LONGITUDINAL STUDY OF GENETIC CAUSES OF INTRAHEPATIC CHOLESTASIS (LOGIC)

Childhood Liver Disease Research Network (ChiLDReN)

AMENDMENT 7

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I. PROTOCOL OVERVIEW (ABSTRACT)

This study will be conducted as part of the National Institutes of Health (NIH)-supported Childhood Liver Disease Research Network (ChiLDReN). ChiLDReN is investigating rare cholestatic liver diseases of childhood: alpha-1-antitrypsin (α1-AT) deficiency, Alagille Syndrome (ALGS), progressive familial intrahepatic cholestasis (PFIC), bile acid synthesis defects (BASD), and mitochondrial hepatopathies (all previously studied by the Cholestatic Liver Disease Consortium [CLiC]); biliary atresia (previously studied by the Biliary Atresia Research Consortium [BARC]); neonatal hepatitis; and cystic fibrosis liver disease, which is studied by a branch of ChiLDReN known as the Cystic Fibrosis Liver Disease (CFLD) Network.

This longitudinal observational study will investigate the natural history and progression of four genetic causes of intrahepatic cholestasis of childhood, including: (1) α1-AT deficiency; (2) ALGS; (3) PFIC or benign recurrent intrahepatic cholestasis (BRIC); and (4) BASD. In this study, we will collect defined data elements in a uniform fashion at fixed intervals for up to 20 years in a relatively large number of patients with these rare disorders. In addition, a biobank of participant specimens and DNA samples will be established for use in ancillary studies to be performed in addition to this study. By comparing outcome measures between the four liver diseases (i.e., using each disorder as a disease-control for the other disorders), the full impact of each disorder can best be determined in comparison with the other liver diseases. Using the longitudinal database in this fashion, this study will provide an improved understanding of the effects of the cholestatic liver during childhood irrespective of the underlying etiology, as well as to the pathophysiology, outcome, and complications of each of the disorders. This initial characterization will allow calculation of sample sizes for future therapeutic interventional clinical trials and provide the baseline to which interventions should be compared.

II. SPECIFIC AIMS

The following hypotheses will be tested in this longitudinal study of the four cholestatic liver diseases:

II.A. Hypotheses

1. Each of the four intrahepatic cholestatic diseases will have unique phenotypic features and a characteristic natural history.
2. Genotypic differences in participants with each of the cholestatic diseases may influence the disease phenotype and progression.
3. Poor growth and decreased bone mineral density in patients with cholestatic liver diseases is variably dependent on the degree of cholestasis, body composition, and/or the severity of liver synthetic dysfunction.
4. Early biomarkers will be predictive of outcome in cholestatic liver diseases.

To test these hypotheses, the following Specific Aims will be addressed. Several of the Specific Aims pertain to all four diseases, and others will be tested in specific disorders. Another important feature of this study will be the collection and storage of specimens in the National Institute of Diabetes Digestive and Kidney Diseases (NIDDK) specimen repository that will be utilized in future Ancillary Studies of ChiLDReN (to be proposed) to further address the pathophysiology and outcomes of these four liver diseases.

II.B. Specific Aims

Aims related to all four liver diseases

1. To determine the **clinical phenotype** and **natural history** of each of the four liver diseases during childhood and early adulthood.
2. To determine the frequency of **poor growth** and its predictors in all four diseases.
3. To **develop a repository** of serum, plasma, urine, tissue, and DNA specimens, that will be used in future Ancillary Studies to determine circulating biomarkers that predict disease progression and outcomes, to identify new genetic causes of the disorders, and to identify modifier genes that influence the incidence, severity and progression of liver disease among genetically affected individuals.

Aims related to specific diseases

4. To determine **genotype-phenotype** relationships in ALGS and PFIC (or BRIC) disorders.
5. To determine the frequency of decreased bone mineral density in ALGS and PFIC (or BRIC) disorders.

III. BACKGROUND

III.A. CLiC and ChiLDReN

In late 2004, CLiC was initially funded by the Office of Rare Disorders, the National Center for Research Resources, and the NIDDK of the NIH and was established to study five rare, genetic disorders resulting in intrahepatic cholestasis in children. The initial NIH funding for CLiC extended through June 2009. CLiC was composed of 11 Clinical Sites (including one site at Saint Louis University that is focused specifically on α1-AT deficiency enrollment), four Biologic Core facilities, and a Data and Technology Coordinating Center (DTCC), located in the United States and the United Kingdom. In November 2008, the Scientific Data Coordinating Center (SDCC) was moved to the University of Michigan. In June 2009, CLiC was incorporated into the new, NIH-funded ChiLDReN research network (Appendix I). Currently the ChiLDReN consortium consists of 13 clinical sites and an SDCC, with responsibility shared between the University of Michigan and Arbor Research Collaborative for Health. The objective of CLiC, which has now been encompassed by ChiLDReN, was to investigate the pathophysiology, etiology, natural history, diagnosis, treatment, and outcomes of five genetic causes of intrahepatic cholestasis, including mitochondrial hepatopathies. The mitochondrial hepatopathy study is now a separate study protocol within ChiLDReN. The goal of the Longitudianl Study of Genetic Causes of Intrahepatic Cholestasis (LOGIC) protocol is to perform a longitudinal study of each of the other four disorders and to develop a DNA repository and tissue/serum/plasma/urine repository to aid in current and future studies of these disorders. By systematically studying clinical aspects and the
underlying pathologic processes, the ChiLDReN investigators believe that a better understanding will be gained of how the childhood liver is injured in cholestasis and in the specific ChiLDReN diseases,

and that new strategies for treating these disorders can be developed.

III.B. Clinical Significance of Genetic Causes of Intrahepatic Cholestasis

Genetic causes of intrahepatic cholestasis compose a unique subset of rare diseases that need a more concentrated national effort to better understand the molecular and cellular basis of these disorders; the natural history and the medical, developmental, and social consequences on the child and family; and to develop new therapeutic approaches. The four rare liver disorders that will be the focus of this study are all considerations in evaluating infants with a cholestatic disorder (jaundice, conjugated hyperbilirubinemia, and elevated serum bile acid concentrations), a condition that occurs in one of 2,500 live births. These four disorders, which as a group account for approximately 20%-30% of all infants presenting with neonatal cholestasis, cause significant morbidity and growth problems during childhood, lead to progressive cholestasis (impaired bile flow), hepatic fibrosis, and ultimately cirrhosis of the liver with its incumbent complications (including death), and are among the leading indications for liver transplantation in childhood. Each of these disorders involves perturbations in different intracellular pathways of protein and lipid metabolism, targeting different key organelles, yet producing similar, but individualized clinical features. For example, α1-AT deficiency involves retention of an abnormally folded protein in the endoplasmic reticulum (ER) of liver cells. This pathologic state is associated with mitochondrial dysfunction and quality control mechanisms, including proteasomal degradation, autophagic degradation, and activation of other "stress" signaling pathways, which appear to be critically important in determining whether tissue injury occurs. PFIC involves retention of bile acids that most likely stimulate plasma membrane death receptors and cause direct mitochondrial toxicity-inducing cellular apoptotic machinery. BASDs involve failure to produce protective bile acids and overproduction of toxic bile acids, which may stimulate these same pathways, leading to rapid onset of hepatocellular failure. ALGS involves disordered intrahepatic bile duct development, leading to impaired bile flow and similar intracellular consequences.

Because of the common pathophysiologic feature of *intrahepatic cholestasis*, the injured liver in all of these conditions fails to adequately secrete bile into the intestinal tract, which is essential for normal digestion and excretion of cholesterol and drug metabolites. The consequent very significant malabsorption of dietary fat and fat-soluble vitamins poses a major obstacle to normal nutrition in these growing children. The resulting negative energy and nitrogen balance lead to poor linear growth and decreased lean body mass and subcutaneous adipose tissue stores, having a profound effect on the child's ability to function normally and maintain adequate immune function to protect against common childhood illnesses. Micronutrient deficiencies (e.g., vitamin E, zinc, vitamin A) may produce irreversible neurological, developmental, and retinal deficits if not recognized and promptly treated. In addition, cholestasis-induced pruritus (itching) may be devastating in affected children, interfering with sleep, daily activities, and school performance and causing intractable scratching and skin infections. Cholestasis also directly causes hypercholesterolemia and disfiguring skin xanthoma formation. The jaundice frequently persists, further impacting on the child's physical appearance and self-esteem. Over time, as hepatic fibrosis progresses to cirrhosis, the liver fails to synthesize and release important proteins, lipids, and carbohydrates for the rest of the child's body; remove and metabolize toxins, xenobiotics, and metabolic by-products; and store essential nutrients. These abnormalities may affect cognitive development, brain growth, and linear growth, producing long-term irreversible extrahepatic morbidity.

When irreversible signs of end-stage liver disease or intractable cholestasis develop, children with these cholestatic disorders are frequently evaluated for liver transplantation as their only option for survival, a costly procedure that demands life-long immunosuppression to prevent allograft rejection in almost all cases. In the Studies for Pediatric Liver Transplantation (SPLIT) database, 56 children with ALGS, 54 with α1-AT deficiency, and 25 with PFIC were among the 1761 children who were listed for liver transplantation between 1995 and 2002 at 38 North American liver transplant centers.[1] An additional 42 children with "other" cholestatic and 21 with "other" metabolic disorders may have had these diseases but were undiagnosed. Thus, these four disorders account for almost 10% of all children who are evaluated and listed for liver transplantation in the United States. Unfortunately, current non-surgical treatment for these four diseases is generally inadequate because of a poor understanding of the molecular, biochemical, and cellular basis of liver injury.

Finally, diagnostic procedures for some of these disorders require complex genetic evaluation, surgical liver biopsies, or difficult biochemical testing. The development of more available simple diagnostic testing for these disorders would enhance the ability of caregivers to establish the diagnoses quickly and institute therapies.

In summary, the four rare liver disorders of focus in this study are a serious group of related diseases that demand more rigorous investigation in order to develop a scientific basis for improvements in diagnosis and treatment. The first step in advancing our understanding of these cholestatic disorders and improving our diagnostic capabilities is to develop a *prospective longitudinal database study* that includes a sufficient number of participants for adequate characterization of clinical phenotype; defining the clinical, biochemical, neurodevelopmental, quality of life, and survival outcome; developing and validating biomarkers of disease progression; and the collection of banked genomic DNA and liver biopsy ribonucleic acid (RNA) to be used in Ancillary Studies that will identify new genetic causes, as well as modifier genes involved in these disorders. Furthermore, a longitudinal cohort study of participants with these liver diseases will help accelerate clinical research and progress in understanding the pathogenesis of rare causes of intrahepatic cholestasis by more carefully defining the natural history of each disease and by providing an infrastructure in which to investigate better means of diagnosis and of treatment. The rarity of these diseases and the difficulty in timely diagnosis makes it difficult to accumulate an adequate number of participants followed for an adequate period of time at a single clinical center. A uniform, standardized, coordinated approach to evaluation and follow-up will reduce the number of participants needed to ensure adequate statistical power.

III.C. Current State and Gaps in Knowledge in Individual Cholestatic Diseases

The **four genetic causes of intrahepatic cholestasis** to be studied in this protocol share clinical and pathophysiologic features of cholestasis. *In the following, the current state of knowledge about each disease will be summarized, and current scientific and clinical challenges will be outlined.*

1. α1-AT Deficiency

Incidence: Homozygote PIZZ: 1 in 2000 live births in the United States, especially in populations of Northern European extraction, less in Hispanic and African American populations. Compound Heterozygote PISZ less common.

Number of Affected Individuals in United States: 100,000 (personal communication, John

Walsh, President of Alpha-1 Foundation).

The classical form of α1-AT deficiency (PIZZ) is an inborn error of metabolism that has been associated with chronic liver disease and hepatocellular carcinoma, as well as adult-onset emphysema. Although it is a rare disease, it is the most common genetic cause of liver disease in children and the most frequent genetic/metabolic disease for which children undergo liver transplantation. α1-AT deficiency is also the prototype genetic cause of destructive lung disease/emphysema. α1-AT, the archetype of the Serpin supergene family, is the principal blood-borne inhibitor of destructive neutrophil proteases including elastase, cathepsin G, and proteinase.[2] This glycoprotein is secreted by hepatocytes and is considered an acute-phase reactant because its plasma levels increase during the host response to inflammation/tissue injury. In homozygous PIZZ α1-AT deficiency, mutant α1-ATZ is retained as a polymer in the ER of liver cells.[3,4] Homozygotes are predisposed to premature development of pulmonary emphysema by a loss-of-function mechanism, in which lack of α1-AT in the lung permits uninhibited proteolytic damage to the connective tissue matrix.[5,6] Cigarette smoking markedly increases the risk and rate of development of emphysema.[7] One mechanism for this environmental risk factor involves the functional inactivation of residual α1-AT by phagocyte-derived active oxygen intermediates.[5,6] However, a growing body of evidence suggests that other environmental factors and genetic traits affect the incidence and severity of lung disease among α1-AT-deficient individuals.[8] It is still not entirely clear whether heterozygotes for α1-ATZ are predisposed to lung disease.

Homozygotes for α1-ATZ are at risk for liver disease. However, in contrast to the pathobiology of lung disease, liver injury in this deficiency appears to involve a gain-of-function mechanism, whereby retention of the mutant α1-ATZ molecule within the ER triggers a series of events that are eventually hepatotoxic. The strongest evidence for a gain-of-function mechanism comes from studies in which mice transgenic for mutant human α1-ATZ develop liver injury with many of the histopathologic hallmarks of the human condition.[9,10] Because there are normal levels of anti-elastases in these mice, as directed by endogenous genes, the liver injury cannot be attributed to a loss of function.

Landmark nationwide prospective newborn screening studies done by Sveger in Sweden have documented an extraordinary variation in the phenotypic expression of liver disease among homozygotes that were identified shortly after birth. In these studies, only 10%-15% of the PIZZ population developed clinically significant liver disease over the first 20 years of life.[11,12] These data indicate that other genetic traits and/or environmental factors predispose a subgroup of PIZZ individuals to liver injury. Because only a subgroup of homozygotes develops clinical liver disease, and because there is an inherent bias in ascertainment in other clinical studies of α1-AT deficiency, it has been very difficult to determine whether heterozygous (PIMZ) individuals are at increased risk for liver disease.

