Unraveling the Pathogenesis and Etiology of Biliary Atresia

CARA L. MACK AND RONALD J. SOKOL

Pediatric Liver Center and Liver Transplantation Program, Section of Pediatric Gastroenterology, Hepatology and Nutrition, Department of Pediatrics [C.M., R.J.S.], Pediatric General Clinical Research Center [R.J.S.], University of Colorado School of Medicine and The Children's Hospital, Denver, Colorado, 80218

ABSTRACT

Biliary atresia (BA) is the most common and important neonatal hepatobiliary disorder. Because current treatment is inadequate, there is an urgent need to better understand the etiology and pathogenesis of BA. Two major forms of BA are recognized: an *embryonic* form associated with other congenital anomalies and a *perinatal* form in which bile ducts were presumably formed normally but underwent fibro-obliteration in the perinatal period. There are currently several proposed pathogenic pathways leading to the phenotype of BA, including an immune or autoimmune response to a perinatal insult (*e.g.* cholangiotropic viral infection) and dysregulated embryonic development of the extra- or intrahepatic biliary system. Recent advances in developmental biology, genomics and genetics, and cell immunology and biology, coupled with the development of appropriate animal models, have provided support for these postulated mechanisms. Future investigations combining animal model work and evaluation of clinical specimens holds the promise of identifying

the etiology of BA and providing a scientific basis for treatment and preventative interventions. (*Pediatr Res* 57: 87R–94R, 2005)

Abbreviations

APC, antigen presenting cell
BA, biliary atresia
CD, clusters of differentiation
CMV, cytomegalovirus
IFN, interferon
INV, inversin
LBW, low birth weight
MHC, major histocompatibility complex
OR, odds ratio
RR, risk ratio
RT-PCR, reverse transcriptase polymerase chain reaction
TNF, tumor necrosis factor

BA is an enigmatic cause of neonatal cholestasis, occurring in 1 in 8,000 to 1 in 18,000 live births and presenting typically within the first months of life with jaundice, acholic stools, and hepatomegaly in an otherwise apparently healthy infant (1). Inflammatory obstruction of the extrahepatic bile ducts is accompanied by a characteristic intrahepatic portal lesion. If diagnosed in the first 2–3 mo of life, hepatic portoenterostomy can restore bile flow from the liver into the intestinal tract in 30–80% of patients (1–3). Despite successful surgery, progressive inflammation and fibrosis of intrahepatic bile ducts develops to varying degrees in all patients, leading to biliary cirrhosis in the majority of patients. Consequently, 70–80% of BA patients will eventually require liver transplantation, approximately half in the first 2 y of life (4–6). Thus, BA is the most common indication for liver transplantation in children, responsible for almost 50% of all pediatric liver transplants. It

should be emphasized that it is the intrahepatic biliary lesion that determines overall prognosis and outcome, thus, this disease is no longer called “extrahepatic” BA. Without a better understanding of the etiology and pathogenesis of this intrahepatic sclerosing cholangitic process in BA, little progress can be expected in improving the nontransplantation outcome of patients. Therefore, there has been a renewed interest in recent years in understanding the underlying pathogenetic mechanisms of BA.

It is now apparent that BA is a phenotype resulting from several pathogenic processes that culminate in obstruction of the biliary tree (1,7). The majority (80%) of cases of BA in Western countries are of the *perinatal* or acquired form. These otherwise normal infants are presumably born with a patent biliary system which undergoes progressive inflammation and fibro-obliteration initiated by a perinatal insult. Although the etiology of this form is not completely understood, proposed precipitating factors include infectious, toxic, vascular, and immune mediators. The second form of BA is associated with other congenital anomalies, most commonly involving abdominal situs, and has been dubbed the *embryonic* or fetal form. Defective morphogenesis, caused by mutations in genes regulating biliary development, has been proposed in these cases. In

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Correspondence: Ronald J. Sokol, M.D., Department of Pediatrics, The Children's Hospital, Box B290, 1056 E. 19th Ave., Denver, CO 80218; e-mail: Sokol.ronald@tchden.org

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this review, we will examine recent data supporting the proposed viral and immunologic pathogenesis of the perinatal form of BA, and the potential role for defective genetic regulation of bile duct development in the embryonic form.

VIRAL AND OTHER INFECTIOUS AGENTS

Epidemiology. Epidemiologic studies support a possible infectious etiology of BA. Multiple studies have demonstrated seasonal clustering of cases suggesting environmental exposure to an infectious agent. A population-based study in metropolitan Atlanta (1968–1993) revealed seasonal clustering of BA diagnoses, with rates 3-fold higher in December through March (OR = 2.63 for winter compared with spring) (8). Strickland and Shannon (9) found BA case clustering in northeast Texas ($\chi^2 = 13.89$, $p < 0.001$) and rural locales ($\chi^2 = 25.29$, $p < 0.0001$) and temporal association with fall compared with the remainder of the year ($\chi^2 = 5.96$, $p = 0.015$). A recent study from the state of New York (10) reported seasonal patterns of BA cases that varied by the region of the state. Spring births were at highest risk for BA in New York City and September to November births were at highest risk in other regions of the state. In contrast, epidemiologic studies from Europe have not consistently identified clustering of cases (5,11). Inasmuch as epidemic infections may present at different times of the year in different countries, it is still an open question as to whether case clustering represents a true epidemiologic association.

A second area of epidemiologic research has focused on the birth weight and gestational age of infants with BA. Several studies have revealed an association of low birth weight and shortened gestation with the occurrence of BA. The case-controlled study from New York state demonstrated an increased risk of BA for LBW preterm and LBW term infants (OR = 2.92 and 2.36, respectively, compared with normal weight infants) (10). A similar association was observed in the Atlanta study (8), with term LBW infants having an increased risk compared with term normal birth weight infants (OR = 3.52). Of greatest interest is a cohort study from Sweden that used the Medical Birth Register of Sweden to assess risk of BA (11). In this study, both small for gestational age (SGA) and preterm status conferred an increased risk of BA. SGA infants had a RR of 5.75 compared with that of appropriately sized infants. Infants born between 22–32 wk of gestation had a RR of 6.75 and those born at 33–36 gestational age had a RR of 2.48 compared with term infants. It is important to note that most *in utero* viral infections are associated with intrauterine growth retardation or preterm delivery.

Putative viruses. The ongoing search for a putative virus involved in the pathogenesis of BA has led to conflicting findings regarding at least three different viruses: reovirus, rotavirus, and CMV. Interest in reovirus developed after the observation that infection in weanling mice caused extrahepatic bile duct cyst formation and intrahepatic bile duct pathologic features similar to those of BA (12). Serologic studies initially suggested an increased rate of infection with reovirus in infants with BA and neonatal hepatitis (13,14), however, subsequent studies could not confirm these findings (15,16).

