Childhood Liver Disease Research and Education Network (CHILDREN)

A PROSPECTIVE DATABASE OF INFANTS WITH CHOLESTASIS

Amended Protocol PROBE
Version 01 (Original) February 10, 2004
Version 02 (Amendment 1) February 22, 2005
Version 03 (Amendment 2) September 21, 2005
Version 04 (Amendment 3) January 26, 2006
Version 05 (Amendment 4) January 16, 2007
Version 06 (Amendment 5) April 30, 2010
Version 07 (Amendment 6) September 12, 2013
Steering Committee Approval: January 13, 2004

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Amendment 6: September 12, 2013

Protocol Title page

1. Change Protocol version Version 07 Amendment 6 September 12, 2013
Updates to Working Group
Rationale:
Updates to title page

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2. Insert Amendment 6: September 12, 2013

Rationale:
To include Amendment 6 changes

Design and Outcomes, Section 4.1

Original Text:
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. Subjects diagnosed with biliary atresia will be followed intensively for the first year, at 18 months of age, and then annually up to 10 years of age.

Amended Text:
3. *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. Subjects diagnosed with biliary atresia will be followed intensively for the first year, at 18 months of age, and then annually up to* ***15 years of age****.*

Rationale:
Follow up extended for duration of the next 5 year funding cycle.

Schedule of Evaluations, Section 4.3.1

Original Text:
Follow up: The subject will be followed for 10 years at the times indicated in Table 4.1.
Transplantation: Subjects who receive a liver transplant during their participation in the PROBE study will have annual follow-up visits for up to 10 years.

Amended Text:
4. Follow up: The subject will be followed for **15** years at the times indicated in Table 4.1.

5. Transplantation: Subjects who receive a liver transplant during their participation in the PROBE study will have annual follow-up visits for up to **15** years.

Rationale:
Follow up extended for duration of the new 5 year funding cycle.

Schedule of Evaluations, Table 4.1
6. Table revised to include age-appropriate developmental assessments at specified follow-up intervals
7. Table revised to include age-appropriate developmental assessments at specified follow-up intervals and to include age-appropriate parent and child PedsQL from age 2-15 years.
8. Recommended visit windows after the 2 year visit increased to ± 6months

Rationale:
Follow up extended for duration of the new 5 year funding cycle.

Schedule of Post-Transplant Evaluations, Table 4.2 NEW
9. New table to describe evaluations and intervals for subjects receiving a liver transplant while enrolled in PROBE

Rationale:
Follow up extended for duration of the new 5 year funding cycle.

Section 4.4 Data Collected
10. Item 20 Revise text: Health outcome: quality of life of infant and its effect on the family; from ages 2-15, the parent and child Pediatric Quality of Life Inventory (PedsQL) will be used.

Rationale
Add parent and child report of PedsQL in ages where previously omitted.

11. New text to describe the Lansky Functional Status measure:
The Lansky Functional Status Measure is used to determine the score of a child less than 16 years old that underwent a liver transplant while enrolled in the PROBE study. Children, who might have more trouble expressing their experienced quality of life, require a somewhat more observational scoring system suggested and validated by Lansky et al. This scoring, reported on an ordinal scale from 0-100, provides a rough measure of a patient's well-being including their activity level of play. The child's activity is assessed by the parent/ caregiver at each yearly follow up visit.

Section 7 Data Management
12. Revise text to read: Original case report forms will be securely maintained at the clinical sites. Clean copies of the case report forms will be transmitted monthly to the DCC to be entered into the study database.The forms entered into the database by the

coordinator will not be transmitted to the DCC. Forms with personal identifiers are not sent to the DCC.

Rationale:
To reflect changes in database entry procedures.

Section 7.2 Quality Assurance
13. Change interval of Project Manager/Clinical Monitor visits from yearly to once every two years..

Rationale: To reflect Network changes to frequency of clinical site monitoring of observational studies.

Section 11. References
14. New reference: 78. Lansky SB, List MA, Lansky LL, Ritter-Sterr C, Miller DR. The measurement of performance in childhood cancer patients. Cancer 60 (7) 1987: 1651–6.