Although the Sveger study provides information about the general population of PIZZ individuals, and is the only unbiased longitudinal study of that population, it contains only limited information about the course of α1-AT-deficient children with serious liver disease. Several studies of PIZZ patients referred to subspecialty pediatric liver clinics for significant liver disease have suggested that there is also a high degree of variability in the severity of liver disease.[13-17] This variability in severity has become

particularly important because we now know that children with this deficiency do well after liver replacement (transplantation) therapy. Because liver biopsies were not done, the Sveger study does not address the possibility that 18 year-olds with α1-AT deficiency have persistent subclinical histologic abnormalities and will eventually develop clinical significant liver disease during adulthood. In fact, there is very little known about the development and natural history of liver injury in PIZZ adults.

Although there is evidence from studies of cell culture model systems that the cellular response to retention of mutant α1-ATZ in the ER plays a role in the susceptibility to liver disease in a subgroup of PIZZ individuals,[18] there is only limited information about whether and how much of this is determined by genetic or environmental mechanisms. Clinical studies of this issue have come from biased studies and have produced conflicting results. In one study, the incidence of significant liver disease among siblings at risk was 78%.[16] The other study suggested that the incidence of liver disease in homozygote sibs is 21%.[14] Thus, it is still not clear whether liver disease breeds true in families affected by α1-AT deficiency. If liver diseases did breed true, this would provide substantial support for the presence of another modifier gene that influenced the liver disease phenotype.

Several authors have proposed that co-existing infectious or autoimmune diseases, heterozygosity for other genetic liver diseases, or the presence of unknown modifier genes increase the susceptibility to liver disease and the severity of liver disease in α1-AT deficiency.[19] However, this concept has not yet been validated in a large enough population of homozygous patients to provide statistical significance. The ChiLDReN Network will allow for collection of DNA from an adequate number of affected participants to test this hypothesis in future studies.

2. ALGS

Incidence: 1 in 70,000

Number of Affected Individuals in United States: 2,000-3,000 (estimate)

ALGS is defined as bile duct paucity in association with hepatic, cardiopulmonary, skeletal, ocular, renal, and facial manifestations. Since its description in 1969 by Daniel Alagille,[20] a large and diverse group of abnormalities has been associated with the disorder. The disorder was recognized to be inherited in an autosomal dominant manner, but with variable penetrance and expressivity. In 1997, mutations in *JAGGED1*, a ligand in the *NOTCH* signaling pathway, were shown to cause ALGS.[21,22] The identification and characterization of these mutations has led to a new understanding of the spectrum of ALGS. Prior to the discovery of *JAGGED1*, the diagnosis was made solely by clinical criteria. Data combined from a series of reports estimates the frequency of the manifestations to be bile duct paucity 89%, cholestasis 95%, heart murmur 94%, butterfly vertebrae 68%, posterior embryotoxon 81%, and renal disease 44%.[23-27]
More recent reports have illustrated the importance of other clinical manifestations, including intracranial vascular lesions leading to hemorrhage and stroke, and pancreatic disease.[25,26]

Although a rare liver disorder, ALGS is the most common dominantly inherited etiology of neonatal cholestasis, and among the five most common etiologies overall. It occurs with a frequency of approximately 1 in 70,000 live births in all ethnic and racial groups. Mutations in *JAGGED1* can be found in >90% of patients with clinically defined ALGS, and 50%-70% of mutations are *de novo*.[28,29]

A total gene deletion is seen in 4%. The majority of the *JAGGED1* mutations lead to premature termination of the protein, and only 9% of patients have a missense mutation. Recently, mutations in *NOTCH2* have been found in a small subset of participants.[30,31] Because there is little discernible clinical difference between participants with total gene deletion and those with less extensive mutations, it is presumed that the manifestations of ALGS are due to haploinsufficiency. There are only a few reports of genotype-phenotype correlations in ALGS. The most striking of these is an extensive pedigree with tetralogy of Fallot and cardiac disease due to a missense mutation.[32,33] It is common to have highly variable manifestations in patients with the same genotype, even within a single family. The search for modifying genes that affect the manifestations of the disease has only recently started, with emphasis on the *NOTCH* pathway. The *NOTCH* signaling pathway is an extensive, evolutionarily conserved pathway, with over 35 proteins that are involved in the regulations of cell fate determination.

There are at least five ligands and four *NOTCH* receptors that have been identified in humans. Defects in two other of these genes have been shown to cause human diseases that show some similarity to features of ALGS. Defects in the Notch ligand delta-like3 cause Jarcho-Levin spondylocostal dysostosis, a disorder with primary manifestations in the axial skeleton.[34] Defects in *NOTCH3* cause strokes in CADASIL (cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy disorder) that occur in adulthood.[35] The ability to screen for mutations in family members has allowed an enhanced understanding of the mild end of the clinical spectrum of ALGS. Relatives of probands who carry the same mutation may have few if any evident manifestations typically associated with ALGS. We have recently found that only 11% of 50 relatives of probands with *JAGGED1* mutations had clinical features that would have led to the ALGS diagnosis.[36] Thus, modifying factors (genetic or environmental) are clearly present, but not yet defined.

The mortality of ALGS is highly variable, but significant. Cardiac, hepatic, and central nervous system (CNS) disease account for the majority of deaths. The presence of complex intracardiac disease is the only predictor of excessive early mortality (40% survival at 7 years) and cardiac disease accounts for nearly all deaths in early childhood.[25] Hepatic complications account for most of the later mortality. In several studies, when survival is considered without respect to intracardiac disease, it was found to be 72% at 8.9 years,[27] 87% at 20 years,[26] and 75% at 20 years.[25] Recent series demonstrate significant mortality from intracranial hemorrhage.[25-27] Overall, up to 12% of patients presenting with significant liver disease have developed intracranial hemorrhage, some with minimal head trauma.[25-27] Although the frequency of intracranial vascular abnormalities was almost 30% in one small study,[37] there has been no prospective study ascertaining the true prevalence of these abnormalities and the incidence of hemorrhage or other complications in patients with and without detectable abnormalities. The potential presence of vascular abnormalities in the chest and abdomen has not been systematically investigated in ALGS.

Morbidity of ALGS is also extensive. Patients with hepatic or cardiac involvement may have profound growth failure, severe cholestasis, intense pruritus, osteopenia, pathologic fractures, and impaired quality of life. Although synthetic function is generally preserved in most patients, liver transplantation is ultimately required in 20%-43% of patients with significant hepatic disease, making ALGS one of the most common inherited diseases resulting in transplantation. Patients with ALGS have also required heart, lung and kidney transplantation.

In many patients, the clinical manifestations of cholestasis improve after the first few years of life. The factors that result in this improvement are unknown. Although there does not appear to be regrowth of interlobular bile ducts, excretion of bile documented by nuclear scintiscan does occur. Understanding these phenomena would potentially offer opportunity for development of therapies that stimulate either bile flow or intrahepatic bile duct growth. Portal hypertension occurs in approximately 40% of patients with hepatic disease and is a common indication for transplantation, particularly after the first decade of life. The factors that predispose to this portal hypertension are poorly characterized, but may also involve specific molecular or biochemical factors. Through a combination of genotyping and careful clinical phenotyping, systematic and comprehensive prospective monitoring of a large number of ALGS patients must be performed to identify the specific genetic and environmental factors that predispose to the serious complications of ALGS. The ChiLDReN Network provides this participant base and the scientific and clinical expertise to address this important problem.

3. PFIC

Incidence: 1 in 70,000 live births (estimate)

Number of Affected Individuals in United States: 2,000 (estimate)

PFIC includes a range of important rare inherited disorders that result from defects in cellular pathways involved in bile formation and secretion. PFIC type 1 (PFIC1, also known as Byler Disease) results from mutations in the gene *FIC1*.[38] The mechanism by which defects in *FIC1* protein (a P-type ATPase involved in aminophospholipid transport) expression cause liver disease is not known. A recent report suggests that *FIC1* activates the transcription of the Farnesoid X-Receptor (FXR), an important modifier of bile acid homeostasis genes.[39] Loss of *FIC1* leads to reduced expression of FXR and may lead to enhanced intestinal absorption and diminished hepatic secretion of bile salts. PFIC2 results from defects in the canalicular ATP- dependent bile acid transporter, *BSEP*.[40] Liver disease in PFIC2 is an expected manifestation of a marked reduction in hepatic canalicular bile acid secretion. PFIC3 is the result of defects in a canalicular phospholipid flippase, *MDR3*, which is a key element in canalicular phospholipid excretion.[41,42] It is proposed that the low or absent phospholipid in bile leaves secreted bile salts in a non-micellar state and bile duct injury results from the detergent effects of those bile salts on the bile duct epithelial cells. Familial hypercholanemia has only recently been described and appears to be a complex genetic trait.[43] Defects in both bile acid conjugation (BAAT) and tight junction proteins (ZO-2) appear to be involved. More recently, mutations in the tight junction protein *TJP2* have been reported in association with a low GGT PFIC phenotype.[44] With the increasing availability of advanced genomic sequencing techniques, additional candidate genes have been identified, including *NR1H4*, encoding the nuclear bile acid receptor FXR, and *MYO5B*.[45,46]
The clinical phenotype, natural history, and optimal medical treatment regimens for these disorders are not known. The reported clinical phenotypes for these disorders are based primarily upon the most significant mutations with the most severe clinical presentations. PFIC2 and PFIC3 have been reported to be liver specific disorders, with progression to end-stage liver disease in the first decade of life. In contrast PFIC1 is a systemic disorder with liver, pancreatic and intestinal manifestations. It is typically characterized by intense pruritus, with nutritional deficiencies and variable degrees of severe diarrhea. Progression to end-stage liver disease may take longer than with PFIC 2 and 3. *FIC1* mutations and BSEP mutations have also been

associated with BRIC1 and BRIC2, a more benign liver disease seen primarily in adults, but may also occur in children. In this condition, recurrent episodes of mild to severe cholestasis occur up to several times per year with normal clinical and laboratory status in between, and without significant liver fibrosis or portal hypertension. This disease usually has its onset around the time of puberty, but can start earlier in childhood. Since the full spectrum of liver involvement in *FIC1* and BSEP disease has not been clearly defined, this study will include participants with both PFIC and BRIC clinical presentations, and this protocol will refer to both as PFIC disorders. More careful scrutiny of the liver disease associated with defects in *MDR3* (PFIC3) has identified milder phenotypes manifested by cholestasis of pregnancy,[47] biliary cirrhosis in adults,[48] intrahepatic cholelithiasis,[49] and chronic liver dysfunction.[50] This type of analysis has not been performed for the other forms of progressive intrahepatic cholestasis. Thus, the full range of the clinical phenotype of mutations in genes causing PFIC has yet to be determined. With new PFIC disease genes rapidly being identified, it becomes even more important to characterize the clinical phenotypes and natural history of these genetically distinct PFIC disorders.

There are no prospective descriptions of the natural history of children with genetically well-defined intrahepatic cholestasis. The longitudinal follow-up proposed in this study will permit characterization of the natural history of these disorders, including important data regarding growth, development, the evolution of liver disease, and extrahepatic manifestations. This information will be critical for analysis of potential medical and surgical interventions in these disorders.

Most importantly, the optimal treatment regimens for PFIC (or BRIC) disorders are unknown. Standard therapeutic regimens typically include empiric administration of ursodeoxycholic acid (UDCA) and nutritional support with medium chain triglyceride-containing formulas and fat-soluble vitamin supplementation. There is no evidence that these common-sense therapies alter the natural history of PFIC (or BRIC). In most cases, this approach does not influence the disabling and disfiguring pruritus associated with cholestasis in these children. A number of other agents (cholestyramine, rifampin, antihistamines, corticosteroids, etc.) are used with limited success in treating pruritus. Surgical approaches have been employed in an uncontrolled fashion to try to manage the pruritus in children with progressive intrahepatic cholestasis. The premise is that preventing bile acid absorption (ileal exclusion) or discarding bile (partial external biliary diversion) will decrease the presence of toxic bile acids and reduce liver injury and pruritus. A number of small uncontrolled series have reported clinical benefit with either procedure.[51,52] Following liver transplantation, persistent diarrhea and fatty liver have been described in PFIC.[53] At present, there is no prospective study, or even well-validated retrospective data comparing the utility of partial external biliary diversion versus ileal exclusion in genotypically well-defined patients with PFIC. Given the rare nature of these disorders and the varying decision-making at centers, it is unlikely that this issue can be resolved in the absence of a prospective longitudinal database study.

4. Bile Acid Synthesis and Metabolism Disorders

Incidence: 1 in 100,000 live births (estimate)
Number of Affected Individuals in United States: 1,000 individuals (estimate)

Bile acid synthesis from cholesterol occurs via multiple complex biochemical pathways that are catalyzed by a number of different enzymes located in different sub-cellular compartments of hepatocytes.[54-57] In the last two decades, specific disorders in bile acid synthesis have been identified as a significant cause of cholestatic liver disease and syndromes of fat-soluble vitamin malabsorption. Six defects in enzymes involved in primary bile acid synthesis from cholesterol have now been identified,[54-62] including 3β-hydroxy-C_{27}-steroid dehydrogenase/isomerase (3β- HSD; also called 3 β -hydroxy-C_{27}-steroid oxidoreductase), the Δ^4-3 oxosteroid 5 -reductase (Δ^4-3-oxo-R), the oxysterol-7β-hydroxylase, the sterol 27-hydroxylase (cerebrotendinous xanthomatosis, CTX), the 2-methylacyl-CoA racemase, and the enzymes involved in side-chain conjugation. In addition, a spectrum of bile acid metabolic defects involving impaired side-chain oxidation are known which are secondary to abnormalities in peroxisomal structure and function. These rare genetic autosomal recessive diseases are manifest as progressive neonatal cholestatic conditions that lead to liver failure, and/or fat-soluble vitamin malabsorption syndromes.[63] Diagnosis is accomplished by mass spectrometry of bile acid profiles of urine[64] and is usually done during clinical evaluation of the patient because of an unexplained conjugated hyperbilirubinemia and elevated serum aminotransferases. Unfortunately, accurate analysis of urine bile acids by clinical laboratories is not available at this time, so diagnostic tests are generally provided by research laboratories. In four of the described defects in bile acid synthesis, mutations have been identified in the gene encoding the missing enzyme. Inborn errors in bile acid synthesis account for many additional cases of late onset chronic cholestatic disease and neurological disease.

