenia syndrome and extrahepatic biliary atresia. *J Pediatr* 1982;100:399–401.
- 42 Gershoni-Baruch R, Gottfried E, Pery M, Sahlin A, Etzioni A: Immobile cilia syndrome including polysplenia, situs inversus, and extrahepatic biliary atresia. *Am J Med Genet* 1989;33:390–393.
- 43 Chu AS, Russo PA, Wells RG: Cholangiocyte cilia are abnormal in syndromic and non-syndromic biliary atresia. *Mod Pathol* 2012;25:751–757.
- 44 Boon M, Smits A, Cuppens H, Jaspers M, Proesmans M, Dupont LJ, et al: Primary ciliary dyskinesia: critical evaluation of clinical symptoms and diagnosis in patients with normal and abnormal ultrastructure. *Orphanet J Rare Dis* 2014;9:11.
- 45 Akizu N, Silhavy JL, Rosti RO, Scott E, Fenstermaker AG, Schroth J, et al: Mutations in CSPP1 lead to classical Joubert syndrome. *Am J Hum Genet* 2014;94:80–86.
- 46 Rock N, McLin V: Liver involvement in children with ciliopathies. *Clin Res Hepatol Gastroenterol* 2014;38:407–414.
- 47 Horani A, Brody SL, Ferkol TW: Picking up speed: advances in the genetics of primary ciliary dyskinesia. *Pediatr Res* 2014;75:158–164.
- 48 Kennedy MP, Omran H, Leigh MW, Dell S, Morgan L, Molina PL, et al: Congenital heart disease and other heterotaxic defects in a large cohort of patients with primary ciliary dyskinesia. *Circulation* 2007;115:2814–2821.
- 49 Yuan S, Sun Z: Expanding horizons: ciliary proteins reach beyond cilia. *Annu Rev Genet* 2013;47:353–376.
- 50 Karjoo S, Hand NJ, Loarca L, Russo PA, Friedman JR, Wells RG: Extrahepatic cholangiocyte cilia are abnormal in biliary atresia. *J Pediatr Gastroenterol Nutr* 2013;57:96–101.
- 51 Chardot C, Carton M, Spire-Bendelac N, Le Pommelet C, Golmard J, Auvert B: Epidemiology of biliary atresia in France: a national study 1986–96. *J Hepatol* 1999;31:1006–1013.
- 52 Lachaux A, Descos B, Plauchu H, Wright C, Louis D, Raveau J, et al: Familial extrahepatic biliary atresia. *J Pediatr Gastroenterol Nutr* 1988;7:280–283.
- 53 Gunasekaran TS, Hassall EG, Steinbrecher UP, Yong SL: Recurrence of extrahepatic biliary atresia in two half sibs. *Am J Med Genet* 1992;43:592–594.
- 54 Strickland AD, Shannon K, Coln CD: Biliary atresia in two sets of twins. *J Pediatr* 1985;107:418–420.
- 55 Hyams JS, Glaser JH, Leichtner AM, Morecki R: Discordance for biliary atresia in two sets of monozygotic twins. *J Pediatr* 1985;107:420–422.
- 56 Moore TC, Hyman PE: Extrahepatic biliary atresia in one human leukocyte antigen identical twin. *Pediatrics* 1985;76:604–605.
- 57 Fallon SC, Chang S, Finegold MJ, Karpen SJ, Brandt ML: Discordant presentation of biliary atresia in premature monozygotic twins. *J Pediatr Gastroenterol Nutr* 2013;57:e22–e23.
- 58 Leonard LD, Chao G, Baker A, Loomes K, Spinner NB: Clinical utility gene card for: Alagille Syndrome (ALGS). *Eur J Hum Genet* 2014;22(3).
- 59 Loomes KM, Russo P, Ryan M, Nelson A, Underkoffler L, Glover C, et al: Bile duct proliferation in liver-specific Jag1 conditional knockout mice: effects of gene dosage. *Hepatology* 2007;45:323–330.
- 60 Hayashida M, Nishimoto Y, Matsuura T, Takahashi Y, Kohashi K, Souzaki R, et al: The evidence of maternal microchimerism in biliary atresia using fluorescent in situ hybridization. *J Pediatr Surg* 2007;42:2097–2101.