Protocol Amendments
15. Delete all text related to Amendments 1-5 changes

Rationale:
Optimize document size to contain the current Protocol version only.

PROBE v.7 Amendment 06 INSTITUTIONAL REVIEW BOARD APPROVAL REQUIRED	YES

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 and Education Network (ChiLDREN) clinical site. In order to study the natural history, subjects will be followed until 10 years of age, liver transplantation or, for children without biliary atresia, until complete recovery off of all therapy or 12 months of age, whichever is later.

This study will:

1. collect detailed clinical and demographic information about each subject at enrollment and during follow up,
2. obtain and store blood and urine samples from the subject at diagnosis and during follow up,
3. obtain and store liver and biliary tissue and bile that are removed during diagnosis (i.e., biopsy) or at time of surgery or transplant and that are not needed for diagnostic purposes
4. collect demographic and medical history of parents at enrollment, and
5. obtain and store blood from the biological parents at enrollment.

Some samples of blood, urine, bile and tissue will be stored in repositories for future research. The data and biological specimens will be used for detailed study into the mechanisms and causes of liver problems in young children in order to try to better diagnose and manage these conditions. The subject 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 subjects 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 Johns Hopkins School of Medicine (Baltimore), Children's Memorial Hospital (Chicago), Cincinnati Children's Hospital Medical Center, The Children's Hospital (Denver), Texas Children’s Hospital (Houston), Mount Sinai Medical Center (NYC), Children's Hospital of Philadelphia, Children's Hospital (Pittsburgh), University of California at San Francisco, Washington University, School of Medicine (St. Louis), Riley Hospital for Children (Indianapolis) Children’s Hospital Los Angeles (Los Angeles) Seattle Children’s Hospital (Seattle),

The Hospital for Sick Children, Toronto (Ontario) and Children's Healthcare of Atlanta (Atlanta*).* The data coordinating center is at the University of Michigan, Ann Arbor.

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 is governed by a Steering Committee comprised of the Principal Investigators from each of the participating clinical sites, the Data Coordinating Center Principal Investigator, and the NIDDK Project Scientist.

At the end of the grant period specimens will 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 disease and their families.

2. To establish repositories for blood, urine, bile 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, to outcome and to the success of treatment(s) for the different cholestatic diseases, with special emphasis on biliary atresia.

3. Background

<u>Clinical Significance of Biliary Atresia and Idiopathic Neonatal Hepatitis</u>

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 three months of life[1]. A recent study suggested that the prevalence of biliary atresia may be higher in African-American children than in Caucasian 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 3 weeks of life; whereas the acquired form of biliary atresia generally has onset of jaundice and acholic stools in the 2nd to 4th 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 focus of this project, there are many other causes of neonatal cholestasis that may be investigated by the Childhood Liver Disease and Research Network 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 6 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 Childhood Liver Disease Research and Education 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 age beyond 3-4 months[14]. Consequently, it is absolutely essential that jaundiced infants older than 2 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 approximately a 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 (UNOS Annual Report 2000). It should be pointed out that there is no single liver disease in adults that accounts for as large a proportion of liver transplants. Factors that determine long-term survival without transplantation have not been carefully evaluated. Moreover, quality of life outcome (QOL) 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[4,27].