The common theme in all of these reported defects is an absence or marked deficiency of synthesis of the usual primary bile acids. In response, the liver of these patients synthesizes increased amounts of sterol and bile acid intermediates that are substrates in the pathway proximal to the enzyme defect. These intermediates are subsequently metabolized to C_{24}- or C_{27}- bile acids that retain the basic nuclear structure of the substrates proximal to the missing enzyme. The mechanism of liver injury in these patients is the reduction of normal bile flow caused by the failure to synthesize adequate concentrations of primary bile acids and/or the production of atypical metabolites which are intrinsically hepatotoxic. The liver disease associated with inborn errors in bile acid metabolism is progressive[63] and, if untreated, leads to death due to cirrhosis and liver failure. Long-term survival and clinical improvement of such patients has only been possible with the provision of oral supplements of primary bile acids (cholic acid and UDCA) to stimulate bile flow and lead to down-regulation of bile acid synthesis.[64-67] The only other available therapeutic intervention for most of these defects is liver transplantation. This intervention is limited by the availability of suitable donor organs, high cost, transplant rejection and re-transplantation, as well as a life-long use of anti-rejection medications.

IV. SIGNIFICANCE

The major objectives of this protocol are to conduct a multicenter, longitudinal observational study and develop a repository of DNA and human specimens from participants with four genetic causes of intrahepatic cholestasis, as well as DNA from participant's biological parents. Because there is insufficient characterization of the natural history and clinical course of many chronic intrahepatic cholestatic liver diseases in childhood, a multi-centered effort conducted by experienced clinicians and scientists will be the most effective means of determining the course of these diseases, developing and testing improved diagnostic techniques, discovering genetic causes and modifiers of the diseases,

identifying biomarkers of disease risk and progression, determining predictors of response to therapy and predictors of outcome, and maintaining a cohort of participants for clinical trials of new diagnostic methods and therapeutic approaches. A major advantage of this approach is the utilization of common definitions of disease and tracking participants over time in a uniform manner across multiple clinical sites. The common clinical, biochemical, and genetic database used in the ChiLDReN clinical sites will offer recruitment and retention of adequate numbers of participants followed for a prescribed period of time with these rare liver disorders. The acquisition of DNA on all participants and their biological parents, and a tissue/serum/plasma specimen biobank on all participants entered in this study will make available an important resource for Ancillary Studies that may evaluate etiology, pathogenesis, biomarkers, pharmacogenomics, and genetic modifiers of these disorders.

V. STUDY DESIGN AND METHODS

V.A. Overview (Study Flow)

This is a multi-center longitudinal observational study of four ChiLDReN cholestatic liver diseases, utilizing collection of diagnostic, clinical, and outcome data at defined intervals for up to 20 years. These data will be used to test hypotheses related to these diseases as a group and to specific diseases. The development of the serum, urine and tissue repository, and the maintenance of a DNA bank for genetic analyses, will be an invaluable resource for current and future ancillary investigations into the pathogenesis, genetics, and progression of cholestatic liver injury in the infant and child, and the identification of modifier genes and biomarkers predictive of outcomes. ChiLDReN participants with suspected ALGS and PFIC (or BRIC) will undergo research genotyping at the ChiLDReN Genetics Core. Asymptomatic affected siblings of participants with α1-AT deficiency (but with no evidence of liver disease) will be entered into the database and followed prospectively. All data from this study will be kept in a secure research database at the SDCC of ChiLDReN, located at the University of Michigan and Arbor Research Collaborative for Health in Ann Arbor, Michigan, where the data will be analyzed.

V.B. Study Participants

V.B1. Inclusion Criteria

All current and newly diagnosed patients (based on diagnostic and enrollment criteria in Section V.B4) with α1-AT deficiency, ALGS, PFIC (or BRIC), and bile acid synthesis and metabolism defects, both before and after liver transplant, followed at or referred to each ChiLDReN Clinical Site, will be offered enrollment into this study. Siblings of participants with α1-AT deficiency, who themselves have the underlying disease, but with no evidence of liver disease, will also be offered enrollment. Parents aged 25 and under who have children enrolled in this study may themselves be offered enrollment if they meet entry criteria for Groups 2 or 3 (see Section V.B3 for group descriptions). After informed consent is obtained, participants will be enrolled into this study through five Groups (described in V.B3).

The inclusion criteria are:

1. Children and young adults diagnosed with one of the four cholestatic diseases from birth through 25 years old.
2. Siblings of participants with α1-AT deficiency, who are affected with α1-AT deficiency, but have no evidence of liver disease.
3. Both sexes, all races and ethnic groups.

4. Participant meets the enrollment criteria for one of the four cholestatic liver diseases outlined below in V.B4.
5. Patient and/or parent/legal guardian have the ability to provide written informed consent for enrollment.

V.B2. Exclusion Criteria

Exclusion criteria include:

1. Inability to comply with the longitudinal follow-up described below.

V.B3. Study Groups

Participants with each of the four cholestatic liver diseases (see Enrollment Criteria in Section V.B4) will be enrolled at each ChiLDReN center, including those who are currently followed at the centers, new patients to the ChiLDReN centers, and siblings of patients with specific diseases. At the additional Enrollment Center (Saint Louis University), only participants with α1-AT deficiency will be enrolled. Participants will be enrolled via five Groups in order to facilitate the appropriate collection of data (outlined in Schedules in Tables 3-7). The five Groups are the following:

1. Group 1: Infants < 6 months of age at diagnosis and enrolled in the ChiLDReN (or BARC) PROBE study

Group One applies to infants who were initially enrolled at <6 months of age into the ChiLDReN Prospective Biliary Atresia Epidemiology study (PROBE study; P003). For these PROBE participants, once the definitive diagnosis of a disease studied in LOGIC is established, these participants will be offered enrollment into this LOGIC study, consented for this longitudinal study, and enrolled in this LOGIC study, as well as continued in the PROBE study. Participants may be any age, up to and including 25 years old at the time of diagnosis for a disease studied in LOGIC. Follow-up for both studies will be done concurrently in a seamless fashion, so that the required data elements will be acquired for both studies by the same study coordinator at the same time.

Any additional LOGIC data that are not collected on the PROBE case report forms (CRFs) will be collected on a LOGIC CRF. For periodic reporting and for analyses of Group 1 participants, PROBE data for participants concurrently enrolled in LOGIC will be pooled with LOGIC data. Consent forms and HIPAA forms for both studies will include a statement describing data sharing with the other study.

2. Group 2: Participants from birth through 25 years old at enrollment and not previously enrolled in PROBE study.

Group Two will apply to currently established patients at one of the ChiLDReN Clinical Sites who have one of the ChiLDReN diseases from birth through 25 years of age, or patients with one of these diseases who are newly referred to ChiLDReN Clinical Sites at these ages, and who are not enrolled in the PROBE study. These patients will be offered enrollment into this LOGIC study, consented and followed in Group 2. Affected parents of participants enrolled in the study are eligible for enrollment if they are 25 years old or less.

3. Group 3: Post-liver transplant participants

Group Three will be for participants with ChiLDReN diseases, birth through 25 years old, who have

undergone liver transplantation and are either followed at or referred to the ChiLDReN clinical sites. These participants will have a limited data set collected and limited ChiLDReN follow-up, but will be essential for the studies of genotype-phenotype relationships, of modifier genes, and of natural history of each disease. They will be enrolled for an abbreviated data collection visit and to collect blood (or saliva, if collection of blood is not possible) for preparation of DNA for genetic studies. Affected parents of participants enrolled in the study are eligible for enrollment if they are 25 years old or less and meet Group 3 criteria.

4. Group 4: Screening enrollment

Group Four is a screening group of participants, birth through 25 years old, suspected of having ALGS, PFIC (or BRIC), or BASD, who do not meet complete enrollment criteria for Group 1, 2, or 3. In this Group, ChiLDReN Core laboratories will perform bile acid analysis to detect BASD or genotyping for ALGS or PFIC (or BRIC) on participants that the investigator believes may have one of these diseases, and who would need these tests in order to establish the diagnosis and make the participant eligible for enrollment in Group 1, 2, or 3. Consent will be obtained, a set of brief enrollment CRFs will be filled out, and specimens will be collected from the participant and parents for genotyping (PFIC [or BRIC] or ALGS), or from the participant for urine bile acid analysis. No specimens will be sent to the Repositories for this Group.

5. Group 5: Affected siblings (without evidence of liver disease) of α1-AT deficiency participants who are enrolled in LOGIC.

Group Five is for enrollment of siblings of participants with α1-AT deficiency, birth through 25 years old, who themselves are found to be PIZZ or PISZ upon clinical testing and who do not have evidence of liver disease. Criteria for evidence of liver disease are hepatomegaly or splenomegaly, abnormal hepatic function tests, complications of chronic liver disease, abnormal imaging of the liver (except for fatty liver), or abnormal liver biopsy histology. Enrollment of these participants will be important in order to determine if the liver disease in α1-AT deficiency is concordant in families, supporting a genetic modifier or environmental factor. Affected siblings with evidence of liver disease can be enrolled in Groups 1, 2, or 3.

V.B4. Enrollment Criteria

a. α1-AT Deficiency

Participants must meet both criteria 1 and 2:

1. α1-AT deficiency will be defined as low serum α1-AT concentrations (< lower limit of normal for laboratory) ***and*** the PIZZ or the PISZ phenotype or genotype for participants prior to liver transplantation.

and

2. Participants must also have liver disease associated with α1-AT deficiency. This will be considered present if there is either evidence of neonatal cholestasis (conjugated hyperbilirubinemia and jaundice within the first 3 months of life), chronically elevated (>6 mo.) aspartate aminotransferase (AST), alanine aminotransferase (ALT), or gamma-glutamyltransferase (GGT) above 1.25 times the upper limit of normal, chronic hepatomegaly (clinically measured liver span at mid-clavicular line above the 95 percentile for age present for at least 3 months), clinical findings or complications of portal hypertension or cirrhosis, impaired liver synthetic function, or evidence of inflammation, cholestasis, paucity of interlobular bile ducts, hepatic fibrosis or cirrhosis on liver biopsy, or having undergone liver transplantation for α1-AT deficiency.

For participants after liver transplantation, enrollment criteria will include a history of the above criteria before transplant, or, alternatively, *either* low serum α1-AT level *or* PIZZ or PISZ phenotype or genotype before transplantation *plus* clear histologic evidence of α1-AT deficiency liver disease on the explanted liver (PAS-positive diastase resistant smooth globules in hepatocytes).

Siblings of affected participants, birth through 25 years old, who are also found to be PIZZ or PISZ upon clinical screening, will be offered enrollment into the study. If they have evidence of liver disease, they will be enrolled into Group 1, 2, or 3 as appropriate, based on the inclusion and exclusion criteria for these groups. If they have no evidence of liver disease, they will be enrolled in Group 5 (see V.B3 Group Five for criteria for liver disease). The purpose of including these siblings in the study is to determine if the natural history of the liver disease is concordant in a given family with multiply affected children.

b. ALGS

Participants in this study must both: (1) meet the ALGS Diagnostic Criteria; and (2) have evidence of liver disease (clinical, biochemical, or histological). The ALGS Diagnostic Criteria to be utilized will be those in **Table 1.** These criteria include clinical scenarios in which there is a combination of a family history of ALGS, the presence of paucity of interlobular bile ducts on liver biopsy, the identification of a *JAGGED1* or *NOTCH2* mutation, and clinical criteria (symptoms or signs). The specific clinical criteria are history or presence of the following.

Cardiac: Heart murmur (with further studies to clarify), pulmonary valvular or pulmonary arterial stenosis), pulmonary atresia, tetralogy of Fallot, atrial septal defect (ASD), or ventricular septal defect (VSD).

Ocular: Posterior embryotoxon or other anterior chamber defect, retinal pigmentary anomalies.

Vertebral: Butterfly vertebrae.

Characteristic facial features: Broad forehead, deep-set eyes, pointed chin in child (preteen) or prognathism in adults, triangular face.

Evidence of cholestasis (one or more of the following):

a. Fasting total serum bile acid > 3x ULN for age, *or*
b. Direct bilirubin > 2 mg/dl, *or*
c. Fat soluble vitamin deficiency otherwise unexplainable, *or*
d. γGTP > 3x ULN for age, *or*
e. Intractable pruritus explainable only by liver disease.

Renal: Functional defects (such as renal tubular acidosis), renal insufficiency, renal vascular hypertension, vesicoureteral reflux, and/or structural defects (agenesis, small kidneys, renal cysts, renal artery stenosis, dysplastic kidneys).

Siblings or parents (if 25 years of age or less) of ALGS participants will also be offered enrollment into the study if they meet the ALGS Diagnostic Criteria (Table 1) and if they have evidence of liver disease. They will be enrolled into Group 1, 2, or 3, as appropriate.

Table 1. ALGS D iagnostic Criteria

ALGS family history[a]	Paucity	*JAGGED1*[d] or *NOTCH* 2 mutation	Number of clinical criteria required
Present or absent	Present	Identified[b]	Any or no features
None (proband)	Present	Not identified[c]	3 or more features
None (proband)	Absent or unknown	Not identified	4 or more features
None (proband)	Absent or unknown	Identified	1 or more features
Present	Present	Not identified	1 or more features
Present	Absent or unknown	Not identified	2 or more features
Present	Absent or unknown	Identified	Any or no features

Major clinical criteria include cholestasis, consistent cardiac, renal, ocular disease, butterfly vertebrae, or characteristic "Alagille" facies of childhood or adulthood. (See V.B4b for details.)

