Childhood Liver Disease Research Network

(ChiLDReN)

A Prospective Database of Infants with Cholestasis

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For a list of sites, sponsor information and principal investigators, please refer to the study website: www.ChildrenNetwork.org

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(ChiLDReN)

Protocol Signature Sheet Instructions

ATTN: STUDY COORDINATOR

Print one copy of the protocol signature sheet on page 3. Original signature of the Principal Investigator and date of signature is required. Return a digital copy to the SDCC using the ChiLDReN-Monitors@ArborResearch.org email address and the original signed copy ***must*** be maintained in your regulatory files.

ChiLDREN PROBE Amendment 8 Version 09

PROTOCOL AMENDMENT 8 SIGNATURE SHEET

A Prospective Database of Infants with Cholestasis
Date of Protocol: 02/10/2004
Date of Protocol Amendment 1: 02/22/2005
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Date of Protocol Amendment 6: 09/12/2013
Date of Protocol Amendment 7: 12/12/2017
Date of Protocol Amendment 8: 09/19/2019

I hereby confirm that I have read and understand **Protocol Amendment 8** and all its attachments and that these documents contain all the details necessary to perform the trial. Unclear passages were clarified in a discussion with the lead investigator of the study.

When necessary to delegate tasks, I undertake to delegate them only to qualified personnel, to inform the personnel about the study and their duties and to supervise the conduct of the study. I agree that the NIDDK and their authorized representatives should have free access to all study documents at their request, to ascertain that the study is conducted in accordance with the protocol. This includes the informed consent.

I agree to the **Protocol Amendment 8** in all details and will perform the study in accordance with the amended protocol, the Declaration of Helsinki, the ICH Note on 'Good Clinical Practice', and local regulations.

Information related to the investigation should only be transferred to third persons after the written consent of the Childhood Liver Disease Research Network Steering Committee has been obtained. This does not apply if the information transfer is mandatory (e.g. submission to ethical committee). Despite the above, it is general policy of the NIDDK to encourage publication of results from clinical investigations. Submission of manuscript for publication will be decided upon by the ChiLDReN Steering Committee.

_____ _____
(Signature of Primary Investigator) (Date)

_____
Print Name of Primary Investigator
Investigator Copy (Original to be filed in the Site Regulatory Binder)

Contents

PROBE v.9 Amendment 08 INSTITUTIONAL REVIEW BOARD APPROVAL REQUIRED	YES

PROBE v.9 Amendment 08 REVISION OF INFORMED CONSENT REQUIRED	YES

1. Objectives

The primary objectives of this research are to establish (1) a database containing clinical information and (2) a repository of blood and tissue samples from children with neonatal liver diseases such as biliary atresia and neonatal hepatitis to facilitate research in these important liver problems in children. Examples of the use of this database and repository are to study the pathogenesis and natural history of biliary atresia and neonatal hepatitis or to evaluate patterns of cellular gene and protein expression in tissue specimens and plasma by viral, genomic, and proteomic techniques.

The study population will consist of infants, both male and female, with cholestasis who are less than or equal to 180 days old at the time of diagnosis at a Childhood Liver Disease Research Network (ChiLDReN) clinical site. In order to study the natural history, participants will be followed through the age of 20, liver transplantation, or for children without biliary atresia, until complete recovery, off of all therapyor 12 months of age, whichever is later.

This study will:

1. Collect detailed clinical and demographic information about each participant at enrollment and during follow–up.
2. Obtain and store blood samples from the participant at diagnosis and during follow-up.
3. Obtain and store liver and biliary tissue that is removed during diagnosis (i.e., biopsy) or at time of surgery and that are not needed for diagnostic purposes.
4. Collect demographic and medical history of parents at enrollment.

Some samples of blood and tissue will be stored in repositories for future research. The data and biological specimens will be used for detailed study of the mechanisms and causes of liver problems in young children to better diagnose and manage these conditions. The participant will receive standard-of-care treatment and will not be restricted in type of treatment or from changes in treatment, such as newer treatments as they are developed. The participants may not directly benefit from participation in this research, but in the future other children with similar problems may benefit from new information that may lead to better medical care.

The clinical sites participating in this study are Ann & Robert H. Lurie Children's Hospital (Chicago), Cincinnati Children's Hospital Medical Center, The Children's Hospital (Denver), Texas Children's Hospital (Houston), Children's Hospital of Philadelphia, Children's Hospital of Pittsburgh of UPMC (Pittsburgh), University of California at San Francisco, Riley Hospital for Children (Indianapolis), Children's Hospital Los Angeles (Los Angeles), Seattle Children's Hospital (Seattle), The Hospital for Sick Children, Toronto (Ontario), Children's Healthcare of Atlanta (Atlanta), and the University of Utah (Salt Lake City). The Scientfic Data Coordinating Center (SDCC) is at the University of Michigan and Arbor Research Collaborative for Health both in Ann Arbor, Michigan.

The study is funded by the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK), which is part of the National Institutes of Health. The ChiLDReN Network is governed by a Steering Committee comprised of the Principal Investigators from each of the participating clinical sites, the SDCC Principal Investigators, and the NIDDK Project Scientists.

At the end of the grant period specimens will be kept in repositories under contract to NIDDK for future use by investigators using a peer review process.

2. Specific Aims

1. To establish a prospective database with demographic and clinical information about infants with cholestatic liver disease and their families.

2. To establish repositories for DNA, blood and tissue samples from these children and their first degree relatives.

3. To prospectively follow these children over time to characterize the natural history of the disease.

4. To identify risk factors (such as, environmental, infectious and genetic risk factors) related to onset, outcome, and to the success of treatment(s) for the different cholestatic diseases, with special emphasis on biliary atresia.

3. Background

Clinical Significance of Biliary Atresia and Idiopathic Neonatal Hepatitis

Neonatal cholestatic disorders are a group of hepatobiliary diseases occurring within the first three months of life in which bile flow is impaired and characterized by conjugated hyperbilirubinemia, acholic stools, and hepatomegaly. Overall, 1 in 2500 live births is affected with a neonatal cholestatic disorder. The two most common causes of neonatal cholestasis are biliary atresia and idiopathic neonatal hepatitis. Other causes include a variety of metabolic and genetic diseases, known infections, progressive familial intrahepatic cholestatic disorders, paucity of interlobular bile ducts, and many others. Biliary atresia is the most common of these disorders, occurring in approximately 1 in 8000 to 1 in 15,000 live births, and characterized by complete fibrotic obliteration of the lumen of the extrahepatic biliary tree within the first three months of life[1]. A recent study suggested that the prevalence of biliary atresia may be higher in African American children than in white children[2]. Fibrous obliteration may involve the entire extrahepatic biliary system or any part of the system, with injury and fibrosis of intrahepatic bile ducts as well (hence the term "extrahepatic" has been dropped in recent years from the name of this disorder). Biliary atresia is most likely a clinical phenotype resulting from a number of prenatal or perinatal insults to the hepatobiliary tree, although the etiologic factors and pathogenesis of the obliteration of the biliary tree are poorly understood[3]. In approximately 10-20% of patients with biliary atresia, another major congenital anomaly is present, suggesting that defective development of the bile duct system caused the biliary atresia[4]. In particular, the polysplenia syndrome (polysplenia, midline liver, interrupted inferior vena cava, situs inversus, preduodenal portal vein and malrotation of the intestine) is present to some degree in 8% to 12% of all children with biliary atresia[5]. Biliary atresia associated with other congenital anomalies has been termed the "fetal" or "embryonic form", although it may be a common phenotype of multiple prenatal etiologies[6]. For some cases, it has been proposed that these anomalies are caused by abnormal expression of genes (somatic or inherited mutations) that regulate bile duct development, such as those that determine laterality of thoracic and abdominal organ development (association with polysplenia syndrome). One such gene might be the human homologue to mouse inv, which, when mutated, leads to altered development of the biliary tree in mice who develop situs inversus[7]. Alternatively, an intrauterine insult may interrupt normal development of multiple organs, including the biliary tree. The more common (70-80% of cases) form of biliary atresia is not associated with other congenital anomalies and

has been termed the “perinatal” or “acquired form”, in which it is believed that various perinatal or postnatal events trigger progressive injury and fibrosis of a normally developed biliary tree[8]. Clinically, the fetal form of biliary atresia is associated with jaundice and acholic stools within the first three weeks of life; whereas the acquired form of biliary atresia generally has onset of jaundice and acholic stools in the second to fourth weeks of life, following a period of normally pigmented stools. Despite these potential disparate etiologies, the clinical phenotype of these two forms of biliary atresia may appear identical until other congenital anomalies are discovered upon clinical investigation.

Idiopathic neonatal hepatitis is a descriptive term used for cases of prolonged neonatal cholestasis in which the characteristic “giant cell hepatitis” lesion is present on liver biopsy, and in which no other infectious, genetic, metabolic, or obstructive cause is identified[9].In various series, idiopathic neonatal hepatitis may comprise up to 30-40% of all cases of neonatal cholestasis. Over the past two decades, patients believed to have idiopathic neonatal hepatitis were later found to have newly discovered metabolic diseases (such as alpha-1 antitrypsin deficiency, progressive familial intrahepatic cholestasis, neonatal iron storage disease, inborn errors of bile acid synthesis) and newer viral infections (e.g., parvovirus or HHV6 infection). Up to 20% of cases of idiopathic neonatal hepatitis are progressive, appear to be familial, and have a worse prognosis. These cases may indeed be caused by novel genetic or metabolic disorders, which are yet to be defined. The clinical presentation of idiopathic neonatal hepatitis and biliary atresia are similar, although patients with biliary atresia tend to appear well nourished, whereas those with idiopathic neonatal hepatitis are frequently small for gestational age and failing to thrive. In both conditions, jaundice, acholic stools, dark urine, and hepatosplenomegaly develop within the first three months of life, and conjugated hyperbilirubinemia with elevation of hepatocellular and canalicular enzymes is found during laboratory evaluation[9].

Although biliary atresia and idiopathic neonatal hepatitis are the main foci of this project, there are many other causes of neonatal cholestasis that may be investigated by ChiLDReN potentially leading to new knowledge and understanding of hepatocyte and biliary physiology and pathophysiology[10]. Recent identification of the genetic and molecular causes of several forms of progressive familial intrahepatic cholestasis (PFIC) (e.g., mutations in genes coding for BSEP, FIC1, and MDR3)[11] has not only provided explanation for etiology of these rare but devastating disorders, but moreover, has led to the discovery of new bile acid and phospholipid

membrane transporters. This new knowledge has revolutionized our understanding of mechanisms of bile flow, predisposition to gallstone disease, and role of heterozygote states in these and other genes modifying or causing other hepatobiliary diseases[12,13]. Alagille syndrome, alpha-1 antitrypsin deficiency, cystic fibrosis liver disease, TPN-related cholestasis, choledochal cyst and PFIC are other disorders worthy of investigation by this Network. Because infants and young children with other causes of neonatal cholestasis will be required as "disease controls" in the proposed database, a cohort of children with other relevant neonatal liver diseases will be tracked during this study and will available for additional investigation.