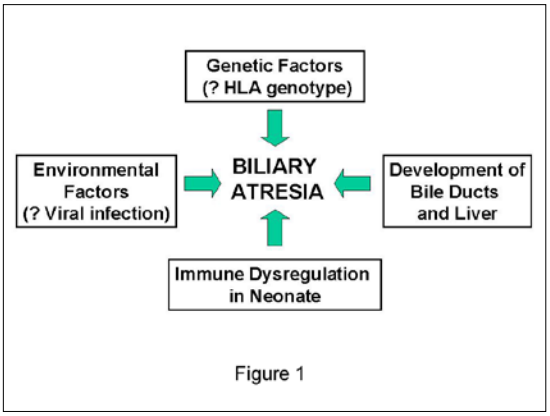

Figure 1

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[28]. 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 3 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[29,30]. 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"[31]. 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[32]. Although a recent study from Sweden[33] 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[34] 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[35]. 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[36,37] and in an infant Rhesus monkey with biliary atresia[38], although other groups could not replicate these findings in infants[39]. Serologic studies of reovirus antibodies in infants with biliary atresia have likewise been inconclusive[36,39,40]. 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[41] 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[42] reported nested RT-PCR evidence of reovirus infection in snap frozen liver or bile duct from 55% of cases of acquired/perinatal form 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[43]. Petersen et al[44] subsequently reported that the administration of interferon-α prior to rotavirus infection prevented the biliary disease and Qiao et al[45] reported an increase in the incidence of bile duct obstruction in normal newborn BALB/c mice compared to SCID (immunodeficient) mice infected with rotavirus, indicatingthe role of the immune system in this mouse model. Riepenhoff-Tolte et al[46] 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[47] 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[42].
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[48,49]. However, Domiati-Saad et al[50] 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[51] 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[52] 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[53].

Silviera et al[54] 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[55], and in Japan with A33, B44, and DR6[56]. 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[57] demonstrated lymphocytes in the connective tissue of the portahepatis in biliary atresia patients, and Bill et al[58] pointed out the relationship of intramural mononuclear inflammatory cells with epithelial cell necrosis in bile duct remnants. In 1995, Ohya et al[59] 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[60]. 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 groups[55,61,56] 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[62] 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[63]. Davenport[62] 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[64] 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[65] 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[65]. 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 the Childhood Liver Disease Research and Education Network. 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[66] 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[53] 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 Childhood Liver Disease and Education 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[67] and Mitchell et al[68] 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 the Childhood Liver Disease and Research Network 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[69]. Pickett et al[70] 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[71]. 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[7,72]. 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 malformation[1,73]. 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 Childhood Liver Disease Research and Education 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. Subjects diagnosed with biliary atresia will be followed intensively for the first year, at 18 months of age, and then annually up to 15 years of age. Other subjects diagnosed 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 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 subjects with liver problems. This research involves the collection of diagnostic, clinical and outcome data concerning the subject, which is kept without identification (coded) in a national research database of infants with liver disease. Samples of blood and urine 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, any liver biopsy specimen in excess of that needed for diagnostic use will be sent to the tissue repository. When a portoenterostomy or liver transplant occurs, sections of the liver, biliary remnant and bile specimens, if removed in the course of surgery and in excess of that needed for diagnostic use, will be sent for the repository. These specimens will be used in investigations into the mechanisms and causes of the liver damage that occur in the subject'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 15 years. All data from this study will be kept in a secure research database at the data coordinating center.

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 Subjects

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 subject's parent(s)/guardian(s) willing 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 1500 g may be included in the database. The investigator should request permission for a protocol exemption when biliary atresia is suspected. 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. The investigator should request permission for a protocol exemption for these cases. 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

Subjects 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. Printed information about the study and 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.

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:

1. **Recruitment**: Following diagnosis of cholestasis in an infant less 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.
2. **Baseline**: Once informed consent is obtained, the coordinator may abstract information from the subject's medical chart and meet with the parent(s)/guardian(s) to complete the intake and history forms (see below for details).
3. **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 subjects.
4. **In-patient / Discharge**: For in-patients, data will be collected from the time of surgery or diagnosis to the time of discharge.
5. **Follow up:** The subject will be followed for 15 years at the times indicated in Table 4.1.
6. **Transplantation:** Subjects who receive a liver transplant during their participation in the PROBE study will have annual follow-up visits for up to 15 years.