[a]Family history = ALGS present in a first degree relative
[b]Identified = *JAGGED1 or NOTCH2* mutation may have been identified in clinical or research laboratory
[c]Not identified = Not identified on mutation screening, or not screened for
[d]*JAGGED1* mutation = Mutation, whole gene deletion, or deletion of chromosome 20p, which includes J*AGGED1* locus

c. PFIC (or BRIC)

PFIC (or BRIC) inclusion criteria for LOGIC enrollment:

Definite PFIC (or BRIC):

1. Documented confirmed two mutant alleles in *ATP8B1*, *ABCB11, ABCB4, TJP2* or other genes to be

described that will be shown to be confirmed causes of PFIC.

or

Presumed PFIC (or BRIC):

Participants must meet both 2 and 3:

2. History or presence of chronic liver disease (one or more of the following):
    a. Duration of biochemical or clinical abnormalities of >6 months, *or*
    b. Clinical/pathologic stigmata of chronic liver disease, *or*
    c. Sibling of known individual affected by PFIC or BRIC (predicted to be chronic).

    d. Recurrent and episodic cholestatic disease occurring on more than two occasions with episodes separated by at least 3 months and without other known cause.

and

3. History or presence of cholestasis (one or more of the following):
    a. Fasting total serum bile acid > 3x ULN for age, *or*
    b. Direct bilirubin > 2 mg/dl, *or*
    c. Fat soluble vitamin deficiency otherwise unexplainable, *or*
    d. γGTP > 3x ULN for age, *or*
    e. Intractable pruritus explainable only by liver disease

PFIC exclusion criteria for LOGIC enrollment (for participants enrolled by criteria 2 and 3 above, but not criteria 1):

1. Confirmed diagnosis of other chronic cholestatic liver disease, such as biliary atresia, cystic fibrosis, autoimmune liver disease, extrahepatic biliary obstruction/disease, autosomal recessive polycystic kidney disease (ARPKD), hepatic veno-occlusive disease, chronic allograft rejection, BASD, α1-AT deficiency, ALGS, mitochondrial defect, large duct primary sclerosing cholangitis (PSC), or PSC in the setting of inflammatory bowel disease, or immunodeficiency. (It should be noted, this does not exclude patients from being enrolled into ChiLDReN in one of the other three disease categories.)
2. Short bowel syndrome/total parenteral nutrition (TPN)-related disease
3. Chronic known infectious hepatitis (e.g. Hepatitis C, Hepatitis B, etc.)
4. Chronic known or strongly suspected drug toxicity (e.g., Augmentin-related cholestasis)
5. Acquired immunodeficiency syndrome
6. Acute liver failure
7. Extrahepatic portal vein obstruction, congenital hepatic fibrosis or congenital portosystemic shunt.

Note: Definite and presumed cases of PFIC (or BRIC) will be treated similarly in this study.

d. BASDs

Enrollment criteria for BASDs will be one or both of the following:

1. Biochemical evidence of a BASD documented by Fast Atom Bombardment-Mass Spectrometry (FAB-MS) or GC-MS analysis of urine or serum.

or

2. Two genetic mutations in one of the enzymes in the bile acid synthesis pathway are identified.

Exclusion criteria:
1. Peroxisomal enzyme or structural defect producing a recognized syndromic disorder, such as Zellweger Syndrome, Refsum's Syndrome, Neonatal Adrenoleukodystrophy, or Smith-Lemli-Opitz Syndrome.

e. Exceptions to the Inclusion/Exclusion Criteria

Infants and children highly suspected of having one of the four LOGIC diseases by the local Principal Investigator (PI), but who do not meet enrollment criteria, and do not have genetic or biochemical evidence of one of the LOGIC diseases through Group 4 testing, may be included in this study by the following process. The PI will request permission for a protocol exception by a written request to the Exception Committee. This committee will be composed of three of the Site PIs, an NIH representative, and a SDCC member. The committee will review the request and will decide by majority vote to either allow the participant to be enrolled or deny enrollment based on the likelihood that the participant will have one of the LOGIC diseases. If the participant is subsequently found to have a diagnosis of another disease explaining the cholestatic liver disease, then they will be withdrawn from this study. Members of the committee will recuse themselves for any potential participant at their own center.

V.B5. Human Participants Considerations

A. Risks to Study Participants

1. Involvement of Participants

Participants will be eligible for participation if they meet the inclusion and exclusion criteria for one of the four LOGIC diseases (see V.B4) and fall into one of the five enrollment Groups of this study (see V.B3). Their parents will also be asked to participate by providing family history and blood specimens. Participants of all ethnicities, races, and both sexes will be offered enrollment.

2. Planned Duration of the Entire Study

This study is expected to last up to 20 years.

3. Duration of Participation for Each Participant

Each participant's participation in this research study will be up to 20 years for Groups 1, 2, and 5. Previously, siblings of participants with ALGS who were affected, but without evidence of liver disease, were enrolled in Group 5. Since only three ALGS siblings were enrolled in Group 5, we will not continue to follow these participants prospectively. Participants in Group 4 will be screened and then offered enrollment in Group 1, 2, or 3, if found to meet enrollment criteria. Group 3 participants will be seen once during this study.

4. Source(s) of Research Material(s)

Historical information, physical examination findings, laboratory data, and blood for DNA. If an inadequate amount is obtained for DNA extraction, a redraw request may be made. In the event that collection of whole blood for DNA collection is not possible or contraindicated, saliva will be collected.

5. Potential Risks to Participants
There are minimal physical and psychological risks from being in this study. These risks include psychological risks to the family and child gaining additional knowledge about the child's liver disease or about possible associations of family or genetic factors with the child having developed liver disease. The risks of venipuncture for the DNA bank are pain, bruising, or superficial phlebitis. The risks of genetic information being revealed by any future investigations in the Network are very slight as the blood samples will be de-identified prior to being deposited in the repository. 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.

6. Alternative Treatments Considered
This is an observational study without alteration in routine clinical care. Therefore, there is no alternative treatment.

B. Adequacy of Protection Against Risks
1. Plan to Protect Participants/Mitigate Risks
The study anticipates no excessive risks to the participants, except the possible pain associated with blood draws. EMLA cream may be applied to sites of all blood draws and intravenous lines to minimize pain with these procedures. Blood draws on participants will be scheduled to occur when venipuncture will be performed for clinical indications, whenever possible, or will be performed through indwelling IV lines, placed for clinical purposes. Psychological risks will be minimized by careful explanation of the risks and maintaining complete confidentiality and data security.

John Magee, MD, and Robert Merion, MD, PIs of the SDCC for ChiLDReN, have obtained a Certificate of Confidentiality for this study. Thus, the confidentiality of information collected in this study is protected by this Certificate.

2. Recruitment Plan
Participants will be recruited from patients evaluated at, referred to, and followed at the ChiLDReN clinical sites (Appendix I). The investigator or clinical research coordinator will recruit the parent(s) or guardian(s) during clinic visits, phone contact, 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. In addition, families or patients may contact ChiLDReN sites directly via contact information provided on the ChiLDReN Network website (www.childrennetwork.org). Information about this study, along with a hyperlink to the ChiLDReN Administrative Core, will also be posted on the websites of the relevant Patient Advocacy Groups that are members of the ChiLDReN Network, including the Alagille Syndrome Alliance, the Alpha-1 Foundation, the Children's Liver Alliance for Support Services, the American Liver Foundation, and the Children's Liver Disease Foundation.

3. Informed Consent Plan
All potential participants identified by the local PI and/or designee that meet the inclusion/exclusion

criteria will be given the opportunity to participate. Parents/guardians/ participants will be given the consent/assent forms to review and ask questions about the study. Parents/guardians/participants will be asked to summarize in their own words what participation in this research study involves, and that they are comfortable with the risks and benefits of participating in the research study. Any additional questions they have will also be answered prior to signing the consent/assent. Once the consent/assent form is signed, a copy will be provided to the parent/guardian/participant. All participants will be consented by the PI and/or designee, who have received appropriate training regarding human participant protection and HIPAA compliance, as established by the institutional regulatory requirements. Non-English speaking participants will be able to participate in the study, and institutional review board (IRB) requirements will dictate if a consent form in English may be used.

4. Special Consent/Assent Plan
Non-English-only speaking populations will not be excluded from this study. IRB regulations will be followed regarding the need for translation of the consent form, and other applicable forms (e.g., quality of life).

C. Potential Benefits

1. Possible Health Benefits to Participants
The participant 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.

2. Describe Any Incentives or Rewards Offered for Participation
For each scheduled follow-up visit, the parents/guardians or participants 18 years of age or older will receive up to $20 to reimburse them for parking, meals, or other expenses that they may have related to the visit. IRB approval will be needed for this payment.

3. Importance of the Knowledge to be Gained From This Research
A better understanding of the etiology and pathogenesis of the four LOGIC genetic causes of intrahepatic cholestasis may lead to improvements in diagnosis, treatment, and prevention of these disorders in the future, resulting in decreased morbidity and mortality. The DNA and specimen repository may prove instrumental in identifying new genetic causes and modifier genes for these disorders, as well as identification of biomarkers predictive of disease phenotypes and outcomes. Improved therapy for these diseases could lead to reduced need for liver transplantation and more availability of donor livers for children with other end-stage liver diseases.

V.B6. Study Enrollment and Recruitment

A. Recruitment Protocol

In order to maximize recruitment of participants with these rare liver diseases, recruitment strategies will be used:

1. Current and Future Clinical Site Patients. All patients currently followed at the ChiLDReN clinical sites (plus those with α1-AT deficiency followed at the Saint Louis University enrollment site), and those to be newly diagnosed, who meet the diagnostic criteria for the four rare cholestatic liver disorders, will be offered enrollment into this study, both before and after liver transplant. It is estimated that approximately 30-60 new patients will be referred to or diagnosed at the clinical sites with all of these diseases each year. For Group 5, siblings (who are PIZZ or PISZ) of known α1-AT deficiency participants will be offered enrollment if they have no evidence of liver disease. Affected siblings with α1-AT deficiency or ALGS and evidence of liver disease will be offered enrollment into Groups 1, 2, or 3.

For patients followed at or referred to the ChiLDReN Centers, one of the investigators or clinical research coordinators will speak to the potential participant (if 18 years of age or older), the parent(s) or guardian(s) by telephone, 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. The consent form will be given to the family.
The IRB-approved consent form 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 participant, family, or guardian consents to enrollment into the study, written informed consent will be obtained from the parents or guardians. For potential participants 18 years of age and older, consent will be obtained from the participant.

2. Advertising Strategy for Voluntary Enrollments.
If the family or patient is interested in participation, then the PI or Research Coordinator will contact the patient's current physician in order to explain the nature of this study. The study coordinator may forward the family a copy of the IRB-approved Consent Form for review and discussion.

ChiLDReN studies will be registered in the Clinical Trials.gov website. All notices advertising any ChiLDReN studies will have IRB approval and interested families/patients will be given their choice of participation in any of the ChiLDReN clinical sites. Participation at the ChiLDReN Clinical Site closest to the family will be encouraged.

3. Duration of Enrollment. The majority of participants in this study were enrolled during Years 1 through 4 and are now in longitudinal follow-up. We anticipate continued enrollment of approximately 50 new participants per year for newly diagnosed or referred patients with the ChiLDReN diseases. Thus, we will continue to enroll participants throughout the duration of the study.

B. Number of Participants

Throughout the duration of this study, the plan is to enroll at all of the Clinical Sites: 500 participants

with ALGS, 600 with α1-AT deficiency (of which up to 30 may be siblings of participants), 500 with PFIC (or BRIC), and 75 with BASDs. This will total **1,675** participants at the end of the study.
The ethnicity and racial categories of the participants are outlined in **Table 2**.

Table 2. Planned Enrollment

	Sex		Total
	Female	**Male**	
Ethnic Categories			
Hispanic or Latino	115	147	262
Not Hispanic or Latino	626	787	1413
Ethnic Categories: Total of All Participants	741	934	1675
Racial Categories			
American Indian/Alaska Native	11	6	17
Asian	27	36	63
Native Hawaiian or Other Pacific Islander	3	3	6
Black or African American	62	93	155
White	591	713	1304
More than One Race	47	82	129
Racial Categories: Total of All Participants	741	934	1675

V.B7. Data Collection and Study Visit Schedule
This is an observational longitudinal study which will involve collection of clinical information, family history, physical findings, laboratory tests, clinically indicated radiologic and imaging evaluations, and clinically indicated treatments and their outcomes. In addition to these standard of care procedures, , blood (or saliva) will be collected for DNA extraction, storage and use is future studies. Whole blood (or saliva) for DNA will also be collected from both biologic parents (when available). Whole blood (or saliva) for DNA will be collected from affected siblings of α1-AT deficiency participants. There are five types of enrollment Groups in this study, with the data to be collected at each specific study visit for each Group outlined in Tables 3-7.

The types of study visits for each Group are outlined below.

A. Group One – < 6 month old infant at time of enrollment into PROBE study (Table 3)
The types of study visits within this Group are:
1. Baseline LOGIC visit: After a LOGIC diagnosis is established in an infant or child who was originally enrolled as an infant < 6 months of age in the PROBE study, the family will then be offered enrollment into the LOGIC study. This may occur before or after 6 months of age, depending on when the diagnosis of the LOGIC disease is made. Enrollment can occur during hospitalization or as an outpatient. At least one parent or guardian must sign written informed consent before data collection can begin. The coordinator will abstract information from the participant's medical chart and meet with the parent(s)/guardian(s) to complete the enrollment CRFs for LOGIC that contain data elements not already

collected for PROBE. Data already gathered on participants in the PROBE database will be pooled with the LOGIC database for analysis and reporting.2. Follow-up: The participant will be followed at yearly intervals for up to 20 years including after liver transplantation, or until death. Annual follow-up will begin at 12 months of age and be scheduled within 6 months of the participant's birthday as per the PROBE study protocol. Pertinent data about interval history including major illness, complications, hospitalizations, surgery, and new diagnoses will be collected, as well as growth and anthropometric parameters,

3. Liver transplantation or abdominal surgery: Pertinent data about interval history and liver function, as well as indication and type of liver transplantation or surgery, will be collected.