Diagnosis, Treatment, and Outcome – Current Limitations

In both biliary atresia and idiopathic neonatal hepatitis, infants may present with jaundice in the first 12 weeks of life, progressive loss of pigmentation in their stools, and development of hepatomegaly and splenomegaly[10]. Biliary atresia is more commonly found in infant girls who were appropriate for gestational age at birth and appear to be thriving. Idiopathic neonatal hepatitis is more common in infant males who were small for gestational age, with signs of failure to thrive. Liver biopsy in biliary atresia generally shows bile ductular proliferation, canalicular and cellular bile stasis, portal or periportal fibrosis with the presence of bile plugs in portal tract bile ducts[4]. Hepatocyte giant cell transformation is found in at least 25% of patients with biliary atresia, particularly if the biopsy is obtained in the first six weeks of life. The liver biopsy in idiopathic neonatal hepatitis shows lobular disarray, a variable inflammatory infiltrate with marked giant cell transformation of individual hepatocytes, individual hepatocyte necrosis and apoptosis, increased extramedullary hematopoiesis, and cellular bile stasis. However, bile plugs in portal tract bile ducts are absent, and bile ductular proliferation is usually minimal or absent. Portal tract fibrosis is occasionally found, but is not extensive[10].

Diagnosis: It is essential that the diagnosis of biliary atresia be established as early as possible in the course of the patient's clinical presentation to allow for a successful portoenterostomy. Delay in diagnosis is a considerable problem in the United States because neonatal jaundice may be incorrectly ascribed to "breast milk jaundice", infants may be seen by health care providers only once or twice by 60 days of age, and physician assistants or nurses unaware of these rare disease may be providing the patient care. The diagnosis is established following exclusion of intrahepatic (infectious, metabolic, genetic, and toxic) causes of cholestasis and choledochal cyst (by ultrasonography). Biliary atresia is then diagnosed at the time of mini-laparotomy by intraoperative cholangiography that fails to demonstrate a lumen in some portion

of the extrahepatic biliary tree, surgical findings and characteristic findings on liver and bile duct histology, in the absence of other known etiologies[10]. A percutaneous liver biopsy prior to laparotomy has a diagnostic accuracy for biliary atresia by experienced pathologists of between 90-95% if an adequate biopsy size is obtained[14,15].

Other diagnostic studies are less accurate in differentiating biliary atresia from intrahepatic causes of cholestasis. No serum or urine biochemical tests differentiate between these two disorders. Imaging studies are also inconclusive. For example, failure of isotope excretion into the small intestine during HIDA hepatobiliary scintigraphy has only a 50-75% specificity for biliary atresia despite over 95% sensitivity[16]. Ultrasonography may show a small, non-distended gallbladder (suggesting biliary atresia) if severe intrahepatic cholestasis is present and, conversely, may show a clear fluid-filled gallbladder remnant in biliary atresia that is indistinguishable from normal. This modality is also not sensitive enough to determine presence or absence of the common hepatic and common bile ducts in small infants. However, recently Choi et al[17] suggest that a unique triangular or tubular echogenic density or triangular cord representing the fibrous cone of the bile duct remnant at the hepatic porta may be a specific ultrasonographic finding for biliary atresia. In addition, ultrasonography may visualize congenital anomalies in the abdomen (the polysplenia syndrome) that strongly suggest biliary atresia. Thus, ultrasonography plays an important role, but is generally not diagnostic for biliary atresia. Early studies suggest that magnetic resonance cholangiography (using T2-weighted turbo spin-echo sequences) may hold promise as a non-invasive method for diagnosis of biliary atresia[18]. Finally, the use of endoscopic retrograde cholangiography (ERC) has been proposed for identification of the extrahepatic biliary tree, although it requires considerable technical expertise and the proper sized side-viewing endoscope is not widely available[19]. Because of the small number of cases at any clinical site, investigation of the utility and appropriateness of each of these newer techniques for evaluating cholestatic infants needs to be conducted in a multi-centered manner, as made possible by the ChiLDReN Network.

The diagnosis of idiopathic neonatal hepatitis is assigned only after infectious, metabolic, genetic, and structural causes of a “giant cell hepatitis” are excluded[10]. Therefore, as newer etiologies are discovered, infants thought previously to have “idiopathic” neonatal hepatitis may have their diagnosis reassigned. For these reasons, there is an urgent need for improved nosologic classification of disorders causing neonatal cholestasis.

Treatment: Optimal therapy for biliary atresia diagnosed before 12 weeks of age is a Kasai

portoenterostomy, in which a Roux-en-Y loop of jejunum is anastomosed to the porta of the liver after a careful surgical dissection to locate patent bile duct remnants in the porta. If performed within the first 60 days of life by experienced surgeons, the portoenterostomy should yield bile drainage[20] from the liver into the intestinal tract in at least 70-80% of cases, resulting in increased pigmentation of the stools and resolution of jaundice[14,21]. If performed between 60 and 90 days of life, approximately 40-50% of patients show bile drainage, and if performed after 12 weeks of life, only 10-20% of patients, at best, show evidence of bile drainage. Thus, many surgeons will not perform the portoenterostomy in infants with biliary atresia who present at ages beyond three to four months[14]. Consequently, it is absolutely essential that jaundiced infants older than two weeks of age be evaluated for conjugated hyperbilirubinemia expediently, undergo an evaluation for causes of conjugated hyperbilirubinemia (if present), and that prompt surgical exploration is performed if the diagnosis of biliary atresia cannot be excluded by diagnostic tests.

Post-operatively, ascending cholangitis and sclerosis of patent intrahepatic bile ducts may lead to progressive biliary cirrhosis and liver failure[22].There is no standardized protocol for postoperative management of biliary atresia patients in the United States. Antibiotic suppression of cholangitis, use of short courses of corticosteroids to treat refractory cholangitis, empiric use of ursodiol to stimulate bile flow, and optimization of nutrition and prevention of fat-soluble vitamin deficiencies are frequently used; however, the efficacy of these approaches has not been determined and there is no uniformity in clinical practice[14,23].

The treatment of idiopathic neonatal hepatitis is largely supportive, involving optimization of nutrition, prevention of vitamin deficiencies, and use of choleretic agents and anti-pruritic agents[24].Therefore, infant formulas containing medium-chain triglyceride oil are preferred, fat soluble vitamin supplements are given, and oral ursodeoxycholic acid or cholestyramine are used to induce choleresis. In up to 20% of cases of idiopathic neonatal hepatitis, patients will show progression to cirrhosis and chronic liver failure and may require liver transplantation. In recent years, many of these patients with progressive neonatal hepatitis have been found to harbor a form of PFIC (Byler's disease), or MDR3 deficiency. Patients with PFIC types 1 and 2 may benefit from partial biliary diversion.

Outcome: If the portoenterostomy is not performed in biliary atresia, 80-90% of children will die (without liver transplantation) from biliary cirrhosis by one year of age, and 100% by two to three years of age. Successful portoenterostomy is associated with an approximately 30-40% 10-year

survival at the best centers in North America and Europe[21,25], whereas 10-year survival following portoenterostomy in patients in Japan may exceed 65%[4].If the portoenterostomy is not successful in establishing bile flow, survival without transplantation is similar to or worse than that of patients not undergoing surgery. Post-operative care following portoenterostomy differs in Japan from that used in the United States[4].In Japan, intravenous bile acid treatment (which is not available in the United States) is administered for up to several months and intravenous and oral corticosteroids are routinely given for at least two months following surgery[4]. Intravenous antibiotics and herbal therapies are also commonly used following biliary atresia surgery in Asian countries. It is not clear if these differences in treatment or other factors (e.g., genetic) are responsible for the improved prognosis in Japan. The majority of surviving patients will develop complications of portal hypertension, such as esophageal variceal hemorrhage[26] which can generally be treated medically and endoscopically. Nevertheless, 70-80% of patients with biliary atresia will require liver transplantation in North America during the first two decades of life, despite initial success with portoenterostomy[14,21]. Consequently, biliary atresia accounts for 40-50% of all liver transplants performed in children[27]. It should be noted that there is no single liver disease in adults that accounts for the large proportion of liver transplants. Factors that determine long-term survival without transplantation have not been carefully evaluated. Moreover, quality of life (QOL) outcome measures in biliary atresia and other cholestatic disorders have not been prospectively analyzed in a large enough cohort using age-specific tools that are now available[4,28].

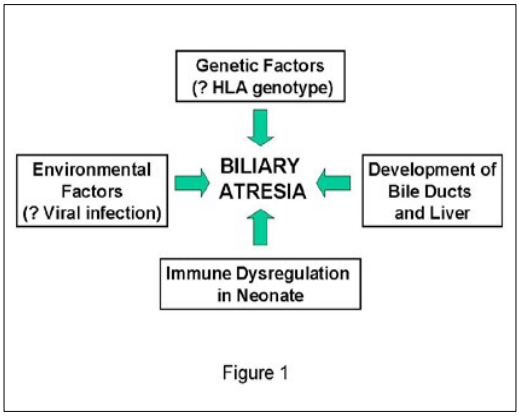

Figure 1

Biliary atresia accounts for about half of the $77 million spent each year on children for liver transplantation and the ensuing hospitalizations in the United States[29]. Liver transplantation amounts to 0.2% of total health care expenditures related to children, even though these children represent 0.0006% of the total pediatric population. Importantly, this disproportionate expenditure for liver transplantation in children could be cut in half if improved therapies for biliary atresia were developed that could abrogate the need for liver transplantation.

Current Theories of Etiology of Biliary Atresia and Neonatal Hepatitis

Our understanding of the etiology and pathogenesis of liver and bile duct injury in biliary atresia

and idiopathic neonatal hepatitis has remained essentially unchanged for the past three decades. Investigation into etiopathogenesis is urgently needed to provide a scientific basis for the development of novel therapeutic strategies. Currently, biliary atresia is believed to be a common phenotypic response of the neonatal liver and bile ducts to a variety of insults. It is proposed that these disorders are caused by various environmental insults (viral, metabolic, vascular) to the development or maturation of the biliary tree (for biliary atresia) or hepatocyte (for idiopathic neonatal hepatitis) that occur in a specific window of time (prenatally to before three months of age) amidst the milieu of a genetic or immunologic susceptibility to either of these diseases (Figure 1). Biliary atresia and idiopathic neonatal hepatitis are not believed to be inherited disorders (except for the 10-20% of familial cases of idiopathic neonatal hepatitis), since HLA- identical twins discordant for biliary atresia have been described, and recurrence of biliary atresia within the same family is exceedingly rare[30,31]. However, this does not exclude the possibility that during fetal development somatic mutations of key genes regulating morphogenesis of these structures may be involved. .Nevertheless, the majority of biliary atresia cases appear to have onset postnatally with normal development of other organs.