Figure 4.1 Schedule of Evaluations

P	RECRUITMENT OR BASELINE	DIAGNOSIS /SURGERY/ DISCHARGE	4 WK POST 3 MO POST 6 MO POST	2 MO POST	12 MO AGE	18 MO AGE	ANNUALLY FROM AGE 2	AT TRANS PLANT	COMPLETE RESOLU-TION w/o BA
Visit windows			±2WKS / ±1MO	±2 WKS	±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	X	**
Parent and Child reported PedsQL ages 2-15 years					Parent		Ages 2-15 yr		**
BSID-II or III (2005)					x		Ages 1- 2 yr		
WPPSI-III (2002)							Ages 3-,5 yr		
WISC-IV							Ages 6, 8, 10, 12, 14yr		
Liver Biopsy / Intra-operative Samples		X						X	
Urine Sample		X	X	‡	X	X	X	X	X
Serum and Plasma Samples		X	X	‡	X	X	X	X	X
Child Blood for DNA***	X Once during the 1st year or at Transplant (if < 1 y of age)								
Parents Medical History	X								

Parent Blood for DNA[#]	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.
**Subjects 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.
*** Subject may have blood drawn for DNA at any visit prior to 1 year of age if the required volume (1-3 ml) can be obtained while keeping within age and/or size specific volume limitations. DNA will be preferentially isolated from these samples obtained prior to 1 year of age. If there is sufficient volume remaining in the sample, an EBV transformed cell line will also be established. In cases where the volume is insufficient to establish an EBV transformed cell line, another blood sample for DNA may be obtained after 1 year of age, again keeping within age and/or size specific volume limitations
[#] Preferred collection is baseline but may be collected at any visit‡ Only to be collected if specimens were not collected at the one month visit.

Figure 4.1 Schedule of POST TRANSPLANT Evaluations

EVALUATIONS	ANNUALLY FROM DATE OF TRANSPLANT
Recommended windows for visits	± 6MO
Follow up Visits	X
QL2P PedsQL	**X** Ages 2-4 yr
QL5P PedsQL; QL5C PedsQL	**X** Ages 5-7 yr
QL8P PedsQL; QL8C PedsQL	**X** Ages 8-12 yr
QL13P PedsQL; QL13C PedsQL	**X** Ages 13-16 yr
Lansky Scale	**X** Ages <16yr

4.4 Data to be Collected

At recruitment/baseline, the following information will be collected (the numbering corresponds to case report forms):

1. Eligibility: fulfillment of the inclusion/exclusion criteria
2. Demographics of infant and parents: Infant: date of birth, sex, birth weight, race and ethnicity; and Parents: date of birth, race and ethnicity, residence, marital status, education, employment status/income, and type of medical insurance
3. 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
4. Pregnancy history of the natural mother: Last pregnancy: Prenatal history, medications, alcohol and tobacco use during pregnancy and after delivery, exposure to pesticides and chemicals during pregnancy, labor 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: Sexually transmitted diseases; Prenatal Tests: Chorionic villi sampling, amniocentesis, results of ultrasounds, Rh sensitivity, blood transfusions.
5. 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, as well as siblings of the infant.
6. 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.
7. Physical 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:

8. 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
9. 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
10. Pathology results of biopsies performed for clinical diagnosis: Grade of fibrosis, cirrhosis, cholestasis, bile duct proliferation, and inflammation.

11. Surgical findings during exploration and/or portoenterostomy: Abdominal anatomy: assessment of intestinal malrotation, liver, portal vein, present 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:

12. 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, metabolic screening, urinalysis and coagulation profile; Medications at discharge; Feeding type at discharge.
13. Samples collected for the repository: blood, urine, liver and bile
14. Final diagnosis

At follow up visits:

20. Physical findings: vital signs, anthropometric measurements, Tanner score from age 8 unless precocious puberty is observed, physical exam of the liver, facial features.
21. Health outcome: quality of life of infant and its effect on the family; from ages 2-15, the parent and child Pediatric Quality of Life Inventory (PedsQL) will be used.
22. Diet and medication record: type of feeding and diet, vitamin and dietary supplements, prescription medications
23. Laboratory findings: Liver function tests, blood CBC, blood chemistry, metabolic screening, urinalysis and coagulation profile
24. History of medical consultations between visits: This is used to determine which of the following 3 forms need to be completed.
25. Interval sentinel events: Complications that occurred between visits – symptoms, lab tests and treatment, as appropriate
26. Surgery: Surgical and diagnostic procedures that occurred between visits
27. Imaging: Findings of ultrasound, hepatobiliary scan, MRCP, etc.
28. Samples collected for the repository: Blood, urine, liver, gall bladder, lymph node and bile specimens. (Tissue specimens are only obtained when the tissue is removed as part of a clinical procedure, biopsy or surgery, and the tissue is in excess of that needed for diagnosis.)
30. Pathology: Findings if any biopsy was conducted: grade, cirrhosis, cholestasis, bile duct proliferation and inflammation and cells
35. Final status: completion of study, lost to follow up, or death