4. Death: At time of death, the LOGIC Final Status CRF should be completed.

B. Group Two – Birth through 25 years old at enrollment and not previously enrolled in PROBE study (Table 4)

The information outlined in Table 4 will be collected on participants enrolled in Group 2 as follows:

1. Baseline LOGIC Visit: Following diagnosis of one of the four LOGIC diseases in a child or young adult from birth through 25 years of age, or for a child or young adult with established diagnosis of a LOGIC disease, the family or participant (if 18 years or older) will be offered enrollment into the study. At least one parent or guardian must sign written informed consent before data collection can begin for participants under 18 years of age. When the child becomes of assent age as required by IRB, they will also be asked to sign the Assent Form. Participants $\geq$ age 18 years will be asked to sign informed consent. Once informed consent is obtained, the coordinator and PI will abstract information from the participant's medical chart and meet with the participant/parent(s)/guardian(s) to complete the intake and history forms. The timeline for follow-up is triggered by the date of enrollment in this study.

2. Follow-up: The participant will be followed at yearly intervals for up to 20 years, until liver transplantation, or death. Pertinent data about interval history including major illness, complications, hospitalizations, surgery, and new diagnoses will be collected, as well as growth and anthropometric parameters,

3. Liver transplantation or abdominal surgery: Pertinent data about interval history and liver function, as well as indication and type of liver transplantation or surgery, will be collected.

4. Death: At time of death, the LOGIC Final Status CRF should be completed.

C. Group Three – Post-Liver Transplant Participants (Table 5)

Group 3 will be for participants with LOGIC diseases, birth through 25 years old, who have undergone liver transplantation and are either followed at or referred to the ChiLDReN clinical sites. Participants previously enrolled in PROBE who have undergone liver transplantation for a LOGIC disease may be enrolled in this group.

The information outlined in Table 5 will be collected on participants enrolled in Group 3 as follows:

Baseline LOGIC Visit: Participants who have undergone liver transplantation for one of the four LOGIC diseases, and who are now age birth through 25 years, will be offered enrollment into this Group. At least one parent or guardian must sign written informed consent before data collection can begin for participants under 18 years of age. When the child becomes of assent age as required by IRB, they will also be asked to sign the Assent Form. Participants ≥ age 18 years will be asked to sign informed consent. Once informed consent is obtained, the coordinator and PI will abstract information from the participant's medical chart and meet with the participant/parent(s)/guardian(s) to complete the enrollment CRFs.

Participants in this group will only have this one Baseline LOGIC visit.

D. Group Four - Screening Enrollment (Table 6)

The information outlined in Table 6 will be collected on participants enrolled in Group 4 as follows:

Baseline LOGIC Visit: A Group 4 consent form will be signed, a brief enrollment CRF will be filled out, and specimens will be collected for a diagnostic study to be performed at a ChiLDREN Core Lab: either genotyping for PFIC (or BRIC) or ALGS (blood drawn on participant and parents), or bile acid analysis for BASD. Research genotyping results will need to be confirmed in a clinical CLIA-approved laboratory (see Section V.B8.E). After these test results are known, and if the participant fulfills eligibility criteria, he/she will be offered full enrollment in Group 1, 2, or 3. However, the genotyping is for research purposes only, so the participant/family will not be given these results. The family will be informed that the results are suggestive and may be positive and that they should seek clinical genotyping in a CLIA-approved laboratory, at their own expense. The bile acid biochemistry testing is being performed in a CLIA-approved laboratory, so the results will be provided to the family. If the test results obtained for the participant are negative, the participant will have completed the study. If the participant does not meet enrollment criteria for Groups 1, 2, or 3 within 2 years of enrollment in Group 4, s/he will be removed from the Longitudinal Study.

E. Group Five – Affected Siblings (Without Evidence of Liver Disease) of α1-AT Deficiency (Table 7)

The information outlined in Table 7 will be collected on participants enrolled in Group 5 as follows:

1. Baseline LOGIC Visit: Group Five is for enrollment of siblings of participants with α1-AT deficiency, birth through 25 years old, who themselves are found to be PIZZ or PISZ upon clinical testing and who do not have evidence of liver disease. Following enrollment of the affected sibling (with liver disease), the participant with no evidence of liver disease will be offered enrollment into Group 5. At least one parent or guardian must sign written informed consent before data collection can begin for participants under 18 years of age. When the child becomes of assent age as required by IRB, they will also be asked to sign the Assent Form. Participants ≥ age 18 years will be asked to sign informed consent. Once informed consent is obtained, the coordinator and PI will abstract information from the participant's medical chart and meet with the participant/parent(s)/guardian(s) to complete the enrollment CRFs.
2. Follow-up: The participant will be followed yearly for up to 20 years, until liver transplantation, or death.

3. Liver transplantation or abdominal surgery: Pertinent data about interval history and liver function, as well as indication and type of liver transplantation or surgery, will be collected.

4. Death: At time of death, the LOGIC Final Status CRF should be completed.

Table 3. Group 1 Schedule of Evaluations – Infants or children who were originally enrolled in PROBE Study at age < 6 months

If a procedure is performed as part of PROBE it will not be repeated for LOGIC. The data will be combined for analysis.

LOGIC Study visit procedures	Baseline LOGIC visit	Yearly follow-up visit 1[a]	Yearly follow-up visit 2-20[a]	At liver transplant or abdominal surgery
Recommended windows for visits		**± 6 mo**	**± 6 mo**	
Informed consent	X			
Eligibility	X			
Intake history/exam	X			
Diagnosis	X	X	X	
Surgical procedure (if performed)	X			X
Parents' medical history	X			
Follow-up visits[b]		X	X	X
Bile acid biochemistry (one-time urine)	X			
Blood for DNA[c]	X[d]	X[d]	X[d]	X[d]

Blood from parents for DNA[c]	X			

a. This study coincides with PROBE annual visits starting at 12 months of age. The PROBE 18-month visit is not included in this LOGIC study. In the PROBE study serum and plasma collected at 2 year old visit and annually up to 10 years and every other year up to 20 years beginning with year 12.
b. Follow-up visits will include interval history, physical examination, and clinically indicated lab and imaging tests.
c. Whole blood collection for DNA extraction is a one-time draw. If an inadequate amount is obtained for DNA extraction, a redraw request may be made. If collection of whole blood for DNA is not possible or contraindicated, procedures will be in place to collect saliva.
d. Performed at baseline visit if possible; otherwise, obtain at 1-year follow-up visit or at a subsequent visit if not collected previously.

Table 4. Group 2 Schedule of Evaluations – Infants or children birth through 25 years old at enrollment and not enrolled in PROBE.

Study visit procedures	Baseline LOGIC visit	Year 1 follow-up	Year 2-20 follow-up	At liver transplant or abdominal surgery
Recommended windows for visits		**± 6 mo**	**± 6 mo**	
Informed consent	X			
Eligibility	X			
Intake history/exam	X			
Diagnosis	X			
Surgical procedure (if performed)	X			X
Follow-up visits[a]		X	X	X
Bile acid biochemistry (one-time Urine)	X			
Blood for DNA[i]	X[b]	X[b]	X[b]	X[b]
Parents' medical history	X			
Blood from parents for DNA[c]	X			

a. Follow-up visits will include interval history, physical examination, and clinically indicated lab and imaging tests.
b. Performed at baseline visit if possible; otherwise, obtain at 1-year follow-up visit or at a subsequent visit if not collected previously.
c. Whole blood collection for DNA extraction is a one-time collection. If an inadequate amount is obtained for DNA extraction, a redraw request may be made. If collection of whole blood for DNA is not possible or contraindicated, procedures will be in place to collect saliva.

Table 5. Group 3 Schedule of Evaluations – Post-Liver Transplant Participants.

Study visit procedures	Baseline[a] LOGIC visit
Informed consent	X
Eligibility	X
Intake history/exam	X
Diagnosis	X
Blood for DNA[b, c]	X
Parents' medical history	X
Blood from parents for DNA[b, c]	X

a. May take place at any age.
b. There is a 12-month window for completion of the blood draws for DNA associated with this visit.
c. Whole blood collection for DA extraction is a one-time draw. If an inadequate amount is obtained for DNA extraction, a redraw request may be made. If collection of whole blood for DNA is not possible or contraindicated, procedures will be in place to collect saliva.

Table 6. Group 4 Schedule of Evaluations – Screening Enrollment Participants.

Study visit procedures	Baseline LOGIC visit (any age)
Informed consent	X
Eligibility	X
Intake history/exam	X
Suspected diagnosis	X
Diagnostic study[a]	X
DNA to genotyping core lab on parents[b]	X

a. Genotyping for suspected PFIC (or BRIC), or ALGS, or urine bile acid analysis for suspected BASD.
b. Only for parents of suspected ALGS or PFIC (or BRIC) participant.

Table 7. Group 5 Schedule of Evaluations – Affected siblings (without evidence of liver disease) of participants with α1-AT deficiency who are enrolled in LOGIC.

Study visit procedures	Baseline LOGIC visit	Year 1-20 follow-up	At liver transplant or abdominal surgery
Recommended windows for visits		± 6 mo	
Informed consent	X		
Eligibility	X		
Intake history/exam	X		
Diagnosis	X		
Surgical procedure (if performed)	X		X
Follow-up visits[a]		X	X
Blood for DNA[c]	X[b]	X[b]	X[b]
Bile acid biochemistry (one-time urine)	X		

a. Follow-up visits will include interval history, physical examination, and clinically indicated lab and imaging tests.
b. Performed at baseline visit if possible; otherwise obtain at 1-year follow-up visit or at a subsequent visit if not collected previously.
c. Whole blood for DNA extraction is a one-time collection. If an inadequate amount is obtained for DNA extraction, a redraw request may be made. If collection of whole blood for DNA is not possible or contraindicated, procedures will be in place to collect saliva.

V.B8. Blood Sample Requirements by Visit and Overall

During this study, DNA extracted from whole blood specimens will be obtained, shipped to, and stored at the NIDDK Repositories for use in future ChiLDReN ancillary studies. This "biobanking" is a critical aspect of this longitudinal study in order to create a resource of DNA, and other specimens from a meaningful number of participants with these rare disorders. In addition, obtaining and storing DNA will allow future studies to investigate genetic causes and influences (modifier genes) in these rare diseases. Genotyping for known causative genes of PFIC (or BRIC) and ALGS will be performed on DNA at the ChiLDReN Genetics Core for participants who have not undergone this testing previously.

A. Research Blood Specimens and Volumes

A.1. Groups 1, 2, 3, and 5

The following types of blood specimens for Groups 1, 2, 3 (blood for DNA only), and 5 will be drawn *for research purposes only* in this study. If whole blood for DNA had been previously collected for the PROBE study, then those samples will satisfy the blood sample requirement for this study (e.g., blood for DNA isolation from the child and parents). Laboratory data obtained for clinical indications will also be recorded on the data collection forms at each study visit. If an inadequate

amount of whole blood for DNA extraction is obtained, a redraw request may be made. If collection of whole blood for DNA is not possible or contraindicated, procedures will be in place for collection of saliva for DNA.

From the biological mother and father (unless already collected for PROBE study) in Groups 1, 2, and 3:

1. 10 ml of whole blood in one 10 ml EDTA vial to be sent to the NIDDK Repository for DNA extraction **OR** 2 ml of saliva collected in a saliva collection kit.

Infant or Child DNA (Groups 1, 2, 3, and 5):
Starting at 1 year of age, 4 ml (< 50 kg) or 10 ml (≥ 50 kg) of whole blood will be collected one time for DNA isolation and storage as described in Tables 8 and 9, below. If participants are <1 year of age at enrollment and 1.0-3.0 ml of blood can be obtained while keeping blood draw volume within the limitations set in Tables 8 and 9, then this volume of blood should be drawn prior to 1 year of age for DNA isolation and storage as in Tables 8 and 9. If collection of whole blood for DNA is not possible or contraindicated, 2 ml of saliva will be collected in a saliva collection kit.

For those participants 50 kg and greater in weight, 10 ml of whole blood in one 10 ml EDTA vial will be sent to the NIDDK Repository for DNA extraction. Participants who are at least 18 years of age, but who weigh less than 50 kg will have 10 ml of whole blood drawn unless the physician specifies that the blood draw amount should be 4 ml, as is standard for children under 50 kg. If an inadequate amount is obtained for DNA extraction, a redraw request may be made. If collection of whole blood for DNA is not possible or contraindicated, 2 ml of saliva will be collected in a saliva collection kit.

Total Research Blood Drawn: The total volume of blood drawn for research-only purposes from participants enrolled in this study is outlined in Tables 8 and 9. These volumes should be within acceptable limits of all IRBs at Clinical Sites.

Table 8. Total Amount of Blood Drawn from Group 1 Participants and Their Parents for Research Purposes (Combined PROBE Study and LOGIC Study)

Participant's visit ***	Amount in ml drawn for research at the visit	Maximum research blood draw in ml within 2-month period	Maximum research blood draw in ml within 3-month period
Initial	4	4	4
4 weeks post-diagnosis	4	8	8
3 months post-diagnosis	4	8	12*
6 months post-diagnosis	4	8**	8 **
12 months of age	8	8	8
18 months of age	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 diagnosis.

** When the 6-month 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.

*** Parents of infants enrolled in Group 1 will have 10 ml of blood drawn one time, at baseline, for DNA.

Table 9. Amount of Blood Drawn for DNA Extraction from Participants and Parents Enrolled in Groups 2, 3, and 5

	Group 2	Group 3	Group 5
Blood for DNA (drawn one time) participant ≤ 50 kg[a]	4 ml	4 ml	4 ml
Blood for DNA (drawn one time) participant ≥ 50 kg[a]	10 ml	10 ml	10 ml
Blood for DNA from parent[a] (one time only)	10 ml	10 ml	None

a. Whole blood colletion for DNA extraction is a one-time collection. If an inadequate amount for DNA extraction is obtained, a redraw request may be made. If collection of whole blood for DNA is not possible or contraindicated, procedures will be in place for collection of saliva for DNA extraction.