Viral Infection: Epidemiologic studies support a possible infectious etiology to biliary atresia and idiopathic neonatal hepatitis. There has been continued demonstration of seasonal clustering of cases suggesting environmental exposure to an infectious agent[2]. In addition, several models of viral infection in newborn mice produce lesions similar to biliary atresia[3], as described below. In 1974, Benjamin Landing, a pediatric pathologist, proposed that biliary atresia, idiopathic neonatal hepatitis and choledochal cyst represented the end result of different primary sites of injury to the hepatobiliary tree by a common insult, and coined the term “infantile obstructive cholangiopathies”[32]. Although Landing proposed involvement of the hepatitis B virus, subsequent studies have shown no association between the common hepatotropic viruses (hepatitis A, B and C) and biliary atresia. More recent attention has focused on the possible role of five viruses.

For many years, cytomegalovirus (CMV) has been proposed as a possible etiologic agent because a modest proportion of infants with biliary atresia and idiopathic neonatal hepatitis have been infected with CMV, as are normal infants[33]. Although a recent study from Sweden[34] showed a higher prevalence of CMV antibodies in mothers of biliary atresia patients, and CMV DNA was present in livers from 50% of infants with biliary atresia, a Canadian group[35] could not demonstrate CMV in bile duct remnants from 12 children with biliary atresia. The role of CMV

has not been explored in a large prospective multi-centered study with proper controls.

The two viruses most commonly implicated are reovirus and rotavirus. Interest in reovirus stemmed from the observation that infection in weanling mice causes pathologic features of the intrahepatic and extrahepatic bile ducts and the liver similar to those of biliary atresia[36].These lesions persisted even after infectious virus or viral antigens could no longer be detected. One group detected reovirus antigens in bile duct remnants from infants with biliary atresia[37,38] and in an infant Rhesus monkey with biliary atresia[39], although other groups could not replicate these findings in infants[40]. Serologic studies of reovirus antibodies in infants with biliary atresia have likewise been inconclusive[37,40,41]. The high incidence of passively transferred maternal anti-reovirus IgG may have confounded these studies. Two groups of investigators have examined hepatobiliary tissues removed from infants with biliary atresia for reovirus RNA. Steele et al[42] failed to detect reovirus RNA in archived, formalin-fixed preserved hepatic tissues of 14 biliary atresia patients, 20 idiopathic neonatal hepatitis patients, and 16 controls, using a nested reverse transcriptase (RT) PCR assay. In contrast, Tyler et al[43] reported nested RT-PCR evidence of reovirus infection in snap frozen liver or bile duct from 55% of cases of acquired/perinatal forms of biliary atresia and in only 8-15% of autopsy controls and infants with other liver diseases under one year of age. The discrepancies between these two studies may lie in the methods of preparation of the tissue, different methods of RNA isolation, and the use of PCR primers for different reovirus genes. If reovirus were shown to be involved, potential anti-viral strategies (e.g., ribavirin) could be entertained. Although the bulk of the evidence favors reovirus as being involved in the etiology of the perinatal form of biliary atresia, this is far from conclusive, and can only be definitively evaluated in a study with large numbers of well-characterized patients and appropriate disease and normal controls.

Recent interest has also focused on Group C rotavirus (another virus of the Reoviridae family) in the etiology of biliary atresia. Group A rotavirus infection was shown to produce extrahepatic bile duct obstruction in newborn mice with hepatic histology similar to biliary atresia[44]. Petersen et al[45] subsequently reported that the administration of interferon-α prior to rotavirus infection prevented the biliary disease and Qiao et al[46] reported an increase in the incidence of bile duct obstruction in normal newborn BALB/c mice compared to SCID (immunodeficient) mice infected with rotavirus, indicating the role of the immune system in this mouse model. Riepenhoff-Tolte et al[47] examined hepatobiliary tissues of human patients for RT-PCR evidence of Group C rotavirus infection. Ten of 18 biliary atresia patients and 0 of 12 liver disease control patients

showed evidence of rotavirus RNA. In contrast Bobo et al[48] failed to detect RNA evidence for rotavirus Groups A, B, or C in tissues from 10 biliary atresia patients using an RT-PCR enzyme immunoassay, however, almost half the patients were over 12 months of age at the time that tissues were obtained. Thus, there is suggestive evidence that rotavirus infection may be involved in up to 50% of cases of biliary atresia, similar to the prevalence of reovirus infection in the study of Tyler et al[43].

The possible role of other viruses has recently been investigated. Human papilloma virus (HPV) was detected by PCR in archived liver tissue from 16 of 18 biliary atresia patients compared to no control patients from Argentina[49,50]. However, Domiati-Saad et al[51] failed to demonstrate evidence of HPV DNA in 19 patients with biliary atresia or idiopathic neonatal hepatitis from the United States, although they did detect HHV6 DNA in several cases of neonatal hepatitis and biliary atresia. The possible role of HPV and HHV6 in biliary atresia is unsettled and requires further investigation.

Finally, Mason et al[52] recently described immunoreactivity to retroviral proteins in serum from patients with biliary atresia, as well as adult cholestatic liver diseases. They attributed this to an autoimmune response to antigenically-related cellular proteins or to an immune response to uncharacterized viral proteins. Further work in this potentially important area in both adult and pediatric biliary disorders is warranted.

Immune Injury in Biliary Atresia: Schreiber et al[53] proposed that biliary atresia was the result of a "multi-hit" pathologic process, in which a viral or toxic insult to biliary epithelium leads to newly expressed antigens on the surface of bile duct epithelia, which, in the proper genetically-determined immunologic milieu, are recognized by circulating T-lymphocytes that elicit a cellular response causing bile duct epithelial injury, eventually resulting in fibrosis and occlusion of the extrahepatic bile duct. Unique aspects of innate and acquired immunity that are present in the neonate may also play an important role in determining why these disorders only present within the first three months of life and in a small percentage of infected infants. In addition, passively acquired maternal factors could potentially affect presentation and immune recognition of antigens and T-cell activation in the neonate, causing liver injury as it does in the neonatal lupus syndrome[54].

Silviera et al[55] reported an association of HLA-B12 (49% biliary atresia patients vs. 23% of controls) and of haplotypes A9-B5 and A28-B35 with biliary atresia. Other groups could not

replicate these findings but reported a relationship of biliary atresia with HLACw4/7[56], and in Japan with A33, B44, and DR6[57]. These disparate results may reflect genetic differences among ethnic populations or chance associations. Since HLA genotypes have been associated with a variety of immune and autoimmune diseases, MHC Class I and Class II genotypes may predispose to biliary atresia or idiopathic neonatal hepatitis, a hypothesis that needs to be investigated in a larger multi-ethnic cohort of patients.

A number of investigators have characterized the nature of the inflammatory infiltrate and associated cytokines present in biliary atresia tissues. In 1977, Gosseye et al[58] demonstrated lymphocytes in the connective tissue of the portahepatis in biliary atresia patients, and Bill et al[59] pointed out the relationship of intramural mononuclear inflammatory cells with epithelial cell necrosis in bile duct remnants. In 1995, Ohya et al[60] further showed that degeneration of intrahepatic bile ducts was associated with lymphocytic infiltration into bile duct epithelial cells in biliary atresia at the time of diagnosis. These initial studies clearly established the possible role of T-cell-mediated bile duct injury in biliary atresia.

In order for T-cells to effectively mediate inflammation, they must encounter antigen presented by a competent antigen-presenting cell (APC). Two signals are required for full T-cell activation from the APC's, including surface expression of self-MHC molecules bearing the antigenic peptide which interacts with the T-cell receptor, and also co-stimulatory molecules (B7-1, B7-2) that interact with CD28 on the T-cell[61]. Adhesion of APC's with T-cells also requires the expression of intracellular adhesion molecules (ICAMs). Helper T-cells (CD4+) recognize antigenic peptides in the context of self-MHC Class II expression, and cytotoxic T-cells (CD8+) recognize antigen in the context of self MHC Class I molecules. Based on this paradigm, several investigators have proposed that bile duct epithelial cells may function as APC's in biliary atresia. Normally, MHC Class I antigens, but not those of Class II, are expressed by bile duct epithelium. However, several group[56,57,62] showed that HLA-DR (MHC Class II molecules) were aberrantly expressed by bile duct epithelium in liver specimens of biliary atresia patients. Davenport et al[63] further demonstrated that CD4+ lymphocytes and natural killer (CD56+) cells predominated in the liver and extrahepatic bile duct in biliary atresia, that the cellular infiltrate was both activated and proliferating, and that ICAM-1 was expressed in sinusoidal endothelium. These data are consistent with the hypothesis that lymphocyte adhesion and T-cell activation and cytotoxicity, at least in part, mediate the extrahepatic bile duct damage and obliteration in biliary atresia.

The Kupffer cell (resident liver macrophage) may also function as an APC cell in the liver. A recent study from Japan demonstrated increased numbers and size of Kupffer cells in liver tissue of biliary atresia patients at the time of diagnosis[64]. Davenport[63] also showed that an increase in CD68+ macrophage infiltration (Kupffer cells) in portal tracts and biliary remnant tissue of biliary atresia patients was predictive of a poor outcome after the portoenterostomy procedure, consistent with the function of activated macrophages to release cytokines, reactive oxygen intermediates, and growth factors that may signal hepatic stellate cells to synthesize and secrete collagen, thereby promoting fibrogenesis and cirrhosis. One other important feature of macrophages is the capability to secrete tumor necrosis factor-α (TNF-α), reactive oxygen species, and nitric oxide which may be involved in the induction of both apoptotic and necrotic intracellular pathways. Along these lines, Funaki et al[65] have shown that apoptosis of intrahepatic bile duct epithelial cells is highly prevalent in biliary atresia liver compared to normal liver or that of patients with choledochal cyst. Moreover, Liu et al[66] reported a relationship between fas ligand (FasL) mRNA in bile duct epithelial cells and the presence of apoptosis in biliary atresia patients. Since FasL is not normally expressed in bile duct epithelial cells, this finding appeared to be specific to biliary atresia. Surprisingly, bile drainage after the portoenterostomy procedure was significantly better in patients with negative signals for FasL on bile duct epithelium than patients with positive signals, suggesting that upregulation of FasL may result in apoptotic fratricide in which bile duct epithelial cells actually injure other similar cells, or perhaps bile duct epithelium are resisting attack by infiltrating lymphocytes by posing a counterattack against Fas-expression lymphocytes[66]. These provocative results emphasize interactions between macrophages, T-lymphocytes, bile duct epithelial cells, hepatocytes, and other cells in the liver which should be extended in future studies conducted by ChiLDReN . The proposed serum and tissue bank outlined in this protocol should provide the specimens needed to conduct a thorough investigation into immune mechanisms in biliary atresia and idiopathic neonatal hepatitis.