Post transplant follow-up data collection will be reduced to a brief interval history including major illness, complications, new diagnoses as well as growth and anthropometric parameters and measures of quality of life and functional status.

Developmental assessments:

For neurodevelopmental testing, at ages 1-2 years, the Bayley Scales of Infant Development-II or III will be performed on English speaking participants. For those subjects who are already enrolled in the PROBE study and have had the Bayley II administered at age 1; they will have the Bayley II administered at age 2. However those subjects either new to the PROBE study or those that have not had the Bayley II administered; they will have the Bayley III administered at age 1 and at age 2.

The Bayley Scales of Infant Development-III (BSID) is the most commonly reported test for preschoolers from 1 month to 42 months of age and provides information in both cognitive (the mental developmental index [MDI] and motor (the psychomotor developmental index [PDI]) domains. The BSID is well standardized and was updated in 1993 (Bayley II) and in 2005 (Bayley III), and its score is based on a mean of 100 and a standard deviation of 15. It has been proven to be a reliable and valid instrument for this age group. It has the advantage of assessing all areas of development. The Mental Development Index (MDI) assesses sensory and perceptual acuity, discrimination and response; acquisition of object constancy, memory, learning and problem solving, vocalizations communication, basis of abstract thinking, habituation, mental mapping and complex language and mathematical concept formation. The Psychomotor Skills Index (PDI) assesses degree of body control, large and fine muscle coordination, finer manipulatory skills of the hands and fingers, dynamic movement, dynamic praxis, postural imitation and stereognosis.

The Wechsler Preschool and Primary Scale of Intelligence-III (WPPSI-III) (2002) is a standardized test of intelligence for children from 2 ½ to 7 years of age. It is the most commonly used intelligence test both in the clinical setting and in research. It takes approximately 45 minutes to administer and yields four summary scores and 14 subtest scores. Scores include full scale, verbal and performance IQ's, as well as, processing speed quotient with means of 100 and standard deviation of 15 points. This test was selected after careful consideration of other tests of intelligence for the following reasons. It covers a wide range of cognitive tasks. There is a large body of data interpreting the meaning of test findings. It will allow comparison of this study population with other populations. Lastly, the WPPSI-III has proven to have moderate to strong reliability and validity in a variety of studies.

The Wechsler Intelligence Scale for Children – IV (WISC-IV) [77] is a standardized test of intelligence for children from 6 to 16 years of age. As one of the Wechsler scales of intelligence, WISC-IV is considered the "gold standard" measure for evaluating intelligence among children in this age range in both clinical work and research. It is the most commonly used intelligence test used both in the clinical setting and in research. The WISC-IV takes approximately 75 minutes to administer and yields four summary scores: and 14 subtest scores. Summary scores include Verbal Comprehension,

Perceptual Reasoning, Working Memory, Processing Speed and Full Scale Score. WISC-IV covers a wide range of cognitive tasks, including such things as word definitions, reasoning questions, finding missing parts in pictures, putting together designs of colored blocks, remembering numbers, and comparing symbols to see if they are alike.

All the proposed neurodevelopment measures are well-known and respected among psychologists working in pediatric settings, allowing for reliable use across the settings for this study. These three tests are well validated and will be given by trained psychologists at sites convenient to the subject. The results of the assessments will be entered into the BARC database for analysis.

The first assessment is recommended to be at 12 months. This is an early assessment that is not yet predictive of all later neuropsychological events but will suggest the prevalence of significant disabilities and trends of strengths and weakness of the population as a whole.

Testing will be performed within 6 months of each birthday. The information obtained will be indexed in the database to the date of birth, since development can change significantly with in a six-month span especially at the one-year mark.