A.2. Group 4

Specimen Collection for Group 4 Participants: Participants enrolled in Group 4 with suspected PFIC (or BRIC) and ALGS will (and their parents may) have blood collected for LOGIC research genotyping. **The blood volume required for genotyping is 4 ml in an EDTA (purple top) tube, regardless of weight/size of participant or parent.**

A. Specimen Repositories

A central repository has been established by the NIDDK, a division of the NIH, for long-term storage for blood, urine, and tissue specimens. Whole blood or saliva for DNA isolation will be shipped immediately to the DNA facility. Otherwise, samples will be shipped via licensed overnight carrier once every month to the NIDDK central repository. The serum, plasma, urine, bile, tissue specimens, and DNA will be stored at these facilities until authorized for release by the ChiLDReN Steering Committee for ChiLDReN-approved ancillary studies, at which time arrangements will be made to ship coded specimens to the appropriate site.

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

Full, detailed instructions for specimen collection and shipping will be in the Manual of Operations for this study.

B. Genetic Core Specimens

On approximately a quarterly basis, the SDCC will identify new DNA samples available in the NIDDK Repository for PFIC and ALGS participants. Those DNA samples will be requested from the NIDDK Repository and will be shipped directly to the appropriate ChiLDReN Genetics Core Lab(s) for genotyping of the relevant genes.

All DNA samples will be shipped to the NIDDK Repository identified with the LOGIC participant-identifier only, and will include no personal identifiers (PHI). Each DNA sample shipped from the NIDDK Repository to the ChiLDReN Core Labs will be coded with a participant-specific Repository ID number and will also include no PHI. The two coded ID numbers will be linked only by the local study coordinators who submitted the samples to the Repository and by the ChiLDReN Core Lab Director(s) who will enter the results into the SDCC database. At no time will personal identifiers be shared outside the local site of enrollment. Therefore, there will be no linking of genotyping for this study to any previous genotyping that may have been completed for a given participant. Results of this research genotyping will NOT be shared with participating families. The genotyping results will be used for study analyses only. The results of the genetic testing will be sent by the Genetics Core Labs to the SDCC coded by the participant study number, for inclusion in the SDCC database. Further details of the genetic testing are located in the Manual of Operations.

For Group 4 participants in which ALGS or PFIC (or BRIC) is suspected, blood will be sent from the Clinical Site to the Rutgers biorepository with Form 4A (Group 4 Genetics Testing Request Form) and DNA will be isolated and then shipped to the appropriate ChiLDReN Genetics Core laboratory. This form will contain only the LOGIC participant identifier and will not contain PHI. Genotyping for the relevant gene will be performed and the research genotyping results sent back to the Study Coordinator and PI at the local site sending the specimen. If the participant does not remain in the study, DNA in all ChiLDReN Genetics Core Labs will be destroyed after this genotyping. Results will not be shared with the family.

Genetic testing (for Groups 1, 2, 3, 4, and 5 participants) is performed as part of the research study of ChiLDReN and will not be used to make clinical decisions. The local PI will recommend to the family that they should obtain appropriate genetic testing on a new blood specimen in a CLIA-approved laboratory. The cost of this clinical genetic testing will be borne by the participant's health insurance or participant's family. The consent forms will specifically instruct families that the results of this research genetic testing will NOT be shared with them.

C. Urine Bile Acid Biochemistry Testing

At enrollment into Groups 1, 2, and 5, urine **(**1-10 ml) will be collected for bile acid analysis by clean catch into a sterile collection cup, cotton balls, or bag depending on the age of the child. Prior to this urine collection, participants should have UDCA therapy (if the participant was receiving this medication) discontinued for 5-7 days, as is the standard clinical practice for urine bile acid testing. This should be of no clinical risk to the participant. UDCA can mask urine abnormalities observed in cholestasis and in BASDs. Samples will be placed in clean vials appropriately labeled, and frozen at -20°C or colder. Stored samples should be batch shipped to the Bile Acid Biochemistry Core at

Cincinnati Children's Hospital Medical Center on a monthly to quarterly basis. Dr. Ken Setchell's laboratory will analyze the urine by Fast Atom Bombardment-Mass Spectroscopy for bile acid composition. Test results will be entered directly into the SDCC database via electronic CRF. Once entered, they will be made available to the local study coordinator and PI. Dr. Setchell's lab is CLIA-approved, but these samples are provided to him with study identifiers only. There will be no other identifying information (such as name, DOB, medical record number) shared with Dr. Setchell. For this reason, study investigators will use his results to determine whether repeat testing (on a clinical basis) is recommended, but his research report will not be included in the medical record of the participant.

D. Specimen Use

The ChiLDReN Steering Committee has developed policies for the approval of ancillary studies, which might require the use of samples in the Repositories. 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 and have received IRB approval by the center conducting the study. 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 clinical medical record.

One of the goals of the NIDDK Repositories is to make samples available for investigations that have not yet been specified. Until the funding for ChiLDReN terminates (current funding is until June 2024, but 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 participant identifiers have been removed from samples in the repositories (i.e., samples are de-identified).

At the end of the funding of ChiLDReN, the study database will be transmitted to the NIDDK Repository with all participant identifiers removed; e.g., dates will be converted to ages.

V.B9. Drug Information

There will be no drug treatment or therapeutic intervention mandated by this study. Participants will be treated by routine local therapy standards, and the treatments given will be recorded in this database.

V.B10. Supportive Care Guidelines

Participants will be cared for by the local routine standard of care for children with the four ChiLDReN diseases.

V.B11. Criteria for Removal from Study

Groups 1 and 2: Participants in this study will be followed for up to 20 years, until liver transplantation, or until death. The participant's parents or guardians may request that the participant be removed from the study at any time. If the participant is age 18 or greater, he or she may request to be removed from the study at any time. In addition, the investigator may withdraw a participant from the study if he/she determines that it is in the participant's best interests. The study will end at the time of death.

Group 5: Participants in this study will be followed for up to 20 years, until liver transplantation, or until death. Previously, affected siblings of ALGS participants who did not have evidence of liver disease

were enrolled in Group 5. Since only 3 ALGS siblings have been enrolled to date in Group 5, these participants will no longer be followed prospectively. The participant's parents or guardians may request that the participant be removed from the study at any time. If the participant is age 18 or greater, he or
she may request to be removed from the study at any time. In addition, the investigator may withdraw a participant from the study if he/she determines that it is in the participant's best interests. The study will end at the time of death.

Groups 3 and 4: Participants will be seen one time after the enrollment visit (if necessary to complete collection of data) in these two groups. The participant's parents or guardians may request that the participant be removed from the study at any time. If the participant is age 18 or greater, he or she may request to be removed from the study at any time. In addition, the investigator may withdraw a participant from the study if he/she determines that it is in the participant's best interests.

Withdrawal from the study will not impact the study participant's future medical or surgical care; however, the grant will not be able to fund any of the testing or procedures once the participant has withdrawn.

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

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

V.B12. Measurements

Most measurements in this study are standard of care that will be performed at local laboratories.

V.B13. Schedule/Description of Measurements

See sections V.B7, V.B8, and V.B12.

V.B14. Outcome Variables

The specific aims of this study are to characterize the natural history of the four LOGIC cholestatic diseases by prospectively evaluating and following participants over time and to identify risk factors related to onset, severity, and outcome of each disease.

The Primary Outcome measures demonstrating disease progression for each of the diseases of the study are:

- Liver transplantation

- Death
- Presence of ascites (or treatment for ascites)
- Growth failure (defined as weight or length Z-score for age < -2)
- Worsening liver function, defined as increase of 10 or more points in the calculated Pediatric End-stage Liver Disease score (PELD) (for <12 years of age) or Model for End-stage Liver Disease score (MELD) (for 12 years of age or older)
- Development of complications of portal hypertension (e.g., variceal hemorrhage)

Secondary Outcomes for each disease will include:

- Jaundice (total serum bilirubin >2.0 mg/dl)
- Listing for liver transplantation
- PELD or MELD (calculated)
- Growth (length and weight Z-score)

V.B15 Data Collection

The following data will be collected on study participants at enrollment and during follow-up. For participants initially enrolled in the PROBE study, following signing of consent for LOGIC, relevant data that has already been collected for PROBE will be pooled with the LOGIC data for analyses. Several specific data elements will be obtained only in participants with specific diseases, which are marked as such in the list below. All laboratory and imaging test results used in this data collection will be those that had been obtained originally for clinical indications, with the exception of urine bile acid analysis, all of which will be done for research purposes and funded by this study or local research funds.

A. Enrollment – Groups 1, 2, and 5

The following data are to be obtained on all participants with a known LOGIC disease or at time of new diagnosis of one of the four LOGIC diseases (Groups 1, 2, and 5).

1. Primary hepatobiliary diagnosis, with confirmation of diagnostic criteria.
2. Demographics: age at entry, date of birth, location and zip code of place of birth, sex, race/ethnicity, area of residence of parents, occupation of father and mother, marriage status of parents, income status of parents, educational level of parents, type of medical insurance of parents
3. Prenatal history: Pregnancy of proband: Illnesses and medications during pregnancy, alcohol and tobacco use during pregnancy, exposure to pesticides and chemicals during pregnancy, radiation exposure, hemorrhage, weight gain, ultrasonography or amniocentesis results (if available), history of in vitro fertilization or other infertility treatment used during pregnancy. Previous pregnancy history: Outcome of each pregnancy, including complications and current status of each live birth. Prenatal tests: Chorionic villi sampling, amniocentesis, results of ultrasounds, Rh sensitivity, blood transfusions.
4. Birth history: birth weight and length, gestational age, PROM, fetal distress, medications used, type of delivery
5. Clinical presentation: detailed history of all symptoms, signs, age at onset, initial interventions
6. Complications of portal hypertension: ascites, variceal hemorrhage, encephalopathy,

spontaneous bacterial peritonitis, hepatopulmonary syndrome or pulmonary hypertension – recorded as sentinel events

7. Complications of cholestasis: pruritus, excoriations, xanthoma, bone fractures, fatigue, jaundice, fat-soluble vitamin deficiency symptoms
8. Associated organ system involvement: cardiovascular, ocular, renal, neuromuscular, gastrointestinal, pancreatic, skeletal, audiologic, hematologic, other
9. Surgical history: prior surgery, findings at surgery
10. Family history: Maternal and paternal sides of family – liver disease, infant deaths, congenital malformations, history related to specific disorders, consanguinity. Ancestral history will include the place of birth of participant's parents, grandparents, great grandparents, and great great grandparents.
11. Social history
12. Current medications and diet history
13. Physical examination: Measurements: Weight, length, head circumference, triceps and subscapular skinfold thickness, mid-arm circumference, liver and spleen size; Appearance: Jaundice, cyanosis, spider hemangiomas, clubbing, palmar erythema, edema, facial features; Assessments of systems: Cardiovascular, abdominal/gastrointestinal/hepatic, musculoskeletal, urogenital. Tanner stage: Self-report if > 8 years old. Neurologic: DTRs, EOMs, truncal and limb ataxia, and gait
14. Laboratory data (if clinically indicated): Liver function tests (conjugated and total bilirubin, albumin, total protein, alkaline phosphatase, AST, ALT), γGT, alpha-fetoprotein, CBC, prothrombin time/INR, PTT, electrolytes, BUN, creatinine, glucose, calcium, phosphate, magnesium, uric acid, urinalysis, blood ammonia, serum cholesterol, lipid profile, total bile acid level, hepatitis B and C serologies (if available), 25-hydroxy vitamin D, alpha tocopherol, total serum lipids, retinol, retinol binding protein
15. Metabolic disease tests (if obtained clinically to establish the diagnosis): Serum α1-AT level and PI phenotype or genotype, sweat chloride analysis, serum and urine amino acids and urine organic acids, T4, TSH, iron, TIBC, ferritin, RBC test for galactosemia (if relevant), very long chain fatty acid levels, transferrin electrophoresis,acylcarnitine profile, urine succinylacetone, urine FAB-MS for bile acid profile (if previously obtained)
16. Genetic testing (if performed previously): Prior genetic testing for PFIC (or BRIC) genes, α1-AT deficiency, *JAGGED1, NOTCH2* or Bile acid synthetic defects
17. Imaging studies: Ultrasonography of abdomen and kidneys, CT or MRI of abdomen (if obtained)
18. Nuclear medicine studies: Hepatobiliary scintigraphy (if performed)
19. Surgery performed: Type of operation, indications, and findings
20. Intraoperative findings for abdominal surgery (if exploratory laparotomy, cholangiogram, partial external biliary diversion or ileal exclusion has already been performed)
21. Genetic Core Testing results: Blood or saliva sent to Repository for DNA isolation and banking, genotyping if performed. *This will be paid for by the study.* Also, genotyping results from other research or CLIA-approved laboratory will be obtained and recorded. *OnlyCLIA-approved laboratory genotyping data will be shared with the participant and family*.
22. Bile Acid Biochemistry Core analysis: Urine will be sent to Bile Acid Biochemistry Core and analyzed; the results obtained will be shared with the Clinical Site that will enter these data into

the CRFs. *This will be paid for by the study*.

Justification: These data fields represent intake data at enrollment or time of diagnosis of the genetic cause of intrahepatic cholestasis. The data will be important for defining baseline characteristics of study participants; evaluating familial, genetic, epidemiologic, and demographic factors that may be associated with these disorders and with severity of illness or disease progression; assessing diagnostic accuracy and utility of clinical, laboratory, and imaging studies; assessing baseline liver function, physical findings, imaging findings, liver histology, bile acid analyses, and genetic results that may be used in the analysis of factors that predict outcomes.