Autoimmunity in Biliary Atresia. Biliary atresia shares features with several autoimmune diseases, such as the female predominance, apparent triggering by viral infection, and aberrant HLA expression in bile duct epithelium. Thus, it has been proposed that tissue injury biliary atresia may represent an autoimmune mediated process. Vasiliauskas et al[67] have reported that 10 of 11 patients with biliary atresia were positive for serum IgG and IgM antineutrophil cytoplasmic antibodies (ANCA), with higher levels of the IgM- ANCA in biliary atresia patients compared with children and adults with other liver diseases. Burch et al[54] studied autoantibodies

in mothers of children with biliary atresia and idiopathic neonatal hepatitis, in order to test the hypothesis that maternal transfer of autoantibodies might be involved in liver and bile duct injury. The results showed that low titer anti-Rho antibodies were more common in mothers of infants with biliary atresia and idiopathic neonatal hepatitis than in controls, and that low titer antinuclear antibodies were also more common in mothers of infants with liver disease. The ChiLDReN Network will provide an ideal venue for future investigation of maternal factors and autoimmunity in biliary atresia.

An exciting advance in understanding risk factors for autoimmunity has been the demonstration of polymorphisms in genes that predict susceptibility of individuals to autoimmune disorders. Recent reports of Bernal et al[68] and Mitchell et al[69] have shown that TNF-α gene polymorphisms are associated with susceptibility to primary sclerosing cholangitis (58% of PSC vs 29% of controls in Bernal's study), raising the possibility that genetic differences in genes that regulate immune function, the inflammatory response, cellular regeneration, and cell survival signals may predispose to biliary injury in various clinical settings. Extending on these observations, it may well be that genes that regulate metabolism and transport of bile acids and phospholipids play a role in protection from bile duct injury in biliary atresia. Serum, DNA, and tissue specimens made available by ChiLDReN will be instrumental in determining if genetic risk factors for autoimmunity play a role in neonatal cholestatic disorders.

Vascular Etiology. A vascular/ischemia etiology for biliary atresia has been proposed based on experimental evidence[70]. Pickett et al[71] demonstrated the development of biliary obstruction following ligation of hepatic vessels in fetal sheep. Intrahepatic and extrahepatic bile ducts receive their blood supply exclusively from the hepatic arterial circulation, such that hepatic artery ischemia may lead to bile duct strictures, particularly following liver transplantation. Several investigators have demonstrated an arteriopathy in branches of the hepatic artery in the extrahepatic biliary tree of biliary atresia patients[72]. It has been proposed that the vasculopathy may be the primary lesion in biliary atresia; however, whether these lesions are primary or secondary to another process remains unclear.

Defective Morphogenesis: Several lines of evidence suggest that certain cases of biliary atresia are caused by defective morphogenesis of the biliary tree. Because anomalies of visceral organ symmetry are associated with biliary atresia, it is of interest that a recessive insertional mutation in the proximal region of mouse chromosome 4 or complete deletion of the inversion (inv) gene in the mouse leads to anomalous development of the hepatobiliary system in this model[7,73].

Important work by Mazziotti et al[7] in the inv mouse suggests that this gene plays an essential role in morphogenesis of the hepatobiliary system. It will be necessary to investigate homologues of this and other related genes in infants with biliary atresia to determine whether inherited or somatic mutations or deletions are responsible for individual cases of biliary atresia.

Intrahepatic bile development depends on interactions between mesenchyme and portal venous radicals. Primitive hepatic precursor cells differentiate into a single layer of cells that soon form a double layer as the primitive bile ductule anlage. Cells then scatter and remodel as a single layer around the lumen to form the portal tract bile duct[8]. Defects in remodeling of this ductal plate lead to the ductal plate malformation that is believed to be responsible for the liver lesion of congenital hepatic fibrosis and other bile duct dysplastic diseases. However, a number of infants with biliary atresia appear to show evidence of the ductal plate malformation on liver biopsy[8], suggesting that interactions between hepatocyte growth factor or scatter factor and receptors such as the c-met oncogene may be defective in cases with biliary atresia and ductal plate malformatiom[1,74]. Abnormalities in induction of hepatocyte growth factor during a critical period for mesenchymal/epithelial signaling or other defects in the intracellular adhesion systems could account for defective bile duct development in biliary atresia and other disorders. Further investigation of this important area of bile duct development is necessary.

Toxin Exposure: Time and space clustering of cases of biliary atresia have led to the proposal that an environmental toxin could be involved in its pathogenesis. Currently, other than infectious agents, no environmental agent has been clearly associated with biliary atresia or idiopathic neonatal hepatitis. It is clear that the etiologies of biliary atresia and idiopathic neonatal hepatitis remain poorly understood and that the future development of new diagnostic, preventative and therapeutic strategies will require a better understanding of the causative factors. The ChiLDReN Network will provide an ideal environment in which to investigate multiple proposed etiologies simultaneously through hypothesis-directed investigations.

4. Study Design

4.1. Design and Outcomes

This is a multi-center project to establish a prospective database of clinical information and a repository of blood and tissue samples from children with diagnoses of neonatal liver diseases, such as biliary atresia and neonatal hepatitis, in order to perform research in these important liver problems. Children will be screened and enrolled at presentation at the participating

pediatric liver sites. Participants diagnosed with biliary atresia will be followed intensively for the first year, at 18 months of age, and then annually up to 20 years of age, or liver transplantation. Other participants diagnosed with ongoing cholestasis will be followed on the same schedule; if there is complete (clinical and biochemical) resolution of their underlying liver disease off all therapy, there will be one follow-up visit within one year (preferably scheduled at the time of the next planned follow-up visit or at 12 months of age, whichever is later) for data collection and to obtain blood samples. The development of a serum and tissue bank of specimens from children with various neonatal cholestatic disorders will be an invaluable tool for current and future investigations into the etiology and pathogenesis of hepatobiliary injury in the infant.

Detailed clinical data, laboratory investigations, liver biopsy specimens, and long-term follow-up of outcomes are part of the normal standard of care with respect to the diagnosis and treatment of the participants with liver problems. This research involves the collection of diagnostic, clinical and outcome data concerning the participant, which is kept without identification (coded) in a national research database of infants with liver disease. Samples of blood will be obtained for later research analysis, whenever possible, at the time of clinically indicated blood draws or when there is IV access for a clinical procedure. When liver biopsy specimens are obtained for diagnostic purposes or at the time of portoenterostomy, any liver biopsy specimen in excess of that needed for diagnostic use will be sent to the repository These specimens will be used in investigations into the mechanisms and causes of the liver damage that occur in the participant's condition. As part of the standard of care, the study will follow-up and record progress of the liver problem by routine clinical examinations and laboratory tests for up to 20 years of age or until liver transplantation. All data from this study will be kept in a secure research database at the SDCC and transferred to the NIDDK data repository after the study ends.

This multi-center network of investigators will address issues of etiology, pathogenesis, natural history, diagnosis, and novel treatments for one of the most devastating pediatric illnesses that occur in the first few months of life, biliary atresia, and related disorders such as idiopathic neonatal hepatitis. Although these disorders are not common, the effects on children, siblings, and their families and the frequent need for liver transplantation, mandate a greater degree of attention by the scientific community than in the past. Moreover, knowledge gained from these investigations has a high likelihood of affecting diagnosis and treatment of adults with cholestatic liver disease and improving our understanding of fundamental processes regulating

bile flow, cytoprotection, and liver and biliary development. The development of a serum and tissue bank of specimens from children with various neonatal cholestatic disorders will be an invaluable tool for current and future investigations into the etiology and pathogenesis of hepatobiliary injury in the infant.

4.2 Enrollment of Participants

The study population to be enrolled will consist of male and female infants less than or equal to 180 days old. All racial and ethnic groups will be included.

4.2.1 Inclusion Criteria

- Infant's age less than or equal to 180 days at initial presentation at the ChiLDReN clinical site.
- Diagnosis of cholestasis defined by serum direct or conjugated bilirubin greater than 20% of total and greater than or equal to 2 mg/dl.
- The participant's parent(s)/guardian(s) able to provide informed written consent.

4.2.2 Exclusion Criteria

- Acute liver failure.
- Previous hepatobiliary surgery with dissection or excision of biliary tissue.
- Diagnoses of bacterial or fungal sepsis (except where associated with metabolic liver disease).
- Diagnoses of hypoxia, shock or ischemic hepatopathy within the past two weeks (If the cholestasis persists beyond two weeks of the initiating event, the infant can be enrolled).
- Diagnosis of any malignancy.
- Presence of any primary hemolytic disease (except when diagnosed with biliary atresia or another cholestatic disease being studied by ChiLDReN).
- Diagnosis of any drug or TPN-associated cholestasis (except when diagnosed with biliary atresia or another cholestatic disease being studied by ChiLDReN).

- Diagnosis with ECMO-associated cholestasis.

- Birth weight less than 1500g (except when diagnosed with biliary atresia).

4.2.3 Exceptions to the Inclusion/Exclusion Criteria

Infants with biliary atresia with a birth weight of less than 1500g may be included in the database. If the diagnosis is subsequently not confirmed, the infant will become ineligible.

Similarly, infants with a hemolytic disorder, or a diagnosis of any drug or TPN-associated cholestasis, who have biliary atresia or another cholestatic disease being studied by ChiLDReN may be included in the database. If the diagnosis is subsequently not confirmed, the infant will become ineligible.

When an eligible diagnosis, such as metabolic liver disease, is suspected but is not yet ascertained at the time of initial evaluation, the infant should be recruited into the database; the infant will become ineligible if the diagnosis subsequently does not confirm cholestatic disease.

4.2.4 Study Enrollment Procedures

Participants will be recruited from patients evaluated at, referred to, and followed at the ChiLDReN clinical sites. The investigator or clinical research coordinator will recruit the parent(s) or guardian(s) during clinic visits or during an inpatient admission to the hospital. The investigator will discuss the study design, benefits and possible risks with the family. A copy of the consent form will be given to the family. The IRB-approved consent will include the purpose of the trial, the responsible parties and investigators, potential benefits, risks of participation, the right to refuse to be in the study, the right to withdraw from the study under no penalty, contact numbers, and information about the responsibility for injury and payment for medical care. If the family consents to entry into the study, written informed consent will be obtained from the parents or guardians and case report forms will be completed. The study will be listed on www.clinicaltrials.gov and the ChiLDReN website www.ChildrenNetwork.org.