Detection of reovirus antigens in the bile duct remnants from an infant Rhesus monkey with BA (17) and from human infants with BA (18) was reported, although the latter findings were not replicated by others (15). Three groups of investigators have examined hepatobiliary tissues removed from infants with BA for reovirus RNA. Steele *et al.* (19) failed to detect reovirus RNA in archived, formalin-fixed preserved hepatic tissues of 14 BA patients using nested RT-PCR. A recent study from Japan likewise failed to identify reovirus RNA in frozen biliary atresia hepatobiliary tissue, however, the average age at the time of collection of the tissue was 8.7 mo (20). In contrast, Tyler *et al.* (21) reported the detection of reovirus RNA by RT-PCR in snap-frozen liver, gall bladder, or bile duct remnants from 55% of cases of BA (average age 2.2 mo) and in only 8–15% of controls. The discrepancies among these studies may lie in the age of the patients at the time of sample acquisition, methods of preparation of the tissue, and the use of PCR primers for different reovirus genes. It is also possible that the proposed perinatal viral infection is cleared from the liver within a relatively short period of time, resulting in the inability to detect virus at the time of diagnosis or later, as has been demonstrated in mouse BA models based on viral infection (see below).

Another virus of the *Reoviridae* family, Group C rotavirus, has received attention. Riepenhoff-Talty *et al.* (22) examined hepatobiliary tissues of BA patients for RT-PCR evidence of Group C rotavirus infection. Ten of 18 BA patients at diagnosis and 0 of 12 liver disease control patients showed evidence of rotavirus RNA. In contrast, Bobo *et al.* (23) failed to detect rotavirus Groups A, B, or C RNA from 10 BA patients and 14 liver disease controls by RT-PCR techniques. However, almost half the patients in that study were over 12 mo of age at the time that tissues were obtained. Rotavirus has also been used to develop a mouse model of BA. Rhesus rotavirus infection produces extrahepatic bile duct obstruction by 2 wk of life despite viral clearance in newborn mice (24,25). Timing of infection was critical, with the highest incidence of cholestasis if infection occurred in the first 12 h postpartum, after which the incidence of disease diminished (26). It was proposed that the rapidly developing immune system of the mouse over the first week of life may be a factor responsible for the critical window of time in which rotavirus infection may cause biliary disease.

Twenty-three patients with BA were evaluated for CMV by analyzing liver histology, CMV-IgM titers, or CMV viral culture. Five patients (24%) were found to be positive for CMV (27). A recent study from Brazil likewise found positive CMV-IgM titers in 28.5% of patients with BA or choledochal cysts (28). A study from Sweden (29) showed a higher prevalence of CMV antibodies in mothers of BA patients and CMV DNA was present in livers from 50% of those infants. However, a Canadian group (30) could not demonstrate CMV in bile duct remnants from 12 children with BA. Finally, other viruses have also been examined including papillomavirus (31,32) and herpes virus-6 (32) with similar conflicting results.

In summary, based on the similarities between the neonatal murine models of reovirus and rotavirus and some human studies showing about a 50% frequency of each of these

viruses in infants with BA, further study of the roles of these and related viruses in the etiology of BA are warranted. The possible role of CMV, papillomavirus, and herpes virus-6 in BA is likewise unsettled and requires further investigation.

There is scant literature regarding the potential role of bacterial infection as a cause of BA. Tracy *et al.* (33) investigated the expression of CD14, a monocyte cell surface receptor activated by bacterial endotoxin [lipopolysaccharide (LPS)], on BA liver tissue obtained at the time of portoenterostomy. Intense staining of CD14 was found on sinusoidal and portal tract macrophages in BA tissue and not found in the portal tracts of control specimens. Furthermore, Ahmed *et al.* (34) compared the *in situ* expression of CD14 between early and late stages of BA. Almost 70% of BA specimens at time of portoenterostomy had extensive CD14 expression, especially on sinusoidal Kupffer cells. The CD14 expression was markedly decreased at the time of transplant. They proposed that potential exposure to portally derived LPS leads to CD14 overexpression in BA resulting in macrophage activation with release of pro-inflammatory cytokines and bile duct damage. This mechanism is theoretical at this point and warrants further investigation.

ROLE OF THE IMMUNE SYSTEM IN BILE DUCT INJURY AND OBSTRUCTION

At the time of diagnosis of BA, the extrahepatic duct remnant is composed of fibrous tissue and scattered lymphocytes (35) whereas intrahepatic ducts are surrounded by a mixed cellular infiltrate composed of lymphocytes, macrophages, and eosinophils (36) (Fig. 1). The role of the immune system in bile duct injury and obstruction is poorly understood at present and is the focus of intense investigation. In the following, the potential role of T-cell-mediated inflammation, macrophage activation, and autoimmune processes are examined.

Figure 1. Lesion present in common hepatic duct removed at time of diagnosis of biliary atresia in an 8 wk-old infant without other congenital anomalies. A narrow hepatic duct lumen is still patent, however, bile duct epithelial cells are undergoing apoptosis and disruption. A prominent lymphocytic infiltrate surrounds the remaining duct, with concentric sclerosis developing around the narrowed lumen. (Hematoxylin and eosin, $\times 100$.)

T-cell-mediated inflammation. T-cell activation requires the interaction of the T cell with an APC-bearing antigen in the context of MHC class I (for cytotoxic CD8⁺ T cells) or MHC class II (for helper CD4⁺ T cells). In the inflammatory environment, naïve CD4⁺ T cells differentiate to either Th1 effector cells (driven by IL-12 and producing IFN- γ , IL-2, TNF- β , and TNF- α) or Th2 effector cells (driven by IL-4 and producing IL-4, IL-5, and IL-10). In biliary atresia, T-cell infiltrates surround and invade intrahepatic bile ducts (37), characterized predominantly by CD4⁺ T cells (38–40), CD8⁺ T cells (41), or a mixture of both (42) (see Fig. 2). Furthermore, increased expression of lymphocyte activation (LFA-1, IL-2 receptor) and proliferation (transferrin receptor) were found in the portal tracts of BA (40,43).