B. Follow-Up Visits – Groups 1, 2, and 5

For infants enrolled initially in PROBE study (Group 1), data will be collected as part of the PROBE protocol at 3, 6, 9, 12, 18, 24 months, and yearly thereafter. The yearly follow-up visits for LOGIC will be timed to coincide with the PROBE scheduled visits, and the LOGIC CRF will collect only data not collected on the PROBE CRFs.

For children and young adults enrolled in LOGIC initially (Groups 2 and 5), the following data are to be obtained yearly following the enrollment visit.

1. Interval history of sentinel events, including illnesses, complications, hospitalizations, surgery, new consultations or diagnoses, number of recurrent episodes of episodic cholestasis
2. Growth and anthropometric parameters
3. Medications: intervals and doses
4. Herbal and complementary/alternative medicine supplements
5. Diet and infant formula
6. Physical findings. Hepatic: liver size and texture, spleen size, spider hemangiomas, clubbing, cyanosis, edema, palmar erythema, encephalopathy, ascites, Tanner stage (self-reported if > 8 years old), DTRs, ataxia
    - Other organ systems of relevance to individual disorders
7. Laboratory data (if clinically indicated): CBC, liver and renal function studies, other chemistries, prothrombin time and PTT, coagulation factors, ammonia, lipid profile, total serum bile acids, cholesterol, alpha fetoprotein (monitor for development of hepatocellular carcinoma), specific biochemical tests for individual diseases (e.g., bile acid composition of serum and urine for children with BASDs on cholic acid treatment)
8. Fat–soluble vitamin levels: 25-hydroxyvitamin D, alpha tocopherol and total lipids, retinol and retinol binding protein
9. Imaging study results: e.g., Doppler abdominal ultrasonography (if performed for clinical indications)
10. Interval sentinel events and complications of portal hypertension: ascites, variceal hemorrhage, encephalopathy, spontaneous bacterial peritonitis, hepatopulmonary syndrome or pulmonary hypertension
11. Interval sentinel events and complications of cholestasis- pruritus, excoriations, xanthoma, bone fractures, fatigue, jaundice, fat-soluble vitamin deficiency symptoms.
12. Interval endoscopic or surgical procedures performed for clinical indications: indications, findings, histological results
13. Date and indications at listing for liver transplantation

14. Date and type of liver transplant: cadaver whole, reduced-size, split donor liver, living donor liver
15. Results of transplantation
16. Other surgical procedures: indications, description of procedure, findings
17. Date and cause of death

Justification: These data fields are essential for defining outcomes in clinical terms, biochemical terms, and survival; determining use of medications, vitamin and herbal supplements and any relationship to outcome; determining frequency of complications and predictors of complications; determining predictors of need for liver transplantation (by linking other tables with these data).

At the time of liver transplantation or abdominal surgery performed for treatment of the liver disease (e.g., partial biliary diversion, ileal exclusion, cholecystectomy), the following data will be obtained:

1. Surgical procedure performed.
2. Interval sentinel events and complications of portal hypertension: ascites, variceal hemorrhage, encephalopathy, spontaneous bacterial peritonitis, hepatopulmonary syndrome, or pulmonary hypertension
3. Interval sentinel events and complications of cholestasis: pruritus, excoriations, xanthoma, bone fractures, fatigue, jaundice, fat-soluble vitamin deficiency symptoms
4. Blood for DNA (if less than 12 months of age)

C. Post-Liver Transplant Enrollment – Group 3

The following data are to be obtained at the single study visit for all post-liver transplant participants (Group 3).

1. Primary hepatobiliary diagnosis
2. Demographics: age at entry, date of birth, place of birth, sex, race/ethnicity, area of residence of parents, occupation of father and mother, marriage status of parents, income status of parents, educational level of parents, type of medical insurance of parents
3. Clinical indications for liver transplant: history of all relevant symptoms, signs, age at onset, initial interventions, sentinel events and treatments that occurred prior to liver transplant
4. Associated organ system involvement at time of liver transplant: cardiovascular, ocular, renal, neuromuscular, gastrointestinal, pancreatic, skeletal, audiologic, hematologic, other
5. Surgical history: prior surgery – type of operation, indications, and findings
6. Liver transplant surgery – type of donor organ
7. Family history: Maternal and paternal sides of family – liver disease, infant deaths, congenital malformations, history related to specific disorders, consanguinity. Ancestral history will include the place of birth of participant's parents, grandparents, great grandparents and great great grandparents.
8. Social history
9. Metabolic disease tests (that were obtained prior to liver transplant to establish the clinical diagnosis)
10. Genetic testing (if performed): prior genetic testing for PFIC (or BRIC) genes, α1-AT, *JAGGED1, NOTCH2,* or bile acid defects
11. Specimens for the repositories: blood or saliva sent to Repository for DNA isolation and banking,

and genotyping if performed at Genetics Core. *This will be paid for by the study*.

D. Screening Enrollment – Group 4

The following data are to be obtained at the single study visit for Screening Enrollment Participants (Group 4).

1. Suspected primary hepatobiliary diagnosis
2. Demographics: age at entry, date of birth, place of birth, sex, race/ethnicity
3. Surgical history: prior surgery
4. Physical examination. Measurements: weight, length, head circumference, liver, spleen; Appearance: jaundice, cyanosis, spider hemangiomas, clubbing, edema, facial features; Assessments of systems: cardiovascular, abdominal/gastrointestinal/hepatic, musculoskeletal, urogenital
5. Justification for need for specialized testing to establish the diagnosis

V.B16. Safety Monitoring

A. Nature of Study

This is an observational, non-interventional study; therefore, no adverse events (AEs) are anticipated. However, any serious AEs will be reported to the SDCC, the NIDDK project officer, and local IRBs or other oversight bodies (e.g., GCRC, CTSA).

B. Study Oversight

The ChiLDReN Steering Committee, the NIH Project Scientist, and the Study Chair will have shared oversight responsibility of this clinical trial. The NIH-appointed Data Safety Monitoring Board (DSMB) has oversight responsibility of the Data Safety Monitoring Plan (DSMP) for this clinical trial. The DSMB will review accrual, patterns, and frequencies of all AEs, and protocol compliance every 12 months. The DSMB makes recommendations to the NIH regarding the continuation of the protocol. Each clinical investigator is responsible for reporting serious unexpected AEs 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 AE, the investigator or designee will log into the ChiLDReN website at the SDCC and complete the Serious Adverse Event Form online. Upon receipt of a serious adverse event (SAE) notification, the system will generate an email to notify the Safety Monitor, the PI at that clinical site, the NIH Program Director, and the SDCC. 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 SDCC. 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 6 months, the SDCC will provide interim reports to the DSMB and the NIH Program Director. The contents of the report are determined by the DSMB. Additions and other modifications to these reports may be requested by the DSMB on a one-time or continuing basis.

C. Definitions

AE. An adverse event (AE) is any unfavorable, harmful, or pathological change in a research

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

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

- Death
- Life-threatening [missing noun- doesn't make sense w/ just life threatening] (participant was at risk of death as a result of the event; does not include hypothetical risk of death if the event had been more severe)
- Inpatient hospitalization or prolongation of existing hospitalization
- Persistent or significant disability or incapacity
- Congenital anomaly or birth defect
- Medical or surgical interventions required to prevent one of the outcomes listed above

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

Expected AE. Since this is an observational study, in general there are no AEs that can be attributed to it, except at the time of blood draws or a breach of confidentiality. The only expected or known risk or AE associated with study procedures is bruising at the time of blood draws that may be attributable to this study.

Venipuncture: The vein in which the needle has been inserted to draw blood may become sore and red. A temporary "black and blue mark" may develop, and rarely fainting may occur.

D. Reporting of Serious AEs

- Within 24 hours (of learning of the event), investigators/subinvestigators must report any SAE that:
  Is considered life-threatening/disabling or results in death of participant
  -OR-
  Is unexpected/unanticipated
- Investigators/subinvestigators must report all other SAEs within 5 working days (of learning of the event).
- All other (suspected) AEs must be reported to the SDCC within 20 working days of the notification of the event or of the site becoming aware of the event.

V.B17. Statistical Considerations

A. Sample Size and Power

Number of participants and rates of accrual were discussed previously in section V.B6.B. As previously

stated in that section, the expected number of participants for this study was originally expected to be 590, updated to 800 in 2010, and to 1150 in 2012. Table 11 provides the newly updated estimated number of 1675 participants by group-disease-specific category with the continuation of ChiLDReN through 2024. It is important to note that in the last grant cycle, three clinical sites were discontinued, and one new site was added. For the discontinued sites, no new longitudinal data or bio-specimens will be collected going forward.

Table 11: Sample Size by Disease and Study Group

	1	2	3	5	Total
	Age: < 6 mo	Age: Birth-25yr	Transplant	A1AT Siblings	
ALGS*	35	350	115	-	500
α1-AT Deficiency*	40	395	135	30	600
Progressive Familial Intrahepatic Cholestasis (or BRIC)	20	350	130	-	500
Bile Acid Synthesis Defects	10	50	15	-	75
Total	105	1145	395	30	1675

* Group 4 not shown because identified pre-clinical cases will be enrolled in Group 1 or 2.

Characterizing the natural history of cholestatic liver disease within these four disease subtypes will be the primary goal of this study, with several formal hypotheses also to be tested. Confidence intervals will be provided when appropriate to reflect the precision of statistical estimates. Estimates associated with ALGS and α1-AT deficiency will be fairly precise and will allow for inferences regarding differences in outcome. There are limitations in recognizing differences between BASDs and the other disease categories. The appropriate analysis for the larger disease groups will be carried through to the smaller disease categories. For example, if model fitting diagnostics support using the Poisson Model for expressing the risk of bone fracture in participants with α1-AT deficiency, we will fit the same model to participants with BASDs. Furthermore, significance levels of covariates will be considered when quantifying the relationships in fitting the model for the large disease categories but will be of little concern for the small disease categories. In this latter case, the size of the coefficients and their similarity to the corresponding coefficients for the other diseases will be the overriding determination in model building process.

Ancillary Study Sample Size Calculation. An additional reason to increase the number of participants in this study in this amendment relates to the stated goal of LOGIC to provide sufficient numbers of participants, human specimens, and DNA to allow for Ancillary Studies (that are not detailed in this protocol) that will have the statistical power to perform genetic analyses and biomarker discovery investigations. For example, GWAS Ancillary Studies are either planned or in process by ChiLDReN investigators to search for genes that will determine the severity of liver disease in ALGS and in α1-AT

deficiency. Using current GWAS methodology or newer Whole Exome Sequencing methodology will require large initial sets of participants for discovery of gene associations or single nucleotide variants, which would then require a large second replication set.[70] Our goal is to enroll 500 participants with ALGS and 600 with α1-AT deficiency, which would provide adequate power to identify gene associations for strong modifier genes in each of these diseases. Previous GWAS studies have used similar numbers of samples and have made important discoveries of gene associations in other liver diseases: genes that were associated with response to Hepatitis C treatment (330 samples allowed detection of genes with odds ratio of >25) and genes that were associated with susceptibility to drug-induced liver injury (240 samples allowed detection of genes with odds ratio of 7.5).

We have performed an example of a power calculation for evaluating modifier genes in α1-AT deficiency.[71] We will base the power analysis on the availability of 600 samples from participants with α1-AT deficiency liver disease, and we will assume that approximately 200 participants will have a phenotype indicating more severe liver disease (cases; liver transplant was required before age 10 years or complications of portal hypertension at any age) and the remainder will not exhibit this phenotype (controls). With 200 case samples and 400 control samples, we performed the following power analysis assuming the genetic effect of the causal variant is additive. We computed powers to detect association for a range of effect sizes (represented by odds ratios) and allele frequencies in the case population. In this analysis, we also assumed a simple allele frequency test based on a 2x2 contingency table is used for association detection and we set the genome-wide significance level for rejecting null hypothesis at p-value < 10^{-6}. The results are presented in Figure 1.[71]

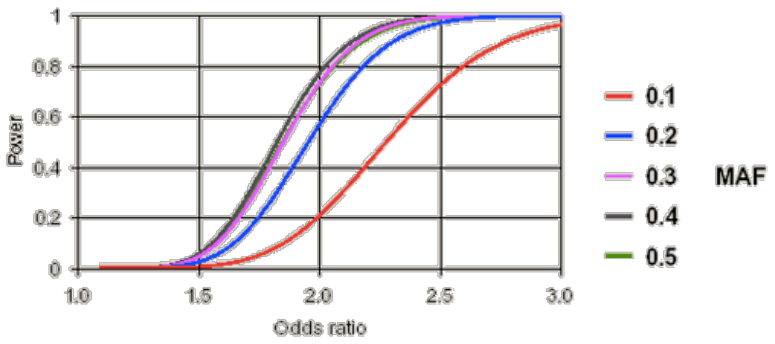

Figure 1: Statistical power to detect association with 400 mild and 200 severe samples. Different curves in the plot represent different allele frequencies.

Figure 1 demonstrates that there is sufficient power (>80%), with 600 samples under the assumptions described above, for minor allele frequencies ≥ 0.30 and OR > 2.06, and for allele frequencies > 0.10 and OR > 2.59.

A second replication set of samples would be needed to perform a GWAS study for α1-AT deficiency liver disease modifier genes. It is proposed that this set could be obtained from other collaborations, using the same disease definition and phenotyping used in this study. For example, the North American Society for Pediatric Gastroenterology Hepatology and Nutrition (NASPGHAN) has agreed to be a

partner in this type of GWAS study for α1-AT deficiency. Nearly all pediatric gastroenterologists in North America are members of this organization which has a history of supporting academic projects. The NASPGHAN membership of over 1,400 pediatric gastroenterologists across North America could be used to enroll participants and gather data and DNA samples from 400 α1-AT deficiency participants that are not enrolled in LOGIC at any of the 13 ChiLDReN sites. It is estimated by ChiLDReN investigators that there exist over 3,000 participants < 25 years of age with α1-AT deficiency liver disease in the United States. Thus, over 2,500 additional participants would be available for potential enrollment by pediatric gastroenterologists in NASPHGAN in order to capture 400 for a replication set to complete a proposed GWAS study.

