**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.

**Ta b l e 1 . ALGS D iagnostic Criteria**

|  |  |  |  |
| --- | --- | --- | --- |
| **ALGS family historya** | **Paucity** | ***JAGGED1d* or*****NOTCH* 2 mutation** | **Number of clinical criteria required** |
| Present or absent | Present | Identifiedb | Any or no features |
| None (proband) | Present | Not identifiedc | 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.)aFamily history = ALGS present in a first degree relativebIdentified = *JAGGED1 or NOTCH2* mutation may have been identified in clinical or research laboratorycNot identified = Not identified on mutation screening, or not screened for***d****JAGGED1* mutation = Mutation, whole gene deletion, or deletion of chromosome 20p, which includesJ*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 DCC 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.