Activated effector T cells produce cytokines that can directly damage epithelial cells or indirectly damage them through stimulation of other immune cells. Recent investigation has

Figure 2. Fluorescent immunohistochemistry reveals an increase in CD8⁺ T cells, CD3⁺/CD4⁺ T cells, and Kupffer cells in the portal tracts in biliary atresia. (Used with permission from Ref. 42.) Frozen sections from normal liver, other neonatal cholestatic liver diseases, and biliary atresia were incubated with MAb, amplified with fluorescein labeled substrate (green), and counterstained with nuclear stain DAPI (blue) (200 $\times$ magnification). Portal tracts were identified by characteristic vessel formation and bile duct epithelial nuclear staining and verified by serial sections with cytokeratin 7 staining, which is specific for bile duct epithelium (not shown). In normal livers, the portal tracts are seen in the center of each photograph. In other cholestatic diseases and biliary atresia the portal tracts are expanded and encompass the entire field at 200 $\times$ magnification. (A) CD8⁺ T cells are more abundant in biliary atresia portal tracts. (B) CD4⁺ cell surface expression is found not only on T cells but also on sinusoidal cells and Kupffer cells. To delineate the CD4⁺ T cells, the tissue was double stained with anti-CD3⁺ antibody (T-cell-specific marker) (red) and CD4⁺ antibody (green). Double-positive cells (CD3⁺CD4⁺) are yellow. There is an increase in the number of CD3⁺CD4⁺ cells in biliary atresia portal tracts. The single-stained red cells (CD3⁺CD4⁻) in biliary atresia reflects the CD8⁺ T cells. Also note the single staining CD4⁺ sinusoidal cells in the parenchyma surrounding the portal tract in normal liver controls. (C) CD68⁺ cells (macrophage/Kupffer cells) are increased in the portal tracts of biliary atresia. Note the CD68⁺ staining of cells in the parenchyma of controls. Figure adapted from Mack *et al.*, *Pediatr Res* 56:79–87, Copyright© 2004 by the International Pediatric Research Foundation, with permission.

revealed that BA is associated with Th1-cell-mediated portal tract inflammation. We have shown that the periductular immune cells in BA produce IL-12, IFN- γ , IL-2, and TNF- α (42). This localized inflammatory profile was unique to BA and was not found in other neonatal cholestatic diseases (42). Bezerra *et al.* (44) used gene expression microarray techniques to analyze BA liver biopsies and observed up-regulation of a number of pro-inflammatory genes (including the Th1 cytokines IFN- γ and osteopontin) and down-regulation of Ig genes (suggesting inhibition of Th2 pathway). This laboratory then went on to use the rotavirus-induced murine model of biliary obstruction to demonstrate the importance of IFN- γ in the development of this bile duct obstruction (45). IFN- γ knock out mice showed increased survival after rotavirus infection and resolution of bile duct obstructive lesions compared with wild-type controls, verifying the critical role of this cytokine in the inflammatory obstructive process. Further investigations of the role of various cytokines in this model and in human BA are warranted in that these may be potential targets for intervention.

There is limited data regarding the identity of the APC responsible for complete T-cell activation in BA. Professional APC include macrophages, dendritic cells, and B cells, whereas nonprofessional APC may include endothelial or epithelial cells (including bile duct epithelium). We and others have shown dramatic increases in portal tract macrophages at the time of diagnosis of BA (discussed below). Furthermore, in our study, portal tract B cells were not increased compared with controls. To date, the potential function of the dendritic cell in the portal tract inflammatory response of BA has not been reported.

Complete T-cell activation requires two signals from the APC: 1) surface expression of self-MHC molecules bearing antigen, which interact with the T-cell receptor; and 2) co-stimulatory molecules B7-1 and/or B7-2, which interact with CD28 on T cells (46). Competent APC also express intercellular adhesion molecules (ICAM) to provide adhesion of APC to the T cell. Investigation of the expression of these molecules in BA has been carried out by standard immunohistochemistry techniques. Strong cell surface expression of HLA-DR (MHC class II) was found on Kupffer cells and to a lesser degree on intrahepatic bile duct epithelium (39,47). Furthermore, Kobayashi *et al.* (48) recently demonstrated increased expression of B7-1 and B7-2 on intrahepatic bile duct epithelium and Kupffer cells in those patients with advanced disease at presentation. Amplified expression of ICAM on bile duct epithelium of BA liver biopsies has also been described (38–40). Thus, there is accumulating evidence for Kupffer cells and bile duct epithelial cells as potential APC in BA.

Macrophage activation. Portal tracts of BA patients are laden with macrophages (42,47,49) and it appears that the intensity of the macrophage infiltrate correlates with a worse prognosis. Kobayashi *et al.* (47) found that patients with no bile flow after the portoenterostomy had markedly increased numbers of portal tract macrophages at diagnosis. Davenport *et al.* (40) reported prominent macrophage staining in the portal tracts of BA patients. The intensity of the staining correlated with macrophage activation, as evidenced by an increase in the lysosome-rich cytoplasmic volume, and with poor outcome after portoenterostomy.

Activated macrophages produce cytokines IL-12 and IL-18, which function together to promote Th1 cellular differentiation. We demonstrated increased mRNA expression of IL-12 in livers of BA patients at diagnosis (42) and Urushihara *et al.* (50) reported macrophage activation and increased serum IL-18 in BA patients. Macrophages also secrete cytotoxic cytokines such as TNF- α , which are markedly up-regulated in BA (42). TNF- α may also, through receptor binding, induce apoptotic or necrotic cell pathways causing persistent bile duct epithelial injury. To that end, Funaki *et al.* (51) showed that almost 50% of intrahepatic bile duct epithelial cells in BA liver were apoptotic compared with only 2.5–3.6% in the control groups. Another cell death pathway is triggered by the interaction of Fas-ligand (FasL) with Fas-bearing cells. FasL is expressed predominantly on activated T cells and monocytes/macrophages. Liu *et al.* (52) reported that Fas was expressed weakly on bile duct epithelium of both BA and normal liver, however, strong expression was found on 25% of BA samples and none of controls. Aberrant FasL expression was present in bile duct epithelial cells in approximately 30% of BA specimens as well as in the majority of portal tract monocytes. Interestingly, FasL expression on bile duct epithelia was associated with poor bile flow postportoenterostomy.