4.3 Clinical and Laboratory Evaluations

4.3.1 Schedule of Evaluations

The following table indicates the schedule of expected visits and times of data and sample collection. The term ‘post’ refers to the period of time following surgery, either a

portoenterostomy (Kasai) or exploratory surgery to rule out biliary atresia, or the period of time following a definitive diagnosis (or intake, whichever is later) at the ChiLDReN clinical center. The term 'age' refers to chronological age. The term 'surgery' refers to the portoenterostomy procedure or the exploratory surgery to rule out biliary atresia.

Infants with cholestasis will be identified at the time of their clinic visit for evaluation or hospital admission. An investigator or research coordinator will approach the parents and/or guardians and explain the study. If a parent or guardian gives written informed consent, they will be asked for a convenient time to meet with the coordinator to complete forms describing the infant's medical history, the mother's pregnancy history, and familial histories. Since these forms are lengthy and it is desirable to obtain information about both parent's family histories, the coordinator will have flexibility in scheduling the completion of forms during the recruitment/baseline phases.

The types of visits are:

- **Recruitment**: Following diagnosis of cholestasis in an infant less than or equal to 180 days, the family will be approached for recruitment into the study. At least one parent or guardian must sign written informed consent before data collection can begin.
- **Baseline**: Once informed consent is obtained, the coordinator may abstract information from the participant's medical chart and meet with the parent(s)/guardian(s) to complete the intake and history forms (see below for details).
- **Surgery/Diagnosis**: The timeline for follow-up is triggered either by the date of the portoenterostomy for patients with biliary atresia or the date that the diagnosis is confirmed for other participants.
- **In-patient/Discharge**: For in-patients, data will be collected from the time of surgery or diagnosis to the time of discharge.
- **Follow-up:** The participant with a native liver will be followed for 20 years of age or up until the time of liver transplant at the times indicated in Table 4.1.

Table 4.1 Schedule of Evaluations for Participants with Native Liver

Evaluation	RECRUITMENT OR BASELINE	DIAGNOSIS /SURGERY/ DISCHARGE	4 WK POST 2 MO POST 3 MO POST	6 MO POST*	12 MO AGE*	18 MO AGE	ANNUALLY FROM AGE 2-	AT TRANSPLANT	COMPLETE RESOLU-TION w/o BA
Visit windows			±2WKS	±1 MO	±1 MO	±2 MO	±6 MO		
Informed Consent	X								
Eligibility	X								
Intake History/Exam	X								
Diagnosis		X							
Surgical Procedure (if performed)		X						X	
Discharge Assessment		X							
Follow-up Visits			X	X	X	X	X		**
Liver Biopsy		X							
Serum and Plasma Samples	X†	X†	X†	X†	X†	X†	X†		X
Parent and Child Blood or Saliva for DNA***	X One time collection								
Parents' Medical History	X								

*The 6 mo post and 12 mo of age visits will be combined when the 6 mo post visit is at 10 mo of age or greater.

**Participants without biliary atresia. will complete one scheduled visit (preferably scheduled at the time of the next planned follow-up visit or at 12 months of age, whichever is later) after complete resolution of their liver disease.

***The one-time DNA collection can occur at any visit. For those participants aged 1 year or less, DNA can only be collected via whole blood (if the required volume (1-3 ml) can be obtained while keeping within age and/or size specific volume limitations). Saliva collection cannot be used for DNA in children less than 1 year of age.

†Serum and Plasma samples will be collected at Baseline, months 1, 3, 6, 12, 18, and 24 along with years 3, 4, 5, 6, 7, 8, 9, 10, 12, 14, 16, 18, and 20.

4.4 Data to be Collected

At Recruitment/Baseline:

CRF 01 Eligibility: Fulfillment of the inclusion/exclusion criteria.

CRF 02 Demographics of infant and parents: Infant (a): Date of birth, sex, birth weight, race and ethnicity; and Parents (b): Date of birth, race and ethnicity, residence, marital status, education, employment status/income, and type of medical insurance.

CRF 03 Medical history of infant: History of medical consultations and presenting symptoms at this visit, and assessment of barriers to access of care related to health insurance.

CRF 04 Pregnancy history of the natural mother: Last pregnancy: Prenatal history, medications, alcohol and tobacco use during pregnancy and after deliverylabor and delivery, complications of the newborn, and antepartum hemorrhaging; Previous Pregnancy History: Outcome of each pregnancy, including complications and current status of each live birth; Additional Maternal Medical History Prenatal Tests: Abnormal ultrasound.

CRF 05 Maternal (biological mother) family history with an emphasis on liver and autoimmune diseases and congenital abnormalities: Whether child's biological mother is living, detailed disease history of all first order biological relatives including the infant's mother, mother's siblings, and infant's maternal grandparents, and siblings of the infant.

CFR 06 Paternal (biological father) family history with an emphasis on liver and autoimmune diseases and congenital abnormalities: Whether child's biological father is living, detailed disease history of all first order biological relatives including the infant's father, father's siblings, and infant's paternal grandparents.

CRF 07 Physical exam: Findings at the intake examination - Measurements: Weight, length, head circumference, skinfold thickness, liver, spleen; Appearance: Jaundice, cyanosis, facial features; Assessments of systems: Cardiovascular, abdominal/ gastrointestinal/hepatic, musculoskeletal, urogenital.

During Diagnosis/Surgery:

CRF 08 Laboratory results: Results of the initial clinical laboratory workup: Liver function tests, CBC with differential, blood chemistry, vitamin levels, metabolic and genetic mutation tests,

serological studies, urinalysis and coagulation profile.

CRF 09 Imaging results of diagnostic studies that were performed: Ultrasound findings of the gallbladder, bile ducts and liver; hepatobiliary scan; MRCP; chest X- ray; Results of any other diagnostic testing.

CRF 11 Surgical findings during exploration and/or portoenterostomy: Abdominal anatomy: Assessment of intestinal malrotation, liver, portal vein, presence of ascites; Hilar biliary anatomy: Assessment of gall bladder and bile duct; Other findings: Post exploration diagnosis, biliary atresia anatomic classification, Hilar dissection, drainage procedure, intraoperative complications.

At Hospital Discharge or Final Diagnosis:

CRF 12 Hospital discharge: Post-operative complications and medical condition at discharge: Complications: E.g., fever, infections, ascites, hemorrhage; Physical examination: General appearance, liver and spleen size, presence of ascites; Laboratory evaluation at weekly intervals post-portoenterostomy (biliary atresia patients only) or at discharge: Liver function tests, CBC with differential, blood chemistry, and coagulation profile.

CRF 13 Discharge medications: Medications at discharge; feeding type at discharge.

Manifest: Samples collected for the repository: blood, liver tissue.

CRF14 Final diagnosis.

At Follow-up Visits:

CRF 20 Physical exam: Vital signs, anthropometric measurements, Tanner score from age eight unless precocious puberty is observed, physical exam of the liver , facial features.

CRF 22 Diet and medication record: Type of feeding and diet, vitamin and dietary supplements, and prescription medications.

CRF 23 Laboratory results: Liver function tests, blood CBC, blood chemistry, and coagulation profile.

CRF 24 Interval history: History of medical consultations between visits: Interval sentinel events: Complications that occurred between visits, symptoms, lab tests, and treatment, as appropriate.

Surgery: Surgical and diagnostic procedures that occurred between visits.

Imaging: Findings of ultrasound, hepatobiliary scan, MRCP, etc.

Manifest: Samples collected for the repository: Blood and/or saliva. .

CRF 35 Final status: Completion of study, lost to follow-up, transplant, or death.

4.5 Specimens to be Collected:

From the biological mother and father:

1. 10 ml of whole blood in one 10 ml NaEDTA vial for DNA extraction to be sent to the NIDDK Central Repository facility.

2. 2 ml of saliva collected in a saliva collection kit in the event that blood collection is not possible or contraindicated.

DNAwill be sent to the NIDDK Central Repository for storage until use. Therefore, a total of 10 ml of whole blood will be removed from each parent.

From the infant during initial work up/diagnosis whenever it is most convenient and least intrusive; i.e., at the time of clinically indicated blood draws or when there is clinically indicated IV access: 4 ml of whole blood will be drawn in two tubes.

1.) 2 ml of whole blood to extract plasma: (~6 aliquots of 0.2 ml each).

2.) 2 ml of whole blood to extract serum: (~6 aliquots of 0.2 ml each).

At the time of initial liver biopsy, exploratory surgery, or portoenterostomy, any liver tissue that is removed as part of the clinical surgical procedure, but is not needed for diagnostic purposes, will be collected for the repository. In participants undergoing a portoenterostomy or other biliary reconstruction at < 40 days of age, a portion of the excised biliary remnant and adjoining gallbladder may also be obtained. Hence, when removed as part of the clinical procedure and based on availability after samples needed for diagnosis, the following may be obtained for the repository:

1. Tissue from the liver

2. Unstained paraffin-embedded slides of the liver

3. Tissue from the biliary remnant, in patients < 40 days old

4. Unstained paraffin-embedded slides of the biliary remnant, in patients < 40 days old

From the infant at follow-up visits: (4 weeks post surgery or post diagnosis if the infant does not have biliary atresia, 3 and 6 months post, and at 12 mo, 18 mo, 24 mo of age, 3, 4, 5, 6, 7, 8, 9, 10, 12, 14, 16, 18, 20 years of age thereafter):

1.) 2 ml of whole blood for plasma

2.) 2 ml of whole blood for serum

The one-time DNA collection can occur at any visit. For those participants aged one year or less, DNA can only be collected via whole blood (if the required volume [1-3 ml] can be obtained while keeping within age and/or size specific volume limitations). Saliva collection cannot be used for DNA in children less than one year of age.

NOTE: *If an inadequate volume is collected for DNA, a redraw of blood sample may be requested.*

Table 4.2 Amount of Blood Drawn from Infants

Visit	Amount in ml drawn for research at the visit	Maximum research blood draw in ml within 2-month period	Maximum research blood draw in ml within 3-month period
Initial	4	4	4
4 weeks post op or post diagnosis	4	8	8
3 months post op or post diagnosis	4	8	12*
6 months post op or post diagnosis	4	8 **	8 **
12 months of age	8	8	8
18 months of age	4	4	4
Ages 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 14, 16, 18, 20	4	4	4

* This would only happen if the three-month post-op or post-diagnosis visit was scheduled before the three-month anniversary of the operation or diagnosis.

** When the six-month post-op or post-diagnosis visit is at 10 months of age or greater, the blood draw at that visit will be that for 12 months of age and there will be no blood draw at 12 months of age.

NOTE: *If whole blood or saliva has not been collected for DNA, it should also be collected at or prior to the time of transplantation.*

NOTE: *Blood volume for clinically indicated tests: Approximately, 6.5 ml of blood may be removed from the child at each visit to evaluate hepatic function, electrolytes and differential. More may be withdrawn to perform additional clinically indicated lab tests.*

NOTE: *If an inadequate volume is collected for DNA, a redraw of blood sample may be requested.*

4.5.1 Specimen Repositories

A central repository has been established by the NIDDK, a division of the National Institutes of Health, for long-term storage of blood and tissue specimens, and a second repository has been established at the NIDDK Central Repository for DNA extraction. Whole blood for DNA extraction will be shipped immediately to the facility at the NIDDK Central Repository. Otherwise, samples will be shipped via licensed overnight carrier once every month to the NIDDK Central Repository.