Assessment Schedule

Age in years ± 6 months	**Assessment**
1, 2	BSID-III
3, 4, 5	WPPSI-III
6, 8,10, 12, 14	WISC - IV
Annually Post Transplant	Lansky

The Lansky Functional Status Measure is used to determine the score of a child less than 16 years old that underwent a liver transplant while enrolled in the PROBE study. Children, who might have more trouble expressing their experienced quality of life, require a somewhat more observational scoring system suggested and validated by Lansky et al. This scoring, reported on an ordinal scale from 0-100, provides a rough measure of a patient's well-being including their activity level of play. The child's activity is assessed by the parent/ caregiver at each yearly follow up visit.

4.4.1 Study Calendar (Diary)

To facilitate tracking of medical consultations and changes in medications between research visits, clinical sites will provide the parent/guardian with a calendar either prepared by the site or one that is commercially available (a weekly or monthly appointment book)). The parent/guardian may choose to use the calendar to record medical consultations and changes in medication and to bring the calendar with them to research visits. When available, information in the calendar will be transferred to case

report forms; the calendar will not be copied and will not be kept by the clinical site as a source document.

4.5 Specimens to be Collected:

From the biological mother and father:

1. 20 ml of whole blood in two 10 ml NaEDTA vials for DNA extraction to be sent to the NIDDK Central Repository facility.
2. 7.5 ml of whole blood to extract plasma: (~10 aliquots of 0.4 ml each)
3. 7.5 ml of whole blood to extract serum: (~10 aliquots of 0.4 ml each)

Both the serum and plasma will be sent to the NIDDK Central Repository for storage until use. Therefore, a total of 35ml 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 2 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)

In addition, urine will be collected in a clean bag:

3. 5 ml of urine

At the time of a liver biopsy, exploratory surgery or portoenterostomy, any biopsy material that is removed as part of the surgical procedure, but is not needed for diagnostic purposes, will be collected for the repository. In patients undergoing a portoenterostomy or other biliary reconstruction, a portion of the excised biliary remnant 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. gall bladder aspirate
4. tissue from the biliary remnant
5. unstained paraffin-embedded slides of the biliary remnant
6. lymph node

From the infant at follow up visits: (4 or 8 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 and annually thereafter):

1. 2 ml of whole blood for plasma
2. 2 ml of whole blood for serum
3. Subject may have blood drawn for DNA at any visit prior to 1 year of age if the required volume (1-3 ml) can be obtained while keeping within age and/or size specific volume limitations. DNA will be preferentially isolated from these samples obtained prior to 1 year of age. If there is sufficient volume remaining in the sample, an EBV transformed cell line will also be established. In cases

where the volume is insufficient to establish an EBV transformed cell line, another blood sample for DNA may be obtained after 1 year of age, again keeping within age and/or size specific volume limitations.

4. 5 ml of urine

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 or 8 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 or 9.2**	8 or 9.2**
12 months of age	9.2	9.2	9.2
18 months of age	4	4	4
Annually from age 2	4	4	4

* This would only happen if the 3-month post-op or post-diagnosis visit was scheduled before the 3-month anniversary of the operation or diagnosis.

** When the 6 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: When a transplant is performed, blood (4 ml) to provide serum and plasma the repository should be drawn at or prior to the time of transplantation. When blood for cell lines (5.2 ml) has as yet not been drawn, and consent was provided, the blood may be drawn either at or prior to the time of transplantation or at a clinical visit subsequent to the transplant during which blood is being drawn for clinical purposes.

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.

From the infant at the time of transplantation:

1. tissue from the native liver
2. unstained paraffin-embedded slides of the native liver

NOTE: Consent for this procedure may be included in the initial written informed consent or obtained by a separate informed consent prior to the procedure to conform with the IRB requirements at the clinical site.