In summary, characterization of the hepatic immune response present at the time of diagnosis of BA (Table 1) forms the basis of the following hypothesis. It is proposed that an initial insult to bile duct epithelia (*e.g.* a cholangiotropic virus) leads to HLA-DR-restricted Kupffer cell (or bile duct epithelium) presentation of antigens (derived from virus or altered self-antigens on bile duct epithelia) to CD4⁺ T cells with subsequent differentiation and proliferation of effector Th1 cells and local release of IL-2, IFN- γ , and TNF- α . IFN- γ -stimulated release by macrophages of TNF- α and nitric oxide could lead to bile duct epithelial cell apoptosis and necrosis, obstruction, and, ultimately, fibrosis. Continued investigations using animal models and human tissues should help better

Table 1. Immune profile of biliary atresia at presentation

Composition of portal tract infiltrate	CD4 ⁺ T cell (38, 40), CD8 ⁺ T cell (39, 40), macrophage (40, 45, 47, 48), NK cells (38)
Amplified cell surface expression	APC: HLA-DR (36, 37, 45), B7-1 (46), B7-2 (46), ICAM-1 (36, 37, 41) Activated T cell: LFA-1 (38), CD25 (38), CD71 (38) Cell death: Fas (50), FasL (50), TUNEL (49)
Cytokine up-regulation	IL-2 (40), IFN- γ (40, 42), TNF- α (40), IL-12 (40), IL-18 (48), IL-8 (42), osteopontin (42)

define the key factors stimulating and perpetuating this immune response.

BA as an autoimmune disease. The progressive nature of BA, despite initial re-establishment of bile flow with a portoenterostomy, suggests that a persistent autoimmune attack directed at bile ducts may play a role (53,54). In BA, it is possible that the initiator of the bile duct injury is a cholangiotrophic virus, leading to the exposure of altered self bile duct epithelial antigens or previously sequestered self bile duct antigens that would then be recognized as "foreign" and elicit a persistent autoimmune-mediated bile duct injury. It is also possible that molecular mimicry, whereby the virus contains antigens that cross-react with self bile duct epithelial antigens, elicits immune responses against both viral and self antigens (54,55).

The Rose and Witebsky criteria (56) used to define an autoimmune disease include evidence of lymphocytic infiltrate, presence of autoantibodies or evidence on an autoantigen, association with other autoimmune disorders in the patient or family, and HLA association. There is clearly a predominance of lymphocytes in BA liver tissue. The search for autoantibodies has yielded preliminary results. Vasilias et al. (57) reported that 10 of 11 patients with BA were positive for serum IgG and IgM antineutrophil cytoplasmic antibodies (ANCA), with higher levels of the IgM-ANCA in BA patients compared with children and adults with other liver diseases. At our center, Burch et al. (58) examined lupus autoantibodies in mothers of children with BA and idiopathic neonatal hepatitis to test the hypothesis that maternal transfer of autoantibodies might be involved in liver and bile duct injury in the infant. Low titer anti-Rho antibodies were more prevalent in mothers of infants with BA and idiopathic neonatal hepatitis than in controls, and low titer antinuclear antibodies were more common in mothers of infants with liver disease. To date, there have been no studies addressing the frequency of other autoimmune diseases in BA patients or in other family members. Interestingly, children with BA who undergo liver transplant have a 2.5% risk of developing *de novo* autoimmune hepatitis after transplantation (59,60), perhaps indicating a genetic predisposition to autoimmune disease.

Multiple inherited genetic polymorphisms are believed to contribute to autoimmune disease susceptibility; the strongest associations are with HLA genes, especially HLA-DR, -DQ, or -DP (MHC-class II equivalent) (46). For example, increased frequencies of HLA B8 and DR3 are found in patients with primary sclerosing cholangitis (61,62) and HLA A1-B8-DR3 and DR4 are risk factors for autoimmune hepatitis (63). Investigators have explored the possibility of a link between HLA type and BA with conflicting results. Rosenthal et al. (64) suggested an increased frequency of HLA-Cw4/7 in BA. Silveira et al. (65) reported a significantly higher prevalence of HLA-B12 and A-Kader et al. (66) from Egypt observed significant increases in both HLA B8 and DR3. Only 55 and 18 BA patients were analyzed, respectively, and serological phenotype techniques were used. More recently, DNA genotyping has been adapted for HLA typing, yielding a greater number of HLA loci and alleles to be tested with much greater accuracy. Using DNA genotyping, Donaldson et al. (67) found no HLA

associations in 101 BA patients from the United Kingdom. It is clear that a large, multicentered study of HLA genotyping (including high resolution of HLA-class II) and a sample size calculated to generate statistically sound results is needed.

MORPHOGENESIS GENES AND BILIARY ATRESIA

Laterality genes. It has been proposed that the embryonic form of BA is caused by defective development of the biliary tree. The association of anomalies of visceral organ symmetry with BA (the polysplenia or BA splenic malformation syndrome) implies that genes that control normal situs development are also key regulators of normal extrahepatic bile duct development. The initial animal model that suggested this class of genes was that of a recessive insertional mutation in the proximal region of mouse chromosome 4 or complete deletion of the inversion (*inv*) gene in the mouse (68,69). This mouse model, with laterality defects in abdominal organ placement, also included anomalous development of the hepatobiliary system. Therefore, the human *INV* gene, recently mapped by Schon and colleagues (70), was examined in 64 patients with heterotaxia. No mutations were found in seven patients with BA and various congenital laterality defects, making it unlikely that the *INV* gene is responsible for the majority of embryonic cases of BA. Interestingly, recessive mutations in *INV* were recently described in children with nephronophthisis type 2 (71), a disease in which abnormal biliary development has been described (72). Moreover the *INV* protein was found to interact with nephrocystin, both being located on the primary cilia of renal tubular cells (71). Thus, gene products with localization to the mechanosensory primary cilium of renal tubular cells and cholangiocytes may produce aberrant biliary development with predominance of ductal plate malformation. In this regard, there is subset of patients with BA who have a ductal plate malformation-like lesion in portal tracts, and who are believed to have a worse prognosis (73). It remains to be seen whether mutations or polymorphisms of genes that code for proteins regulating functions of the primary cilia will play a role in the pathogenesis of BA.

More than 30 other mammalian proteins have now been described that are involved in establishment of left-right patterning during embryogenesis, although few have been examined in BA. Bamford et al. (74) analyzed the *CFC1* gene (which encodes the human CRYPTIC protein) in genomic DNA from 144 cases of familial and sporadic cases of laterality defects. Heterozygous mutations were identified in nine patients, including one with BA and the polysplenia syndrome. Jacquemin et al. (75) subsequently found heterozygous gene mutations in two brothers with laterality defects, one of whom had BA, which were inherited from their unaffected mother. Thus, heterozygous *CFC1* mutations may predispose to BA, but also appear to require a second genetic or environmental factor to produce the BA phenotype. The CRYPTIC protein acts as a cofactor in the Nodal pathway that determines left-right axis development potentially through its function in the transforming growth factor beta signaling pathway (75).