All specimens will include a research study identifier, but otherwise will be de-identified prior to shipment to either repository. A computer log will record all incoming samples at the Central Repository, the storage location, the date, and the type of sample. Receipt of samples will be acknowledged to the originating center and SDCC.

4.5.2 Specimen Use

The ChiLDReN Steering Committee has developed a policy for the approval of ancillary studies – studies that will require use of samples in the repository. ChiLDReN investigators may propose such studies; non-ChiLDReN investigators may propose such studies only if they have a ChiLDReN investigator as a co-investigator. To be approved, these studies must relate to the specific aims of ChiLDReN, namely to study the pathogenesis and natural history of biliary atresia and neonatal hepatitis or to evaluate patterns of cellular gene and protein expression in tissue specimens and plasma by viral, genomic, and proteomic techniques. Examples of studies proposed by ChiLDReN investigators are: Screening for genetic mechanisms for pathogenesis and modifiers of biliary atresia; Study of JAG1 mutations in patients with biliary atresia; Identifying genetic determinants of biliary atresia; Studying the association of perinatal viral infection with biliary atresia and choledochal cyst; Proteomic analysis of neonatal cholestasis; Identifying novel antigens and T and B cell responses in biliary atresia; The association of HLA type and biliary

atresia.

These research studies are not related to clinical care; Tests performed on anonymized samples will not be reported to the parents/guardians nor be included in the medical record. The goal of the NIDDK repositories is to make samples available for investigations that have not been specified. Until the funding for ChiLDReN terminates inMay 2024, all decisions about the use of the samples will be made by the ChiLDReN Steering Committee. After funding for ChiLDReN terminates, the NIDDK will create a peer review mechanism to determine use of the remaining samples. All patient identifiers have been removed from samples in the repositories (i.e., samples are de-identified). The study database will be transmitted to the NIDDK repository with all patient identifiers removed; e.g., dates will be converted to ages.

5. Termination or Withdrawal of Participants

Participants with biliary atresia will be followed intensively for the first year, at 18 months of age, and then annually up to 20 years of age or until the time of liver transplantation. Other participants with cholestasis will be followed on the same schedule; if there is complete (clinical and biochemical) resolution of their underlying liver disease off all therapy, there will be one final follow-up visit within one year (preferably scheduled at the time of the next planned follow-up visit or at 12 months of age, whichever is later) for data collection and to obtain blood samples.

The participant's parents or guardians may request that the participant be removed from the study at any time. In addition, the investigator may withdraw a participant from the study if he/she determines that it is in the participant's best interests.

Note: Upon request of the parents or guardians, samples and data that have been submitted to the NIDDK repository or to the SDCC may be destroyed unless the samples have already been used or the data have been included in reported analyses or unless the linkage between the research identifier and the participant has been destroyed.

When the study ends at a clinical site or the participant completes the study, the linkage between the samples and the participant will be destroyed. Once this linkage has been destroyed, it will no longer be possible to withdraw samples and data from the repository and the database in response to a participant request.

6. Statistical Considerations

General Design Issues: The prospective observational database will collect data on all participants with cholestasis who present at a ChiLDReN clinical site at ≤180 days of age. Since there are many causes of cholestasis, including biliary atresia and neonatal hepatitis, the general design is that of a longitudinal cohort study, stratified by diagnosis.

Specific Aims: The specific aims of this study are to characterize the natural history of different types of cholestatic (liver) disease by prospectively following participants with cholestatic disease over time and to identify risk factors related to onset, to outcome and to the success of treatment(s) of each cholestatic disease, with a special emphasis on biliary atresia.

Primary Outcomes: Disease progression defined by:

1. Transplantation or death.
2. Increase in bilirubin or other biochemical indicators of disease progression.
3. Incidence of complications related to liver malfunction.

Sample Size and Accrual: It is anticipated that the 13 ChiLDReN clinical sites combined will accrue approximately 40 cases of biliary atresia annually and an equal number of cases of patients with cholestasis with a different etiology. If 75% of the eligible participants are recruited into the observational database, there will be 30 cases per year of biliary atresia and a similar number with other diseases. The intention of the observational database is to continue to accrue participants as long as possible. Therefore, the sample size is a function of the available participants and length of accrual. Using data from these participants, ChiLDReN desires to estimate the incidence of the failure of treatment, factors associated with success/failure, etc.

6.1 Data Analyses

Data cleaning. Prior to unblinding any study, the data for each variable will be examined univariately and bivariately to identify potential outliers and deviations from statistical assumptions. The goal is both to identify data values that should be queried back to the clinical site and variables that should be transformed prior to analysis.

Baseline comparisons. When groups are to be compared, the treatment groups will be compared at baseline on demographic variables and baseline values of outcome measures.

Measures at baseline that differ between groups will be included as covariates in the models that are used to analyze the efficacy of the treatment.

Intent-to-treat and per protocol analyses. When groups are compared, the primary analysis will be intent-to-treat. Each participant will be included in the intent-to-treat analysis. All hypotheses will be tested using a two-tailed test even when the hypothesis is stated as one-sided.

Missing data. There are two types of missing data, values that are missing due to an incomplete form and visits that are missing. When a small proportion of the values (<25%) are missing in a scale, the missing values will be imputed. No values will be imputed for missing visits unless the absence of the visit is meaningful, as in the evaluation of compliance with the treatment program. We will use methods of analysis, such as mixed models, that do not require complete data. When items are missing on a form and imputation is necessary, they may be multiply imputed.

Models. Longitudinal studies involve repeated evaluations of the participants, first at screening or baseline, and then at several time points after diagnosis and treatment. When the endpoint is a time-to-event, such as death or time to transplant, a Cox regression model will be fitted to the data. Otherwise, depending on whether the endpoint is dichotomous (improved/not improved) or continuous (bilirubin value), generalized estimating equations or random effects models will be fitted to the data. These models allow the analysis to account for the within-subject correlation during analyses.

7. Data Management

7.1 Case Report Forms

The Statistical Analysis of Biometrical & Educational Research (SABER; formerly known as Biometrics and Outcomes Research Core [BORC]) in the Department of Biostatistics at the University of Michigan and Arbor Research Collaborative for Health are responsible for data management and analysis. Both have operated as the SDCC for several multi-center trials.

Case report forms are developed by the SDCC and study investigators and published on the ChiLDReN password-controlled website. The case report forms do not contain any personal participant identifiers, only birth dates, which are necessary for research purposes.

A combination of web-enabled and centralized data entry and management will be used. After the visit is completed, the research coordinator will enter data in electronic case-report forms via

a web-enabled data management system.

7.2 Quality Assurance

The Clinical Monitors will review data submitted to the SDCC for accuracy and completeness. The Clinical Monitors will communicate with the study coordinators at each site about queries generated by the SDCC and address all questions and concerns regarding the study protocol and problems with data entry or specimen sample shipment. Site visits will be made annually. Interim site visits may be made to centers with low compliance or high error rates. Performance reports will be generated quarterly to investigators and study coordinators at each center, as well as to the Data Safety Monitoring Board and NIDDK.

7.3 Training

The SDCC will maintain a manual of operations to assist study coordinators at each center in following the protocol, entering and transferring data, and collecting, processing and shipping samples. The SDCC will be responsible for training the study coordinators at each center about the study protocol, the completion of source documents, the use of the web-based data entry system, and proper procedures with shipping samples to the Central Repository. The SDCC will review the study protocol and data entry system, and check all regulatory documents prior to site initiation.

8. Adverse Events

An adverse event (AE) is any unfavorable, harmful, or pathological change in a research participant as indicated by symptoms, physical signs, and/or clinically significant laboratory abnormalities that occur in association with the study procedures. This definition includes intercurrent illness, injuries, and exacerbation of pre-existing conditions. Stable pre-existing conditions and elective procedures to address such conditions are not AEs. A change in a laboratory variable is considered an AE if it was considered by the investigators to be clinically significant (that is, if it institutes a diagnostic evaluation or indicates additional therapy is necessary).

Serious AE (SAE) The term serious is based on participant outcome associated with events that could threaten a participant's life or functioning. An event should be considered serious if it results in any of the following:

- Death
- Life-threatening (participant was at risk of death as a result of the event; does not include hypothetical risk of death if the event had been more severe)
- Inpatient hospitalization or prolongation of existing hospitalization
- Persistent or significant disability or incapacity
- Congenital anomaly or birth defect
- Medical or surgical interventions required to prevent one of the outcomes listed above

The phrase 'related to study' implies causality or attribution to the study procedures. For purposes of defining as SAE, if a causal relationship cannot be ruled out, then an AE should be considered 'related to the study procedure(s)'. As noted above, it is very unlikely that any AEs will be attributable to this study.

Since this is an observational study and research procedures (phlebotomy, survey response) present minimal risk, we anticipate few AEs. The only serious adverse events (SAEs) related to the performance of this study are those related to phlebotomy (bruising, syncope). Whenever possible, blood samples will be obtained in conjunction with clinical samples. AEs occurring when samples are collected in the absence of clinical samples, such as the parent blood collections, should be considered to be related to the study procedure(s). The SAE reporting window for each participant begins with the first study procedure and ends 30 days after last study procedure.

SAEs must be reported to the SDCC within 24 hours of the site's awareness of the occurrence. The site should complete the SAE report form in ChiLDReNLink within this time period. Once the form is saved electronically, notification will immediately be sent to the SDCC. All SAEs should be recorded during the time frame specified by the local institutional review board (IRB) authority.

8.1 Data Safety Monitoring Board

The National Institutes of Health have set up a Data Safety Monitoring Board (DSMB) to oversee this study. The DSMB will act in an advisory capacity to the NIDDK to monitor participant safety and evaluate the ability of the ChiLDReN Network to achieve its research goals. Members of the

DSMB are independent of the study investigators and represent disciplines related to liver disease, biostatistics, and epidemiology, as well as possibly having a lay member. The DSMB will meet every six months or more frequently if requested by the Chair of the DSMB or the NIDDK Program Director, either in person or by teleconference.