Similar calculations justify increasing the number of ALGS participants in this study to 500. The highly variable expressivity of the hepatic phenotype in ALGS, even among individuals in the same family, is consistent with the presence of genetic or environmental modifying factors. Dr. Nancy Spinner and colleagues recently published *THBS2* as the first candidate modifier gene influencing liver disease severity in ALGS. This GWAS was completed with only 161 participants, 97 with mild and 64 with severe liver disease. The top ranking SNP reached suggestive but not genome-wide significance in this small study. These results have not yet been replicated in an independent cohort. It is very likely that additional genetic modifier loci exist, but were not identified in this underpowered GWAS. It is therefore crucial that the ChiLDReN consortium continue to enroll ALGS participants to establish a larger cohort for genetic studies and to accrue longitudinal outcome data in this rare multisystemic disorder.

Regarding the PFIC/BRIC group, one of the main goals of the longitudinal study is to determine the clinical phenotypes and natural history of each form of PFIC/BRIC during childhood and early adulthood. Outcomes would include degree of cholestasis, development of complications, progression to portal hypertension, and survival with native liver, in addition to other factors. We hypothesize that each of the PFIC/BRIC diseases will have unique phenotypic features and a characteristic natural history. Since the PFIC/BRIC group encompasses more than six distinct clinical entities (FIC1 disease, associated with mutations in ATP8B1; BSEP disease, associated with mutations in ABCB11; MDR3 deficiency, associated with mutations in ABCB4; *TJP2* deficiency; FXR deficiency, associated with mutations in *NR1H4; MYO5B* deficiency and unclassified, with no detectable mutations in any of these six genes), additional participants will need to be enrolled in each group in order to make reliable statistical comparisons. In addition to the characterization of clinical phenotypes, another goal of the study will be to determine genotype-phenotype relationships. Recent network-wide initiatives are focused on using next generation sequencing techniques (targeted panels and exome sequencing) to identify causative variants in PFIC/BRIC participants without a genetic diagnosis. This approach is likely to yield discoveries of new genetic diagnoses, each of which will need to be characterized in regards to clinical phenotype and natural history.

For example, one goal of the study would be to predict the difference in progression to portal hypertension in the FIC1 participants as compared with the BSEP and MDR3 groups. Portal hypertension is defined by platelet count < 150,000 and spleen palpable > 2 cm below left costal margin, or complication of portal hypertension such as variceal hemorrhage, ascites, or hepatopulmonary syndrome is estimated as 6%-7% in the BSEP and MDR3 groups, and as low as 2%-3% in the FIC1

group. Assuming that we continue recruitment only for the current grant cycle (4 years) and we are able to continue follow-up for an additional 5 years, we would need approximately 50 children in the FIC1 group and about 80 children in the combined BSEP/MDR3 group for 80% power to detect a difference between the FIC1 group and the other two groups combined (alpha=0.05, two-sided), assuming complete follow-up for the entire group. Allowing for: (a) that the estimated 20% of PFIC participants recruited after transplant do not contribute to this analysis; and (b) that approximately 30% of the total PFIC participants are not being genotyped to one of the three groups, we are increasing recruitment to 500 children total (approximately 100 children in each group for the more common disease genes [FIC1, BSEP, MDR3] and 200 participants either with mutations in the newly-identified genes or unclassified). This increase will allow up to a 20% loss in follow-up information over the course of the study, and still provide approximately 80% power.

In this amendment, we also propose to increase the number of participants in the BASD group from 50 to 75. There have recently been new causative genes identified for BASD, and we expect that additional participants will be identified to have BASD diagnoses, and additional genes may be identified by exome sequencing.

B. Statistical Design

The primary source of participants will be prevalence cases (Group 2). The ideal design when assessing the natural history of diseases calls for enrollment of incident cases being followed for long periods of time. Due to the rarity of these diseases such a design is impractical. The natural concern in this hybrid-sampling scheme is whether the study sample will be representative of the entire spectrum of the disease. The specific concern is under-representing the extremes. Specifically, those with the most severe disease may be under- or over-represented at certain ages. For example, if at the very younger ages the most severe cases are over-represented it will tend to bias the age-specific incidence of liver transplant and death at the younger ages and will also bias the cumulative incidence estimates. We do not believe this selection bias will occur but we can never be certain. Alternatively, those with very mild disease could potentially be under-represented at the older ages because they are not routinely going to the participating specialist for treatment. Our belief is that this will not occur because virtually all patients presenting with liver disease and these four disorders are evaluated by a pediatric gastroenterologist and followed throughout childhood.

Each analysis will target a subgroup of the participants. The analysis that focuses on the natural history of the disease will include Group 1, Group 2, and those newly diagnosed cases from Group 4 who are transferred into Groups 1 or 2. Those who have undergone liver transplant will be a separate analytical subgroup and will be used primarily for future analyses of modifier genes that might predict poor outcome.

The revised sample sizes will support some formal hypothesis testing (the hypotheses outlined in Section II.A and II.B) when comparing ALGS and α1-AT deficiency disease groups. For example, one analytical subgroup will be Group 1 and Group 2 focusing on a dichotomous outcome, with approximately 300 participants in each disease group (since Group 3 [post-transplant] participants would not be included). The power ranges from 84% to 93% for an odds ratio effect size of 1.75 when the frequency of the outcome variable in the lower group is within the range of 20% to 70% (α=0.05, 2-sided), and is over

80% for an odds ratio effect size of 2.00 when the frequency drops to 10% or increases to 80% in the lower group. In the fortunate situations when the outcome variable is normally distributed, small effect sizes will be detectable with high statistical power between the two large disease groups.

Comparisons between these two disease groups would have over 85% power to detect differences on the order of 0.25σ. For comparisons between two PFIC subgroups (assuming enrollment of approximately 75 in each of the groups), we would have over 80% power to detect an odds ratio effect size of 3 when the frequency of the outcome in the lower group is in the range of 20%-65% and over 85% power to detect differences on the order of 0.50 σ.

Given the extensive data analyses anticipated, and the importance of this large collection of data on extremely rare diseases, all analyses will be prespecified in an appropriate statistical analysis plan prior to the analysis, to avoid the need for adjustment for multiple hypothesis testing. It is anticipated that much like the replication of GWAS results for A1AT and ALGS, all important associations identified through this study should eventually be confirmed via independent research databases.

C. Analysis Plan

There are five general categories of analysis that will be conducted on the data arising from this study. Each is briefly outlined below.

I. Age-Specific Incidence

Using life table techniques (i.e., Kaplan-Meier) extended to open cohorts, we will compute age-specific conditional probabilities of being event-free of various single or first-event outcomes (e.g., liver transplant). Graphs will be constructed to reveal any patterns of the cumulative incidence by age along with the calculation of 90% confidence intervals using an appropriate variance estimator (e.g., Greenwood's). Furthermore, time-to-event models accounting for staggered age at entry times (open cohort) will be used to evaluate baseline and time invariant covariate effects on risk. The semi-parametric model proposed by Cox will be used when there are sufficient events (50 or more) and appropriate fully parametric models (e.g., Weibull) will be used when the event is rare. Of particular interest will be evaluating the effect of disease on the age-specific incidence rate applying a simplifying assumption of proportional hazards. This will be conducted both while adjusting for other important covariates and not adjusting (univariate analysis). The primary events we will evaluate are: death, liver transplant, ascites, growth failure, worsening liver function, and complications of portal hypertension (Primary Outcomes, as described in V.B14) and dichotomous secondary outcomes (as described in V.B14) (Specific Aim 1 and 2).

II. Continuous Outcomes with Repeated Measures by Age

For continuous outcomes, a variety of models may be considered but most notable will be the linear mixed-effects model. This model requires that the dependent variable is normally distributed. We will accomplish this by constructing a normal Q-plot to of the residuals. If the residuals are skewed we will apply various transformations to allow us to proceed with using the mixed model. The random effect for participant is a convenient way to account for the correlation of the repeated measures within participant. Using the same approach as described in the previous paragraph, we will evaluate various covariates including the disease-type. Outcomes to be evaluated include: growth Z-scores, PELD and

MELD scores, height, weight, serum bilirubin, serum albumin (continuous primary and secondary outcomes described in V.B14) (Specific Aim 1 and 2).

III. Genotype-Phenotype Associations
Genetic analysis conducted on the ALGS and PFIC/BRIC participants will provide additional independent variables to associate with the outcomes already listed above. These variables will represent various functional mutations in *JAGGED1*, *NOTCH2, FIC1*, *BSEP*, and *MDR3* genes (mutations will be grouped by exon, functional nature of the mutation, and type: missense, nonsense, etc.). The modeling will proceed in the same fashion as previously described (Specific Aim 4). Additional dependent variables of interest include: presence of cardiac, renal, vertebral anomalies, abnormal growth parameters, complications of portal hypertension (ascites, esophageal variceal hemorrhage, hypersplenism, spontaneous bacterial peritonitis, pulmonary hypertension or hepatopulmonary syndrome), and presence of intracranial vascular abnormality/hemorrhage.

IV. Family Analysis
The clinical data from the PIZZ/PISZ genotype siblings of participants with α1-AT deficiency will be analyzed to generate hypotheses of other genetic factors that may explain the disparity in the penetrance of liver disease. Specifically, we will calculate the relative frequency of liver disease among non-index case siblings. It can be expected that 10%-20% will develop clinical evidence of liver disease during childhood. We will conduct an exact binomial test to determine if PIZZ siblings have a statistically significant higher frequency of clinical liver disease over the 10 years of this study.

V.B18. Data Management
All study data will be collected via systems created in collaboration with the SDCC and will comply with all applicable guidelines regarding participant confidentiality and data integrity.

A. Registration
Registration of participants on this protocol will employ an interactive data system in which the clinical site will attest to the participant's eligibility as per protocol criteria and that an appropriate informed consent has been obtained. IRB approval for the protocol must be on file at the SDCC before accrual can occur from the clinical site.

The SDCC will use a system of coded identifiers to protect participant confidentiality and safety. When the participant is registered on the study, using the SDCC provided web-based registration system, the system will assign a participant ID number. Only the registering site will have access to the linkage between this number and the personal identifier of the participant. In this fashion, it is possible to protect against data keying errors, digit transposition or other mistakes when identifying a participant for data entry since the numbers should match to properly identify the participant. In this fashion, no personal identifiers would be accessible to the SDCC.

B. Data Entry
The University of Michigan Department of Biostatistics at the University of Michigan and Arbor Research Collaborative for Health are responsible for data management and analysis.

Arbor Research Collaborative for Health has created ChiLDReNLink, a web-based data entry and management system. The ChiLDReNLink system will be used to capture all relevant study data.

The ChiLDReNLink system allows real-time monitoring of study data for protocol adherence, quality assurance, AE reporting, discrepancy reporting, and other trends. All study data will be entered into the electronic data entry system by study coordinators at each study site. These data will be encrypted and transferred to the SDCC and stored on a secure server at Arbor Research Collaborative for Health. Access to the data entry system is limited and requires a unique username and password combination. The servers are backed up daily and physically stored in a locked facility.

CRFs are developed by the SDCC in collaboration with the protocol team, and published on the ChiLDReN password-controlled website. The CRFs do not contain any personal participant identifiers, except dates (such as date of birth) which are necessary for research purposes. As needed, the coordinator prints the forms for each participant. The forms are completed and then countersigned by the investigator or study coordinator.

Original CRFs will be securely maintained at the clinical sites.

C. Data Quality Control

The Project Manager/Clinical Monitor will communicate with the study coordinators at each site about queries generated by the SDCC and address all questions and concerns regarding the study protocol and problems with data entry or specimen sample shipment. Site visits will be made approximately every 12-24 months. 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.

D. Laboratory Data Flow

The SDCC provides sample barcode labels to the clinical sites that allow for specimen tracking to and reconciliation with the NIDDK Biorepositories. A combination of web-enabled and centralized data entry and management will be used to track the flow of laboratory data among clinical sites, Core labs, and the SDCC.

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APPENDIX I

CHILDHOOD LIVER DISEASE RESEARCH AND EDUCATION NETWORK (ChiLDREN) CLINICAL SITES, DATA COORDINATING CENTER, BIOLOGIC CORES AND LOCAL PRINCIPAL INVESTIGATORS

CLINICAL SITES	PRINCIPAL INVESTIGATOR
1. Children's Hospital Colorado, Aurora, Colorado	Ronald J. Sokol, MD
2. Cincinnati Children's Hospital Medical Center	Jorge Bezerra, MD
3. Children's Hospital of Philadelphia	Kathleen Loomes, MD
4. Children's Hospital of Pittsburgh	Robert Squires, MD
5. Ann & Robert H. Lurie Children's Hospital of Chicago	Peter Whitington, MD
6. Baylor College of Medicine, Houston	Benjamin Shneider, MD
7. University of California at San Francisco	Phillip Rosenthal, MD
8. Indiana University, Indianapolis	Jean Molleston, MD
9. University of Washington, Seattle	Karen Murray, MD
10. Hospital for Sick Children, Toronto, Canada	Binita Kamath, MD
11. Emory University, Atlanta	Saul Karpen, MD
12. University of California at Los Angeles	Kasper Wang, MD
13. University of Utah	Stephen Guthery, MD

ENROLLMENT CENTER

1. Saint Louis University, St. Louis	Jeff Teckman, MD

BIOLOGIC CORES

1. Histopathology Core	Kevin Bove, MD	Cincinnati Children's Hospital Medical Center
2. Bile Acid Biochemistry Core	Kenneth Setchell, PhD	Cincinnati Children's Hospital Medical Center
3. Genetics Core	Richard Thompson, MD	King's College Hospital, London, UK

DATA COORDINATING CENTER

University of Michigan, Ann Arbor MI John Magee, MD; Cathie Spino, D.Sc.
Arbor Research Collaborative for Health, Ann Arbor MI Robert Merion, MD

NIH PROJECT SCIENTISTS

NIDDK: Edward Doo,MD; Averell Sherker, MD;
Jay Hoofnagle, MD