ZIC3 encodes a zinc finger transcription factor that translocates to the nucleus and influences left-right axis determina-

tion. In 2004, Ware *et al.* (76) reported eight *ZIC3* mutations in 194 heterotaxia patient samples and calculated that mutations in this gene were responsible for approximately 1% of patients with sporadic heterotaxy. Among the patients with mutations and complex congenital heart disease, two had BA, one abnormal liver lobulation, and one cholelithiasis and cholecystitis. Thus, there appears to be an association between mutations in this gene and isolated cases of BA and heterotaxia. Evaluation of *ZIC3* in a larger population of patients with BA, with and without other congenital anomalies, will be of interest. Other human genes that determine laterality (*LEFTY* and *ACVR2B*) and cell adhesion (*CRELD1* and *NKX2.5*) have been associated with a small percentage of human situs defects (76). Interestingly, Zhang *et al.* (77) recently reported no differences in mRNA expression of a panel of laterality genes in liver from embryonic *versus* perinatal cases of BA. Future investigations of these and other genes in coming years may shed light on whether inherited or somatic mutations or deletions are responsible for individual cases of BA.

Intrahepatic bile duct development and BA. Normal development of intrahepatic bile ducts is controlled by interactions between mesenchyme and portal venous radicals, forming the ductal plate. The ductal plate is derived from a subset of hepatoblasts that differentiate into a single-layered ring of biliary precursor cells that surround portal venous branches and their encompassing mesenchyme within the liver (reviewed in 78,79), occurring by 5 wk gestation. Remodeling of the ring of cells occurs over the next 6–7 wk. This single layer of cells becomes partly bi-layered followed by the development of focal dilated areas between the two cell layers, which soon give rise to the portal tract bile ducts. Abnormal remodeling of the ductal plate leads to the ductal plate malformation that is present in congenital hepatic fibrosis and other bile duct dysplasias. Interestingly, a number of infants with BA show evidence of the ductal plate malformation on liver biopsy (73). Polymorphisms in *HNF6*, *HNF1-B*, *JAGGED1*, *PKDH1*, or other related genes that regulate remodeling of the ductal plate (80–82) could act as susceptibility factors or modifier genes necessary, but not sufficient, for the development of BA. In this regard, Kohsaka *et al.* (83) identified *JAGGED1* missense mutations in 9 of 102 patients with BA, who had no phenotypic features of Alagille syndrome. Remarkably, prognosis was worse in the group with mutations, suggesting that *JAGGED1* could be a modifying factor in BA. However, other factors known to influence prognosis in BA, such as age at time of portoenterostomy, were not elucidated in the report. Thus, it is not clear whether these patients had an unusual form of BA caused by mutations in *JAGGED1*, whether the phenotype reported was a unique presentation of Alagille syndrome, or whether the gene functioned as a modifying factor in BA. These observations remain to be confirmed but support the possibility that predisposition to BA may have a genetic basis.

Just how a defect in ductal plate remodeling could lead to atresia of the extrahepatic biliary tree is not intuitively obvious. The hypothesis of Tan and colleagues may shed light on this quandary. Tan *et al.* (84) have demonstrated the presence of extravasated bile in bile duct remnants of BA patients, proposed to have originated from breaks in the biliary mucosa at

the porta hepatitis. The supposition is that there is a vulnerable stage of human biliary development between 11 and 13 wk of gestation, when failure of remodeling of the ductal plate structures could lead to disturbances in the normal development of the mesenchymal cuff that surrounds developing hilar bile ducts. Alternatively, connections of the hepatic ducts (derived from tubulization of the ductal plate structures) with the extrahepatic bile ducts could be perturbed. Either of these alterations could potentially cause hilar bile ducts to rupture at the initiation of bile flow at 12–13 wk of gestation. Extravasated bile, with its detergent properties, into adjacent periductal tissues would then lead to protracted inflammation and sclerosis in the submucosa, causing secondary obliteration and obstruction of the more distal extrahepatic bile duct. This hypothetical mechanism was first described by Rolleston and Hayne (85) in 1901, who coined the term “descending cholangitis.”

SUMMARY

Recent advances in developmental biology, genetics, genomics, and cell biology have opened the door to a better understanding of the underlying mechanisms causing BA. A unifying hypothesis is provided in Figure 3, illustrating the proposed stereotypic response of the infant biliary tree to a variety of insults, both prenatal and postnatal. Embryonic BA may be the result of mutations in genes controlling normal bile

Figure 3. Proposed pathways for pathogenesis of two forms of BA. *Perinatal* BA may develop when a perinatal insult, such as a cholangiotropic viral infection, triggers bile duct (BD) epithelial cell injury and exposure of self- or neo-antigens that elicit a subsequent immune response. The resulting inflammation induces apoptosis and necrosis of extrahepatic BD epithelium resulting in fibro-obliteration of the lumen and obstruction of the BD. Intrahepatic bile ducts may also be targets in the ongoing TH1 immune (autoimmune?) attack and the cholestatic injury, resulting in progressive portal fibrosis culminating in biliary cirrhosis. *Embryonic* BA may be the result of mutations in genes controlling normal bile duct formation or differentiation, which secondarily induces an inflammatory/immune response within the common bile duct and liver after the initiation of bile flow at approximately 11–13 wk of gestation. Secondary hepatocyte and intrahepatic bile duct injury ensue either as a result of cholestatic injury or as targets for the immune (autoimmune?) response that develops. The end result is intrahepatic cholestasis and portal tract fibrosis, culminating in biliary cirrhosis. Other major factors may be the role played by genetic predisposition to autoimmunity and modifier genes that determine the extent and type of cellular and immune response and the generation of fibrosis.

duct formation or differentiation, which secondarily induces an inflammatory response within the liver that injures and scleroses intrahepatic bile ducts. Perinatal BA may develop when a perinatal insult triggers bile duct epithelial injury and a subsequent immune response that damages both extrahepatic and intrahepatic bile ducts. Other major factors may be the role played by genetic predisposition to autoimmunity and modifier genes that determine the extent and type of cellular and immune response and the generation of fibrosis. Over coming years, multi-institutional and interdisciplinary collaborations will set the stage to provide the needed patient samples and technology to unravel the pathogenesis of this uniquely neonatal disorder, with the hope of developing a scientific rationale for innovative treatment and preventative approaches.