8.2 Reporting of Serious Adverse Events (SAEs)

Each clinical investigator is responsible for reporting serious unexpected adverse events to the IRB at their institution, to the Safety Monitor, and to the NIH Program Director in an expedited manner. In general, when first informed of an SAE, the investigator or designee will log into the ChiLDReN website and complete the Serious Adverse Event Form online. Upon receipt of an SAE notification the system will generate an email to notify the Safety Monitor, the Principal Investigator at that clinical site, the NIH Program Director, and the SDCC. The Safety Monitor will log in and read the report. If there are questions, the Safety Monitor will contact the site and request clarification. After clarification is received, the Safety Monitor will summarize the case and report it to the Chair of the DSMB, the NIH Program Director, and theS SDCC. The Safety Monitor, the Chair of the DSMB and the NIH Program Director will determine if the case should be reported to the IRBs at all the institutions participating in the trial. The Chair of the DSMB or the NIH Program Director can convene an emergency meeting of the DSMB as necessary.

Every six months, the SDCC will provide interim reports to the DSMB and the NIH Program Director. The contents of the report are determined by the DSMB. Additions and other modifications to these reports may be requested by the DSMB on a one-time or continuing basis. Interim data reports generally consist of two parts; Part 1 (Open Session Report) provides information on study aspects such as accrual, baseline characteristics, and other general information on study status. Part 2 (Closed Session Report) may contain data on study outcomes, including safety data. The DSMB will meet every six months to discuss the findings and to make a recommendation to the NIH regarding continuation, modification, or termination of the study. Additional reports or meetings of the DSMB may be required at the discretion of the Chair of the DSMB or the NIH Program Director.

9. Costs and Payments to Participants

In addition to the collection of routinely obtained clinical data and the results of routine laboratory investigations, this research includes acquiring an extra blood sample (4-8 ml depending on the visit), special handling of the liver biopsy and surgical specimens, and samples of blood from the

parents. There will be no cost to the patient or their insurance for any research-related data collection, including the developmental assessment or special laboratory investigation. The expenses for the storage and handling of the extra research blood samples and research handling of the liver sample are covered by the research.

For each scheduled follow-up visit the parents or guardians will receive a small stipend in the form of cash, gift certificate, or check, (to be determined by the clinical site and consistent with each clinical site's regulatory authority) as reimbursement for parking, meals, or other expenses related to the visit.

10. Ethical Concerns and Informed Consent

There are minimal physical and psychological risks from being in this study. For the *database study*, the risks of venipuncture at the time of the blood draws are pain, bruising, or superficial phlebitis.

The risks of genetic information being revealed by any future investigations in the Network are very slight since the blood samples will be de-identified prior to being deposited in the repository; only a research study number will be included in the database and all dates will be converted to ages by the SDCC prior to transmission to the repository or to any laboratory conducting genetic studies. As the study is ongoing, the clinical site will maintain a link between the research study number and the participant's identity. However, identity information will not be contained in any data file transmitted to the SDCC or to the repository. When the study ends (or ChiLDReN ceases to exist), each clinical site will destroy the linkage between the research study number and the participant's identity.

Loss of confidentiality risks include: that knowledge of a genetic risk may be emotionally stressful to a family member; that this might change eligibility for new health, disability, or life insurance; that there may be unforeseen paternity issues; and that genetic testing may reveal information regarding health risks to other family members living or not yet born. The tissue in the repository will be extra tissue removed at the time of clinically indicated surgery or liver biopsy and will not compromise the clinical care of the patient.

Methods Taken to Reduce Patient Risks: The study anticipates no excessive risks to the participants except the possible pain associated with blood draws. EMLA cream may be applied to sites of all blood draws and intravenous lines to minimize pain with these procedures.

Psychological risks will be minimized by careful explanation of the risks and by maintaining complete confidentiality and data security.

Informed Consent: A common template for the informed consent form will be used by all of the centers, modifying the content or format as necessary to meet the requirements of their respective institutional human subjects committees. The participant will retain a signed consent form; one will be retained for the participant's chart; and one will be included in the research records.

References

1. Balistreri WF, Grand R, Hoofnagle JH, Suchy FJ, Ryckman FC, Perlmutter DH, Sokol RJ. Biliary atresia: current concepts and research directions. Summary of a symposium. Hepatology 1996; 23:1682-92.
2. Yoon PW, Bresee JS, Olney RS, James LM, Khoury MJ. Epidemiology of biliary atresia: a population-based study. Pediatrics 1997; 99:376-82.
3. Sokol RJ, Mack C. Etiopathogenesis of Biliary Atresia. Semin Liv Dis 2001; 21:517-524.
4. Ohi R. Biliary atresia. A surgical perspective. Clin Liver Dis 2000; 4:779-804.
5. Karrer FM, Hall RJ, Lilly JR. Biliary atresia and the polysplenia syndrome. J Pediatr Surg 1991; 26:524-7.
6. Desmet VJ. The Cholangiopathies. In: Suchy FJ, Sokol RJ, Balistreri WF, eds. Liver Disease in Children. Philadelphia: Lippincott, Williams & Wilkins, 2001:39-62.
7. Mazziotti MV, Willis LK, Heuckeroth RO, LaRegina MC, Swanson PE, Overbeek PA, Perlmutter DH. Anomalous development of the hepatobiliary system in the Inv mouse. Hepatology 1999; 30:372-8.
8. Desmet VJ. Congenital diseases of intrahepatic bile ducts: variations on the theme "ductal plate malformation". Hepatology 1992; 16:1069-83.
9. Balistreri WF. Liver disease in infancy and childhood. In: Schiff ER, Sorrell MF, Maddrey WC, eds. Schiff's Diseases of the Liver. Philadelphia: Lippincott-Raven Publishers, 1999:1357-1512.
10. Suchy FJ. Approach to the infant with cholestasis. In: Suchy FJ, Sokol RJ, Balistreri WF, eds. Liver Disease in Children. Philadelphia: Lippincott, Williams & Wilkins, 2001:187-194.
11. Jacquemin E. Progressive familial intrahepatic cholestasis. Genetic basis and treatment. Clin Liver Dis 2000; 4:753-63.
12. Jacquemin E, De Vree JM, Cresteil D, Sokal EM, Sturm E, Dumont M, Scheffer GL, Paul M, Burdelski M, Bosma PJ, Bernard O, Hadchouel M, Elferink RP. The wide spectrum of multidrug resistance 3 deficiency: from neonatal cholestasis to cirrhosis of adulthood. Gastroenterology 2001; 120:1448-58.
13. Jacquemin E, Cresteil D, Manouvrier S, Boute O, Hadchouel M. Heterozygous non-sense mutation of the MDR3 gene in familial intrahepatic cholestasis of pregnancy. Lancet 1999; 353:210-1.
14. Balistreri WF, Bove KE, Ryckman FC. Biliary atresia and other disorders of the extrahepatic bile ducts. In: Suchy FJ, Sokol RJ, Balistreri WF, eds. Liver Disease in Children. Philadelphia: Lippincott Williams & Wilkins, 2001:253-274.
15. Zerbini MC, Gallucci SD, Maezono R, Ueno CM, Porta G, Maksoud JG, Gayotto LC. Liver biopsy in neonatal cholestasis: a review on statistical grounds. Mod Pathol 1997; 10:793-9.
16. Gilmour SM, Hershkop M, Reifen R, Gilday D, Roberts EA. Outcome of hepatobiliary scanning in neonatal hepatitis syndrome. J Nucl Med 1997; 38:1279-82.

17. Choi SO, Park WH, Lee HJ, Woo SK. 'Triangular cord': a sonographic finding applicable in the diagnosis of biliary atresia. J Pediatr Surg 1996; 31:363-6.
18. Guibaud L, Lachaud A, Touraine R, Guibal AL, Pelizzari M, Basset T, Pracros JP. MR cholangiography in neonates and infants: feasibility and preliminary applications. AJR Am J Roentgenol 1998; 170:27-31.
19. Wilkinson ML, Mieli-Vergani G, Ball C, Portmann B, Mowat AP. Endoscopic retrograde cholangiopancreatography in infantile cholestasis. Arch Dis Child 1991; 66:121-3.
20. Chardot C, Carton M, Spire-Bendelac N, Le Pommelet C, Golmard JL, Auvert B. Epidemiology of biliary atresia in France: a national study 1986-96. J Hepatol 1999; 31:1006-13.
21. Karrer FM, Price MR, Bensard DD, Sokol RJ, Narkewicz MR, Smith DJ, Lilly JR. Long-term results with the Kasai operation for biliary atresia. Arch Surg 1996; 131:493-6.
22. Lilly JR. Experimental investigations into the etiology of cholangitis following operation for biliary atresia. J Pediatr Surg 1978; 13:559-60.
23. Karrer FM, Bensard DD. Neonatal cholestasis. Semin Pediatr Surg 2000; 9:166-9.
24. Feranchak A, Ramirez R, Sokol RJ. Medical and Nutritional Management of Cholestasis. In: Suchy FJ, Sokol RJ, Balistreri WF, eds. Liver Disease in Children. Philadelphia: Lippincott, Williams & Wilkins, 2001:195-238.
25. Laurent J, Gauthier F, Bernard O, Hadchouel M, Odievre M, Valayer J, Alagille D. Long-term outcome after surgery for biliary atresia. Study of 40 patients surviving for more than 10 years. Gastroenterology 1990; 99:1793-7.
26. Miga D, Sokol RJ, MacKenzie T, Narkewicz MR, Smith D, Karrer FM. Survival after first esophageal variceal hemorrhage in patients with biliary atresia. J Pediatr 2001; 139:291-6.
27. Smith CM, Davies DB, McBride MA. Liver transplantation in the United States: a report from the Organ Procurement and Transplantation Network. Clin Transpl. 2000:19-30.
28. Schweizer P, Lunzmann K. Extrahepatic bile duct atresia: how efficient is the hepatoporto-enterostomy? Eur J Pediatr Surg 1998; 8:150-4.
29. Bucuvalas JC, Ryckman FC, Atherton H, Alonso MP, Balistreri WF, Kotagal U. Predictors of cost of liver transplantation in children: a single center study. J Pediatr 2001; 139:66-74.
30. Hyams JS, Glaser JH, Leichtner AM, Morecki R. Discordance for biliary atresia in two sets of monozygotic twins. J Pediatr 1985; 107:420-2.
31. Schweizer P, Kerremans J. Discordant findings in extrahepatic bile duct atresia in 6 sets of twins. Z Kinderchir 1988; 43:72-5.
32. Landing BH. Considerations of the pathogenesis of neonatal hepatitis, biliary atresia and choledochal cyst--the concept of infantile obstructive cholangiopathy. Prog Pediatr Surg 1974; 6:113-39.
33. Tarr PI, Haas JE, Christie DL. Biliary atresia, cytomegalovirus, and age at referral. Pediatrics 1996; 97:828-31.
34. Fischler B, Ehrnst A, Forsgren M, Orvell C, Nemeth A. The viral association of

neonatal cholestasis in Sweden: a possible link between cytomegalovirus infection and extrahepatic biliary atresia. J Pediatr Gastroenterol Nutr 1998; 27:57-64.