- 61 Kobayashi H, Tamatani T, Tamura T, Kusafuka J, Yamataka A, Lane G, et al: Maternal microchimerism in biliary atresia. *J Pediatr Surg* 2007;42:987–991; discussion 991.
- 62 Kaewkiattiyot S, Honsawek S, Vejchapipat P, Chongsrisawat V, Poovorawan Y: Association of X-prolyl aminopeptidase 1 rs17095355 polymorphism with biliary atresia in Thai children. *Hepatol Res* 2011;41:1249–1252.
- 63 Zeng S, Sun P, Chen Z, Mao J, Wang J, Wang B, et al: Association between Single Nucleotide Polymorphisms in the ADD3 Gene and Susceptibility to Biliary Atresia. *PLoS ONE* 2014;9:e107977.
- 64 Cheng G, Tang CS, Wong EH, Cheng WW, So MT, Miao X, et al: Common genetic variants regulating ADD3 gene expression alter biliary atresia risk. *J Hepatol* 2013;59:1285–1291.

- 65 Citterio L, Tizzoni L, Catalano M, Zerbini G, Bianchi G, Barlassina C: Expression analysis of the human adducin gene family and evidence of ADD2 beta4 multiple splicing variants. *Biochem Biophys Res Commun* 2003; 309:359–367.
- 66 Cui S, Leyva-Vega M, Tsai EA, Eauclaire SF, Glessner JT, Hakonarson H, et al: Evidence from human and zebrafish that GPC1 is a biliary atresia susceptibility gene. *Gastroenterology* 2013;144:1107–1115.e3.
- 67 Davit-Spraul A, Baussan C, Hermeziu B, Bernard O, Jacquemin E: CFC1 gene involvement in biliary atresia with polysplenia syndrome. *J Pediatr Gastroenterol Nutr* 2008;46: 111–112.
- 68 Shimadera S, Iwai N, Deguchi E, Kimura O, Fumino S, Yokoyama T: The inv mouse as an experimental model of biliary atresia. *J Pediatr Surg* 2007;42:1555–1560.
- 69 Arikan C, Berdeli A, Ozgenç F, Tumgor G, Yagci RV, Aydogdu S: Positive association of macrophage migration inhibitory factor gene-173G/C polymorphism with biliary atresia. *J Pediatr Gastroenterol Nutr* 2006; 42:77–82.
- 70 Uemura M, Ozawa A, Nagata T, Kurasawa K, Tsunekawa N, Nobuhisa I, et al: Sox17 haploinsufficiency results in perinatal biliary atresia and hepatitis in C57BL/6 background mice. *Development* 2013;140:639–648.
- 71 Kohsaka T, Yuan ZR, Guo SX, Tagawa M, Nakamura A, Nakano M, et al: The significance of human jagged 1 mutations detected in severe cases of extrahepatic biliary atresia. *Hepatology* 2002;36:904–912.
- 72 Lee HC, Chang TY, Yeung CY, Chan WT, Jiang CB, Chen W-F, et al: Genetic variation in the vascular endothelial growth factor gene is associated with biliary atresia. *J Clin Gastroenterol* 2010;44:135–139.
- 73 Lee HC, Chang TY, Yeung CY, Chan WT, Jiang CB, Chen WF, et al: Association of interferon-gamma gene polymorphisms in Taiwanese children with biliary atresia. *J Clin Immunol* 2010;30:68–73.
- 74 Miethke AG, Huppert SS: Fishing for biliary atresia susceptibility genes. *Gastroenterology* 2013;144:878–881.
- 75 Zhao D, Long XD, Xia Q: Recent advances in etiology of biliary atresia. *Clin Pediatr (Phila)* 2014; DOI: 10.1177/0009922814548841.
- 76 Karpen SJ, Trauner M: The new therapeutic frontier – nuclear receptors and the liver. *J Hepatol*, 2010, pp 455–462.
- 77 Karpen SJ: Nuclear receptor regulation of hepatic function. *J Hepatol* 2002;36:832–850.