REFERENCES

- Sokol RJ, Mack C, Narkewicz MR, Karrer FM 2003 Pathogenesis and outcome of biliary atresia: current concepts. *J Pediatr Gastroenterol Nutr* 37:4–21
- Ohi R 2000 Biliary atresia. A surgical perspective. *Clin Liver Dis* 4:779–804
- Schweizer P 1986 Treatment of extrahepatic biliary atresia: results and long-term prognosis after hepatic portoenterostomy. *Pediatr Surg* 1:30–36
- Karrer FM, Bensard DD 2000 Neonatal cholestasis. *Semin Pediatr Surg* 9:166–169
- Chardot C, Carton M, Spire-Bendelac N, Le Pommelet C, Golmard JL, Auvert B 1999 Epidemiology of biliary atresia in France: a national study 1986–96. *J Hepatol* 31:1006–1013
- Karrer FM, Price MR, Bensard DD, Sokol RJ, Narkewicz MR, Smith DJ, Lilly JR 1996 Long-term results with the Kasai operation for biliary atresia. *Arch Surg* 131:493–496
- Perlmutter DH, Shepherd RW 2002 Extrahepatic biliary atresia: a disease or a phenotype? *Hepatology* 35:1297–1304
- Yoon PW, Bressee JS, Olney RS, James LM, Khoury MJ 1997 Epidemiology of biliary atresia: a population-based study. *Pediatrics* 99:376–382
- Strickland AD, Shannon K 1982 Studies in the etiology of extrahepatic biliary atresia: time-space clustering. *J Pediatr* 100:749–753
- Caton AR, Druschel CM, McNutt LA 2004 The epidemiology of extrahepatic biliary atresia in New York State, 1983–98. *Paediatr Perinat Epidemiol* 18:97–105
- Fischler B, Haglund B, Hjerm A 2002 A population-based study on the incidence and possible pre- and perinatal etiologic risk factors of biliary atresia. *J Pediatr* 141:217–222
- Bangaru B, Morecki R, Glaser JH, Gartner LM, Horwitz MS 1980 Comparative studies of biliary atresia in the human newborn and reovirus-induced cholangitis in weanling mice. *Lab Invest* 43:456–462
- Morecki R, Glaser JH, Cho S, Balistreri WF, Horwitz MS 1982 Biliary atresia and reovirus type 3 infection. *N Engl J Med* 307:481–484
- Glaser JH, Balistreri WF, Morecki R 1984 Role of reovirus type 3 in persistent infantile cholestasis. *J Pediatr* 105:912–915
- Brown WR, Sokol RJ, Levin MJ, Silverman A, Tamaru T, Lilly JR, Hall RJ, Cheney M 1988 Lack of correlation between infection with reovirus 3 and extrahepatic biliary atresia or neonatal hepatitis. *J Pediatr* 113:670–676
- Dussaix E, Hadchouel M, Tardieu M, Alagille D 1984 Biliary atresia and reovirus type 3 infection. *N Engl J Med* 310:658
- Rosenberg DP, Morecki R, Lollini LO, Glaser J, Cornelius CE 1983 Extrahepatic biliary atresia in a rhesus monkey (*Macaca mulatta*). *Hepatology* 3:577–580
- Morecki R, Glaser JH, Johnson AB, Kress Y 1984 Detection of reovirus type 3 in the porta hepatis of an infant with extrahepatic biliary atresia: ultrastructural and immunocytochemical study. *Hepatology* 4:1137–1142
- Steele MI, Marshall CM, Lloyd RE, Randolph VE 1995 Reovirus 3 not detected by reverse transcriptase-mediated polymerase chain reaction analysis of preserved tissue from infants with cholestatic liver disease. *Hepatology* 21:697–702
- Saito T, Shinozaki K, Matsunaga T, Ogawa T, Etoh T, Muramatsu T, Kawamura K, Yoshida H, Ohnuma N, Shirasawa H 2004 Lack of evidence for reovirus infection in tissues from patients with biliary atresia and congenital dilatation of the bile duct. *J Hepatol* 40:203–211
- Tyler KL, Sokol RJ, Oberhaus SM, Le M, Karrer FM, Narkewicz MR, Tyson RW, Murphy JR, Low R, Brown WR 1998 Detection of reovirus RNA in hepatobiliary tissues from patients with extrahepatic biliary atresia and choledochal cysts. *Hepatology* 27:1475–1482
- Riepenhoff-Talty M, Gouvea V, Evans MJ, Svensson L, Hoffenberg E, Sokol RJ, Uhnho I, Greenberg SJ, Schakel K, Zhaori G, Fitzgerald J, Chong S, el-Yousef M, Nemeth A, Brown M, Piccoli D, Hyams J, Ruffin D, Rossi T 1996 Detection of group C rotavirus in infants with extrahepatic biliary atresia. *J Infect Dis* 174:8–15
- Bobo L, Ojeh C, Chiu D, Machado A, Colombani P, Schwarz K 1997 Lack of evidence for rotavirus by polymerase chain reaction/enzyme immunoassay of hepatobiliary samples from children with biliary atresia. *Pediatr Res* 41:229–234
- Riepenhoff-Talty M, Schackel K, Clark HF, Mueller W, Uhnho I, Rossi T, Fisher J, Ogra PL 1993 Group A rotaviruses produce extrahepatic biliary obstruction in orally inoculated newborn mice. *Pediatr Res* 33:394–399
- Petersen C, Biermanns D, Kuske M, Schakel K, Meyer-Junghanel L, Mildnerberger H 1997 New aspects in a murine model for extrahepatic biliary atresia. *J Pediatr Surg* 32:1190–1195
- Czech-Schmidt G, Verhagen W, Szavay P, Leonhardt J, Petersen C 2001 Immunological gap in the infectious animal model for biliary atresia. *J Surg Res* 101:62–67
- Tarr PI, Haas JE, Christie DL 1996 Biliary atresia, cytomegalovirus, and age at referral. *Pediatrics* 97:828–831
- Oliveira NL, Kanawaty FR, Costa SC, Hessel G 2002 Infection by cytomegalovirus in patients with neonatal cholestasis. *Arq Gastroenterol* 39:132–136
- Fischler B, Ehrnst A, Forsgren M, Orvell C, Nemeth A 1998 The viral association of neonatal cholestasis in Sweden: a possible link between cytomegalovirus infection and extrahepatic biliary atresia. *J Pediatr Gastroenterol Nutr* 27:57–64