In children where consent for DNA and cell lines has been obtained, at the time of transplantation or surgery the following may be obtained:

3. Skin from the surgical incision to establish cell lines and extract DNA.

NOTE: When an investigator believes that it is unlikely that whole blood will be available at either time (either due to the health of the child or due to the risk of loss-to-follow up), skin from the incision (approximately 1cm in length and 3 mm in width) may be removed and sent to the repository to establish cell lines and extract DNA. This method should be used rarely since the success rate for establishing cell lines from skin fibroblasts is much lower than establishing cell lines when whole blood is used.

4.5.1 Specimen Repositories

A central repository has been established by the National Institute of Diabetes & Digestive and Kidney Diseases (NIDDK), a division of the National Institutes of Health, for long-term storage for blood, urine and tissue specimens and a second repository has been established at the NIDDK Central Repositoryfor cell lines and DNA extraction. Whole blood for cell lines 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, and the date, and the type of sample. Receipt of samples will be acknowledged to the originating center.

4.5.2 Specimen Use

The ChiLDREN Steering Committee has developed a policy for the approval of ancillary studies – studies that will require the 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 that have been proposed by ChiLDREN investigators (but as yet none are approved) 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 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 (current funding is until July 2014 and extension is possible), all decisions about the use of the samples will be made by the ChiLDREN Steering Committee. After funding for ChiLDREN terminates, NIDDK will set up a peer review mechanism to determine the 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 Subject Participation

Subjects with biliary atresia will be followed intensively for the first year, at 18 months of age, and then annually up to 15 years of age. Other subjects 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 subject's parents or guardians may request that the subject be removed from the study at any time. In addition, the investigator may withdraw a subject from the study if he/she determines that it is in the subject'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 data coordinating center 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 subject has been destroyed.

When the study ends at a clinical site or the subject completes the study, the linkage between the samples and the subject 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 subject request.

6. Statistical Considerations

General Design Issues:
The prospective observational database will collect data on all subjects 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 subjects 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 15 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 subjects 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 subjects as long as possible—therefore, the sample size is a function of the available subjects and length of accrual. Using data from these subjects, ChiLDREN desires to estimate the incidence of the failure of treatment, factors associated with success/failure, etc.

In the table below we indicate the relationship between sample size and effect size at a fixed power. We assume that we will be comparing infants with biliary atresia to control infants when computing effect size and difference in proportions and that we are trying to identify association of covariates with outcomes for infants with biliary atresia (one sample only) in the last column. All estimates are for 80% power using a two-tailed tests at a 5% level of significance. The effect size is the expected difference between two groups divided by the standard deviation. The difference of proportions is computed for the case where one group has a proportion of 50%; the difference will be smaller when the groups have proportions nearer to zero or one.

For example, it is possible that patients with biliary atresia have a 40% rate of reovirus compared to 20% in the controls. With 85 subjects per group, or after approximately 3 years of patient accrual, there will be 80% power to observe this difference. (This differs from the table below, because we used a worst case scenario, differences from 50%, to compute the entries in the table.) Factors that are hypothesized to affect success of the portoenterostomy are age (in weeks) at the time of the portoenterostomy and the number of episodes of cholangitis after the portoenterostomy; if the association between these factors and length of time to transplant or death is 0.3 or greater, we will be able to identify this correlation with 80% power when 90 subjects with biliary atresia have been studied.

The sample size needed to test the specified effect size or difference in proportions when comparing two groups or to identify the specified correlation in a single group.

Sample size per group	Exp. number of years to attain the sample size	Effect size, if continuous	Difference in proportions (from 0.5), if discrete	Correlation (one sample only)
60	2	0.52	0.25	0.36
90	3	0.42	0.21	0.30
120	4	0.37	0.18	0.26
150	5	0.33	0.16	0.23

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 subject 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 in itself 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 subjects, 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 take the within-subject correlation into account during analyses.

7. Data Management

7.1 Case Report Forms

The Biometrics and Outcomes Research Core (BORC) in the Department of Biostatistics at the University of Michigan is responsible for data management and analysis. It is the data coordinating center for several multi-center trials.

Case report forms are developed by the data coordinating center (DCC) and published on the ChiLDREN password-controlled website. The case report forms do not contain any personal subject identifiers, except dates, such as date of birth, which are necessary for research purposes. As needed, the coordinator prints the forms for each subject. The forms are completed and then countersigned by the investigator or study coordinator.