35. Jevon GP, Dimmick JE. Biliary atresia and cytomegalovirus infection: a DNA study. Pediatr Dev Pathol 1999; 2:11-4.
36. Bangaru B, Morecki R, Glaser JH, Gartner LM, Horwitz MS. Comparative studies of biliary atresia in the human newborn and reovirus-induced cholangitis in weanling mice. Lab Invest 1980; 43:456-62.
37. Morecki R, Glaser JH, Cho S, Balistreri WF, Horwitz MS. Biliary atresia and reovirus type 3 infection. N Engl J Med 1982; 307:481-4.
38. Morecki R, Glaser JH, Johnson AB, Kress Y. Detection of reovirus type 3 in the porta hepatis of an infant with extrahepatic biliary atresia: ultrastructural and immunocytochemical study. Hepatology 1984; 4:1137-42.
39. Rosenberg DP, Morecki R, Lollini LO, Glaser J, Cornelius CE. Extrahepatic biliary atresia in a rhesus monkey (Macaca mulatta). Hepatology 1983; 3:577-80.
40. Brown WR, Sokol RJ, Levin MJ, Silverman A, Tamaru T, Lilly JR, Hall RJ, Cheney M. Lack of correlation between infection with reovirus 3 and extrahepatic biliary atresia or neonatal hepatitis. J Pediatr 1988; 113:670-6.
41. Glaser JH, Balistreri WF, Morecki R. Role of reovirus type 3 in persistent infantile cholestasis. J Pediatr 1984; 105:912-5.
42. Steele MI, Marshall CM, Lloyd RE, Randolph VE. Reovirus 3 not detected by reverse transcriptase-mediated polymerase chain reaction analysis of preserved tissue from infants with cholestatic liver disease. Hepatology 1995; 21:697-702.
43. Tyler KL, Sokol RJ, Oberhaus SM, Le M, Karrer FM, Narkewicz MR, Tyson RW, Murphy JR, Low R, Brown WR. Detection of reovirus RNA in hepatobiliary tissues from patients with extrahepatic biliary atresia and choledochal cysts. Hepatology 1998; 27:1475-82.
44. Riepenhoff-Talty M, Schaekel K, Clark HF, Mueller W, Uhnoo I, Rossi T, Fisher J, Ogra PL. Group A rotaviruses produce extrahepatic biliary obstruction in orally inoculated newborn mice. Pediatr Res 1993; 33:394-9.
45. Petersen C, Bruns E, Kuske M, von Wussow P. Treatment of extrahepatic biliary atresia with interferon-alpha in a murine infectious model. Pediatr Res 1997; 42:623-8.
46. Qiao H, DeVincentes A, Alashari M, Klemenich C, Duffy L, Rossi T, Riepenhoff- Talty M. Pathogenesis of rotavirus-induced bile duct obstruction (a model for human biliary atresia) in normal Balb/c and CB17 scid mice with severe combined immunodeficiency (SCID). Pediatr Res 1999; 45:116A.
47. Riepenhoff-Talty M, Gouvea V, Evans MJ, Svensson L, Hoffenberg E, Sokol RJ, Uhnoo 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. Detection of group C rotavirus in infants with extrahepatic biliary atresia. J Infect Dis 1996; 174:8-15.
48. Bobo L, Ojeh C, Chiu D, Machado A, Colombani P, Schwarz K. Lack of evidence

for rotavirus by polymerase chain reaction/enzyme immunoassay of hepatobiliary samples from children with biliary atresia. Pediatr Res 1997; 41:229-34.

49. Drut R, Drut RM, Gomez MA, Cueto Rua E, Lojo MM. Presence of human papillomavirus in extrahepatic biliary atresia. J Pediatr Gastroenterol Nutr 1998; 27:530-5.
50. Drut R, Gomez MA, Drut RM, Cueto RE, Lojo M. [Human papillomavirus, neonatal giant cell hepatitis and biliary duct atresia]. Acta Gastroenterol Latinoam 1998; 28:27-31.
51. Domiati-Saad R, Dawson DB, Margraf LR, Finegold MJ, Weinberg AG, Rogers BB. Cytomegalovirus and human herpesvirus 6, but not human papillomavirus, are present in neonatal giant cell hepatitis and extrahepatic biliary atresia. Pediatr Dev Pathol 2000; 3:367-73.
52. Mason AL, Xu L, Guo L, Munoz S, Jaspan JB, Bryer-Ash M, Cao Y, Sander DM, Shoenfeld Y, Ahmed A, Van de Water J, Gershwin ME, Garry RF. Detection of retroviral antibodies in primary biliary cirrhosis and other idiopathic biliary disorders. Lancet 1998; 351:1620-4.
53. Schreiber RA, Kleinman RE. Genetics, immunology, and biliary atresia: an opening or a diversion? J Pediatr Gastroenterol Nutr 1993; 16:111-3.
54. Burch JM, Sokol RJ, Narkewicz MR, Reichlin M, Frank MB, MacKenzie T, Lee LA. Antibodies in mothers of children with liver disease. J Pediatr Gastroenterol Nutr, 2003;37:262-7.
55. Silveira TR, Salzano FM, Donaldson PT, Mieli-Vergani G, Howard ER, Mowat AP. Association between HLA and extrahepatic biliary atresia. J Pediatr Gastroenterol Nutr 1993; 16:114-7.
56. Broome U, Nemeth A, Hultcrantz R, Scheynius A. Different expression of HLA-DR and ICAM-1 in livers from patients with biliary atresia and Byler's disease. J Hepatol 1997; 26:857-62.
57. Kobayashi H, Puri P, O'Brian DS, Surana R, Miyano T. Hepatic overexpression of MHC class II antigens and macrophage-associated antigens (CD68) in patients with biliary atresia of poor prognosis. J Pediatr Surg 1997; 32:590-3.
58. Gosseye S, Otte JB, De Meyer R, Maldague P. A histological study of extrahepatic biliar+y atresia. Acta Paediatr Belg 1977; 30:85-90.
59. Bill AH, Haas JE, Foster GL. Biliary Atresia: histopathologic observations and reflections upon its natural history. J Pediatr Surg 1977; 12:977-82.
60. Ohya T, Fujimoto T, Shimomura H, Miyano T. Degeneration of intrahepatic bile duct with lymphocyte infiltration into biliary epithelial cells in biliary atresia. J Pediatr Surg 1995; 30:515-8.
61. Abbas A, Lichtman A, Pober J. Cellular and Molecular Immunology. Philadelphia: W.B. Saunders, 2000.
62. Nakada M, Nakada K, Kawaguchi F, Wakisaka M, Kashimura T, Yamate N, Maeyama S, Uchikoshi T. Immunologic reaction and genetic factors in biliary atresia. Tohoku J Exp Med 1997; 181:41-7.
63. Davenport M, Gonde C, Redkar R, Koukoulis G, Tredger M, Mieli-Vergani G, Portmann B, Howard ER. Immunohistochemistry of the liver and biliary

tree in extrahepatic biliary atresia. J Pediatr Surg 2001; 36:1017-25.

64. Urushihara N, Iwagaki H, Yagi T, Kohka H, Kobashi K, Morimoto Y, Yoshino T, Tanimoto T, Kurimoto M, Tanaka N. Elevation of serum interleukin-18 levels and activation of Kupffer cells in biliary atresia. J Pediatr Surg 2000; 35:446-9.
65. Funaki N, Sasano H, Shizawa S, Nio M, Iwami D, Ohi R, Nagura H. Apoptosis and cell proliferation in biliary atresia. J Pathol 1998; 186:429-33.
66. Liu C, Chiu JH, Chin T, Wang LS, Li AF, Chow KC, Wei C. Expression of fas ligand on bile ductule epithelium in biliary atresia--a poor prognostic factor. J Pediatr Surg 2000; 35:1591-6.
67. Vasiliauskas E, Targan S, Cobb L, Vidrich A, Rosenthal P. Biliary atresia - an autoimmune mediated disorder?, Hepatology. The American Association for The Study of Liver Diseases. Postgraduate course and 46th annual meeting. Chicago, Illinois, November 3-7, 1995. Abstracts, Chicago, IL, Oct, 1995. Vol. 22. W.B. Saunders Company.
68. Bernal W, Moloney M, Underhill J, Donaldson PT. Association of tumor necrosis factor polymorphism with primary sclerosing cholangitis. J Hepatol 1999; 30:237- 41.
69. Mitchell SA, Grove J, Spurkland A, Boberg KM, Fleming KA, Day CP, Schrumpf E, Chapman RW. Association of the tumour necrosis factor alpha -308 but not the interleukin 10 -627 promoter polymorphism with genetic susceptibility to primary sclerosing cholangitis. Gut 2001; 49:288-94.
70. Klippel CH. A new theory of biliary atresia. J Pediatr Surg 1972; 7:651-4.
71. Pickett LK, Briggs HC. Biliary obstruction secondary to hepatic vascular ligation in fetal sheep. J Pediatr Surg 1969; 4:95-101.
72. Ho CW, Shioda K, Shirasaki K, Takahashi S, Tokimatsu S, Maeda K. The pathogenesis of biliary atresia: a morphological study of the hepatobiliary system and the hepatic artery. J Pediatr Gastroenterol Nutr 1993; 16:53-60.
73. Yokoyama T, Copeland NG, Jenkins NA, Montgomery CA, Elder FF, Overbeek PA. Reversal of left-right asymmetry: a situs inversus mutation. Science 1993; 260:679-82.
74. Schmidt C, Bladt F, Goedecke S, Brinkmann V, Zschiesche W, Sharpe M, Gherardi E, Birchmeier C. Scatter factor/hepatocyte growth factor is essential for liver development. Nature 1995; 373:699-702.
75. Wechsler, D. 2003. Manual for the Wechsler Intelligence Scale for Children-IV. San Antonio, TX: The Psychological Corporation.
76. Varni JW, Burwinkle TM, Katz ER, Meeske K, Dickinson P. The PedsQL™ in pediatric cancer: Reliability and validity of The Pediatric Quality of Life Inventory™ Generic Core Scales, Multidimensional Fatigue Scale, and Cancer Module. Cancer 2002; 94;2090-2106.

Please add:

Berauer JP, Mezina AI, Okou DT, Sabo A, Muzny DM, Gibbs RA, Hegde MR, Chopra P, Cutler DJ, Perlmutter DH, Bull LN, Thompson RJ, Loomes KM, Spinner NB,

Rajagopalan R, Guthery SL, Moore B, Yandell M, Harpavat S, Magee JC, Kamath BM, Molleston JP, Bezerra JA, Murray KF, Alonso EM, Rosenthal P, Squires RH, Wang KS, Finegold MJ, Russo P, Sherker AH, Sokol RJ, Karpen SJ; Childhood Liver Disease Research Network (ChiLDReN). Identification of Polycystic Kidney Disease 1 Like 1 Gene Variants in Children With Biliary Atresia Splenic Malformation Syndrome.

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