- Jevon GP, Dimmick JE 1999 Biliary atresia and cytomegalovirus infection: a DNA study. *Pediatr Dev Pathol* 2:11–14
- Drut R, Drut RM, Gomez MA, Cueto Rua E, Lojo MM 1998 Presence of human papillomavirus in extrahepatic biliary atresia. *J Pediatr Gastroenterol Nutr* 27:530–535
- Domati-Saad R, Dawson DB, Margraf LR, Finegold MJ, Weinberg AG, Rogers BB 2000 Cytomegalovirus and human herpesvirus 6, but not human papillomavirus, are present in neonatal giant cell hepatitis and extrahepatic biliary atresia. *Pediatr Dev Pathol* 3:367–373
- Tracy TF Jr, Dillon P, Fox ES, Minnick K, Vogler C 1996 The inflammatory response in pediatric biliary disease: macrophage phenotype and distribution. *J Pediatr Surg* 1:121–125
- Ahmed AF, Nio M, Ohtani H, Nagura H, Ohi R 2001 *In situ* CD14 expression in biliary atresia: comparison between early and late stages. *J Pediatr Surg* 36:240–243
- Gosseye S, Otte JB, De Meyer R, Maldague P 1977 A histological study of extrahepatic biliary atresia. *Acta Paediatr Belg* 30:85–90
- Bill AH, Haas JE, Foster GL 1977 Biliary Atresia: histopathologic observations and reflections upon its natural history. *J Pediatr Surg* 12:977–982
- Ohya T, Fujimoto T, Shimomura H, Miyano T 1995 Degeneration of intrahepatic bile duct with lymphocyte infiltration into biliary epithelial cells in biliary atresia. *J Pediatr Surg* 30:515–518
- Dillon PW, Belchis D, Minnick K, Tracy T 1997 Differential expression of the major histocompatibility antigens and ICAM-1 on bile duct epithelial cells in biliary atresia. *Histohistochemistry* 128:33–40
- Broome U, Nemeth A, Hultcrantz R, Scheynius A 1997 Different expression of HLA-DR and ICAM-1 in livers from patients with biliary atresia and Byler's disease. *J Hepatol* 26:857–862
- Davenport M, Gonde C, Redkar R, Koukoulis G, Tredger M, Mieli-Vergani G, Portmann B, Howard ER 2001 Immunohistochemistry of the liver and biliary tree in extrahepatic biliary atresia. *J Pediatr Surg* 36:1017–1025
- Ahmed AF, Ohtani H, Nio M, Funaki N, Shimaoka S, Nagura H, Ohi R 2001 CD8+ T cells infiltrating into bile ducts in biliary atresia do not appear to function as cytotoxic T cells: a clinicopathological analysis. *J Pathol* 193:383–389
- Mack CL, Tucker RM, Sokol RJ, Karrer FM, Kotzin BL, Whittington PF, Miller SD 2004 Biliary atresia is associated with CD4+ Th1 cell-mediated portal tract inflammation. *Pediatr Res* 56:79–87
- Dillon P, Belchis D, Tracy T, Cilley R, Hafer L, Krummel T 1994 Increased expression of intercellular adhesion molecules in biliary atresia. *Am J Pathol* 145:263–267
- Bezerra JA, Tiao G, Ryckman FC, Alonso M, Sabla GE, Shneider B, Sokol RJ, Aronow BJ 2002 Genetic induction of proinflammatory immunity in children with biliary atresia. *Lancet* 360:1653–1659
- Shivakumar P, Campbell KM, Sabla GE, Miethke A, Tiao G, McNeal MM, Ward RL, Bezerra JA 2004 Obstruction of extrahepatic bile ducts by lymphocytes is regulated by IFN-gamma in experimental biliary atresia. *J Clin Invest* 114:322–329
- Abbas AK, Lichtman AH, Pober JS 2000 Cellular and Molecular Immunology. WB Saunders, Philadelphia, pp vii, 553
- Kobayashi H, Puri P, O'Brian DS, Surana R, Miyano T 1997 Hepatic overexpression of MHC class II antigens and macrophage-associated antigens (CD68) in patients with biliary atresia of poor prognosis. *J Pediatr Surg* 32:590–593
- Kobayashi H, Li Z, Yamataka A, Lane GJ, Miyano T 2003 Role of immunologic costimulatory factors in the pathogenesis of biliary atresia. *J Pediatr Surg* 38:892–896
- Tracy TF Jr, Dillon P, Fox ES, Minnick K, Vogler C 1996 The inflammatory response in pediatric biliary disease: macrophage phenotype and distribution. *J Pediatr Surg* 31:121–126
- Urushihara N, Iwagaki H, Yagi T, Kohka H, Kobashi K, Morimoto Y, Yoshino T, Tanimoto T, Kurimoto M, Tanaka N 2000 Elevation of serum interleukin-18 levels and activation of Kupffer cells in biliary atresia. *J Pediatr Surg* 35:446–449
- Funaki N, Sasano H, Shizawa S, Nio M, Iwami D, Ohi R, Nagura H 1998 Apoptosis and cell proliferation in biliary atresia. *J Pathol* 186:429–433
- Liu C, Chiu JH, Chin T, Wang LS, Li AF, Chow KC, Wei C 2000 Expression of fas ligand on bile ductule epithelium in biliary atresia—a poor prognostic factor. *J Pediatr Surg* 35:1591–1596
- Schreiber RA, Kleinman RE 1993 Genetics, immunology, and biliary atresia: an opening or a diversion? *J Pediatr Gastroenterol Nutr* 16:111–113
- Sokol RJ, Mack C 2001 Etiopathogenesis of biliary atresia. *Semin Liver Dis* 21:517–524
- Eagar T, Tompkins S, Miller S 2001 Helper T-cell subsets and control of the inflammatory response. In: Rich R, Fleisher T, Shearer W, Kotzin B, Schroeder H Jr (eds) *Clinical Immunology Principles and Practice*. Mosby, London, pp 16.11–16.12
- Rose NR, Bona C 1993 Defining criteria for autoimmune diseases (Witebsky's postulates revisited). *Immunol Today* 14:426–430
- Vasiliasukas E, Targan S, Cobb L, Vidrich A, Rosenthal P 1995 Biliary atresia—an autoimmune disorder? *Hepatology* 22:87