A combination of web-enabled and centralized data entry and management will be used. After the case report forms for a visit are completed, the research coordinator will enter a limited set of data into a web-enabled data management system.

Original case report forms will be securely maintained at the clinical sites. Clean copies of the case report forms will be transmitted monthly to the DCC to be entered into the study database.The forms entered into the database by the coordinator will not be transmitted to the DCC. Forms with personal identifiers are <u>not</u> sent to the DCC.

7.2 Quality Assurance:

The Project Manager/Clinical Monitor will review all data submitted to the DCC for accuracy and completeness. The Project Manager/Clinical Monitor will communicate with the study coordinators at each site about queries generated by the DCC and address all questions and concerns regarding the study protocol and problems with data entry or specimen sample shipment. Site visits will be made at 2 year intervals. 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 DSMB.

7.3 Training

The Project Manager will develop 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 Project Manager/Clinical Monitor 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. Test runs of data entry into the web-based data entry system as well as sample shipment will be organized prior to site initiation. The Project Manager/Clinical Monitor will review the study protocol and data entry system, and check all regulatory documents prior to site initiation. A meeting for all investigators and study coordinators may be held in conjunction with the initiation of the study. In-service training for all study coordinators

will be held quarterly via conference calls to review frequently encountered questions regarding the protocol, data entry or sample processing.

8. Adverse Events

Since this is an observational study, in general there are no adverse events that can be attributed to it except at the time of blood draws. Whenever possible, blood samples will be obtained in conjunction with clinical samples. When not possible, such as for the parents, the bruising at the time of blood draws may be attributable to this study.

Although mechanisms for reporting serious adverse events have been established, it is anticipated that there will not be any serious adverse events that can be attributed to this study – there will be serious adverse events that are expected clinically in this study population. The only serious adverse events related to the performance of this study are those related to phlebotomy.

An adverse event (AE) is any unfavorable, harmful or pathological change in a research subject as indicated by symptoms, physical signs and/or clinically significant laboratory abnormalities that occur in association the study procedures. This definition includes intercurrent illness, injuries, exacerbation of pre-existing conditions. Stable pre-existing conditions and elective procedures to address such conditions are not adverse events. A change in a laboratory variable is considered an adverse event 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).

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

- Death,
- Life-threatening (patient 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 adverse events will be attributable to this study.

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 Data and Safety Monitoring Board (DSMB) will act in an advisory capacity to NIDDK to monitor patient safety and evaluate the ability of the ChiLDREN 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

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 a serious adverse event, the investigator or designee will log into the ChiLDREN website and complete the Serious Adverse Event Form online. Upon receipt of a 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 DCC. The Safety Monitor will log in and read the report. If he has questions, he will contact the site and request clarification. After clarification is received, he will summarize the case and report it to the Chair of the DSMB, the NIH Program Director and the DCC. The Safety Monitor, the Chair of the DSMB and the NIH Program Director will determine whether 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 three months, the data coordinating center 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 taking an extra blood (4-11 ml depending on the visit) and urine, special handling of the liver biopsy and surgical specimens, and samples of blood from parents. There will be no costs to the patient or their insurance for any research-related data collection, including the developmental

assessments, or special laboratory investigation. The expenses for the storage and handling of the extra research blood and urine 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 the equivalent of $15-20 (amount and method of payment – cash, gift certificate or check – to be determined by the clinical site) to reimburse them for parking, meals or other expenses that they may have 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 Consortium are very slight since the blood samples will be de-identified prior to being deposited in the repository; that is only a research study number will be included in the database and all dates will be converted into ages by the DCC prior to dissemination to the repository or to any laboratory conducting genetic studies, While the study is ongoing, the clinical site will maintain a link between the research study number and the subject's identity. However, this information will not be contained in any data file that is transmitted to the DCC 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 subject's identity.

If there is a loss of confidentiality, the 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 members of the family who are 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 patients, 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 subject will retain a signed consent form; one will be retained for the subject's chart; and one will be included in the research records.

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