58. Burch J, Sokol RJ, Narkewicz MR, Reichlin M, Frank B, Marchbank A, Lee L 2000 The Role of maternal antibodies in cholestatic liver disease in infants: a new isolated finding in neonatal lupus? *J Pediatr Gastroenterol Nutr* 31(suppl 2):S108
59. Kerkar N, Hadzic N, Davies ET, Portmann B, Donaldson PT, Rela M, Heaton ND, Vergani D, Mieli-Vergani G 1998 *De-novo* autoimmune hepatitis after liver transplantation. *Lancet* 351:409–413
60. Hernandez HM, Kovarik P, Whittington PF, Alonso EM 2001 Autoimmune hepatitis as a late complication of liver transplantation. *J Pediatr Gastroenterol Nutr* 32:131–136
61. Chapman RW 1991 Role of immune factors in the pathogenesis of primary sclerosing cholangitis. *Semin Liver Dis* 11:1–4
62. Donaldson PT, Manns MP 1999 Immunogenetics of liver disease. In: Benhamou J-P, McIntyre N, Rizzetto M, Rodes J (eds) *Oxford Textbook of Clinical Hepatology*. Oxford University Press, Oxford, pp 173–188
63. Manabe K, Donaldson PT, Underhill JA, Doherty DG, Mieli-Vergani G, McFarlane IG, Eddleston AL, Williams R 1993 Human leukocyte antigen A1–B8-DR3-DQ2-DPB1*0401 extended haplotype in autoimmune hepatitis. *Hepatology* 18:1334–1337
64. Rosenthal P, Woolf GM, Tyan DB 1995 A striking association between HLA-C and biliary atresia. *Gastroenterology* 108:A1158
65. Silveira TR, Salzano FM, Donaldson PT, Mieli-Vergani G, Howard ER, Mowat AP 1993 Association between HLA and extrahepatic biliary atresia. *J Pediatr Gastroenterol Nutr* 16:114–117
66. A-Kader HH, El-Ayyouti M, Hawas S, Abdalla A, Al-Tonbary Y, Bassiouny M, Boneberg A, El-Sallab S 2002 HLA in Egyptian children with biliary atresia. *J Pediatr* 141:432–433
67. Donaldson PT, Clare M, Constantini PK, Hadzic N, Mieli-Vergani G, Howard E, Kelley D 2002 HLA and cytokine gene polymorphisms in biliary atresia. *Liver* 22:213–219
68. Mazziotti MV, Willis LK, Heuckeroth RO, LaRegina MC, Swanson PE, Overbeek PA, Perlmutter DH 1999 Anomalous development of the hepatobiliary system in the *Inv* mouse. *Hepatology* 30:372–378
69. Yokoyama T, Copeland NG, Jenkins NA, Montgomery CA, Elder FF, Overbeek PA 1993 Reversal of left-right asymmetry: a situs inversus mutation. *Science* 260:679–682
70. Schon P, Tsuchiya K, Lenoir D, Mochizuki T, Guichard C, Takai S, Maiti AK, Nihei H, Weil J, Yokoyama T, Bouvagnet P 2002 Identification, genomic organization, chromosomal mapping and mutation analysis of the human *INV* gene, the ortholog of a murine gene implicated in left-right axis development and biliary atresia. *Hum Genet* 110:157–165
71. Otto EA, Schermer B, Obara T, O'Toole JF, Hiller KS, Mueller AM, Ruf RG, Hoefele J, Beekmann F, Landau D, Foreman JW, Goodship JA, Strachan T, Kispert A, Wolf MT, Gagnadoux MF, Nivet H, Antignac C, Walz G, Drummond IA, Benzing T, Hildebrandt F 2003 Mutations in *INVS* encoding inversin cause nephronophthisis type 2, linking renal cystic disease to the function of primary cilia and left-right axis determination. *Nat Genet* 34:413–420
72. Johnson CA, Gissen P, Sergi C 2003 Molecular pathology and genetics of congenital hepatorenal fibrocystic syndromes. *J Med Genet* 40:311–319
73. Low Y, Vijayan V, Tan CE 2001 The prognostic value of ductal plate malformation and other histologic parameters in biliary atresia: an immunohistochemical study. *J Pediatr* 139:320–322
74. Bamford RN, Roessler E, Burdine RD, Saplakoglu U, dela Cruz J, Splitt M, Goodship JA, Towbin J, Bowers P, Ferrero GB, Marino B, Schier AF, Shen MM, Muenke M, Casey B 2000 Loss-of-function mutations in the *EGF-CFC* gene *CFC1* are associated with human left-right laterality defects. *Nat Genet* 26:365–369
75. Jacquemin E, Cresteil D, Raynaud N, Hadchouel M 2002 *CFC1* gene mutation and biliary atresia with polysplenia syndrome. *J Pediatr Gastroenterol Nutr* 34:326–327
76. Ware SM, Peng J, Zhu L, Fernbach S, Colicos S, Casey B, Towbin J, Belmont JW 2004 Identification and functional analysis of *ZIC3* mutations in heterotaxy and related congenital heart defects. *Am J Hum Genet* 74:93–105
77. Zhang DY, Sabla G, Shivakumar P, Tiao G, Sokol RJ, Mack C, Shneider BL, Aronow B, Bezerra JA 2004 Coordinate expression of regulatory genes differentiates embryonic and perinatal forms of biliary atresia. *Hepatology* 39:954–62
78. Vijayan V, Tan CE 1997 Developing human biliary system in three dimension. *Anat Rec* 249:389–398
79. Lemaigre FP 2003 Development of the biliary tract. *Mech Dev* 120:81–87
80. Clotman F, Lannoy VJ, Reber M, Cereghini S, Cassiman D, Jacquemin P, Roskams T, Rousseau GG, Lemaigre FP 2002 The oncut transcription factor *HNF6* is required for normal development of the biliary tract. *Development* 129:1819–1828
81. Coffinier C, Gresh L, Fiette L, Tronche F, Schutz G, Babinet C, Pontoglio M, Yaniv M, Barra J 2002 Bile system morphogenesis defects and liver dysfunction upon targeted deletion of *HNF1beta*. *Development* 129:1829–1838
82. Clotman F, Libbrecht L, Gresh L, Yaniv M, Roskams T, Rousseau GG, Lemaigre FP 2003 Hepatic artery malformations associated with a primary defect in intrahepatic bile duct development. *J Hepatol* 39:686–692
83. Kohsaka T, Yuan ZR, Guo SX, Tagawa M, Nakamura A, Nakano M, Kawasaki H, Inomata Y, Tanaka K, Miyauchi J 2002 The significance of human jagged 1 mutations detected in severe cases of extrahepatic biliary atresia. *Hepatology* 36:904–912
84. Tan CE, Moscoso GJ 1994 The developing human biliary system at the porta hepatis level between 11 and 25 weeks of gestation: a way to understanding biliary atresia. Part 2. *Pathol Int* 44:600–610
85. Rolleston HD, Hayne LB 1901 A case of congenital hepatic cirrhosis with obliterative cholangitis (congenital obliteration of the bile ducts). *BMJ* 1:758–760