Longitudinal Study of Genetic Causes of Intrahepatic Cholestasis (LOGIC)

Manual of Operations (MOO)

Version 3.1

July 27, 2017

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1. General

The Manual of Operations (MOO) is designed to facilitate consistency in protocol implementation and data collection across studies, study participants, and study sites. Further, the MOO provides reassurance to all participants that scientific integrity and study participant safety are closely monitored and it increases the likelihood that the results of the study will be scientifically credible. The MOO is analogous to a toolkit in that it contains information needed for the conduct and operations of the study; it can also be used as a training document.

The MOO is a dynamic document that is updated throughout the course of the study to record changes and refinement of procedures. It is maintained in a format that allows it to be easily updated, both online and in print form for binders. The version number and date should appear on each page of the MOO to track all changes and additions to the document. The MOO may be downloaded from the Childhood Liver Disease Research Network (ChiLDReN) website. The Data Coordinating Center (DCC) will inform the study site via email of any changes in the MOO or any other study-related documents. It is the study sites' responsibility to ensure that their files and binders contain the most current versions. The DCC will have the archived documents available on the website.

1.1 Summary of Study

This longitudinal observational study will investigate the natural history and progression of four genetic causes of intrahepatic cholestasis of childhood, including alpha-1 antitrypsin deficiency (a1-AT), Alagille Syndrome (ALGS), progressive familial intrahepatic cholestasis (PFIC) or benign recurrent intrahepatic cholestasis (BRIC), and bile acid synthesis defects (BAD). This study will be conducted as part of the ChiLDReN study. In this study, we will collect defined data elements in a uniform fashion at fixed intervals for up to twenty years over a relatively large number of patients with these rare disorders. A biobank of patient 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 to 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, outcomes, and complications of each of the disorders. This initial characterization will allow calculation of sample sizes for future therapeutic intervention clinical trials and provide the baseline to which interventions should be compared.

This is a multi-center study utilizing collection of diagnostic, clinical, and outcome data at defined intervals. 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 or transformed cell lines for DNA analyses, will be an invaluable resource for current and future ancillary investigations into the pathogenesis, genetics, and progression of cholestatic liver injury in infants and children, as well as the identification of modifier genes and biomarkers predictive of outcomes. Longitudinal Study of Genetic Causes of Intrahepatic Cholestasis (LOGIC) participants with suspected ALGS and PFIC (or BRIC) will undergo research genotyping at the ChiLDReN Genetics Core Labs. In addition, 20 unstained paraffin embedded slides will be shipped to the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) Repository on all participants undergoing liver biopsy or transplantation. Asymptomatic affected siblings of participants with a1-AT deficiency (but without identifiable 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 LOGIC DCC, located at Arbor Research Collaborative for Health in Ann Arbor, MI where the data will be analyzed.

Some samples of blood, bile, and tissue will be stored in repositories for future research. The data and

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

For more specific information on this study, please see the LOGIC Study Protocol (**Appendix A**).

1.2 Specific Aims

1 To determine the clinical phenotype and natural history of each of the four LOGIC 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.

   i. To determine circulating biomarkers that predict disease progression and outcomes,

   ii. To identify new genetic causes of the disorders, and

   iii. To identify modifier genes that influence the incidence, severity, and progression of liver disease among genetically affected individuals.

4 To determine genotype-phenotype relationships in ALGS and PFIC (or BRIC) disorders.

   i. To determine the frequency of decreased bone mineral density in ALGS and PFIC (or BRIC) disorders.

1.3 Hypotheses Related to Specific Amis

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

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 Specific Aims (Section 1.2) 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 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

1.4 Sponsor

The study is funded by the NIDDK which is part of the National Institutes of Health (NIH). ChiLDReN is governed by a steering committee comprised of the Principal Investigators (PIs) from each participating clinical site, the DCC PI, and the NIDDK project scientist.

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

1.5 Study Organization

1.5.1 Data Coordinating Center

Arbor Research Collaborative for Health is the DCC for ChiLDReN studies. The DCC provides project management, logistical coordination, and statistical leadership for the development, implementation, and analysis of ChiLDReN studies. In addition, the DCC will conduct training in protocol implementation, data management, monitoring, and quality control. The DCC also supports regulatory and technical functions (i.e., ChiLDReNLink). For a list of DCC personnel and their roles and contact information, please refer to the Study Directory located on the website https://ChildrenNetwork.org/Secured/StudyDirectory.aspx.

1.5.2 Clinical Sites and Principal Investigators

Below is a table of the participating centers and current site Principal Investigators.

Center Name (by city)	Principal Investigator
Atlanta	Saul Karpen, MD
Chicago	Estella Alonso, MD
Cincinnati	James Heubi, MD
Denver	Ron Sokol, MD
Houston	Benjamin Shneider, MD
Indianapolis	Jean Molleston, MD
Los Angeles	Kasper Wang, MD
Philadelphia	Kathleen Loomes, MD
Pittsburgh	Robert Squires, MD
San Francisco	Philip Rosenthal, MD
Seattle	Karen Murray, MD
St. Louis-SLU	Jeffrey Teckman, MD
Salt Lake City	Stephen L. Guthery, MD
Toronto	Binita Kamath, MD
DCC	John Magee, MD and Robert Merion, MD

1.5.3 Clinical Sites and Study Numbers

Center Name (by city)	ChiLDReN Center ID[1]	ChiLDReN Center ID[2]
Chicago	02 (B)	F02
Cincinnati	03 (C)	F03
Denver	04 (D)	F04
Philadelphia	06 (F)	F06
Pittsburgh	07 (G)	F07
San Francisco	08 (H)	F08
Houston	10 (J)	F10
Indianapolis	12 (L)	F12
St Louis – SLU	11 (K)	F11
Seattle	13 (M)	F13
Toronto	14 (R)	F14
Salt Lake City	15 (S)	F15
Los Angeles	17 (N)	F17
Atlanta	18 (P)	F18

[1]The two-digit numerical code represents the site ID, the letter code represents a participant ID (for data entry) for those sites whose site IDs contain a two-digit site ID.
[2] These numbers represent the site IDs assigned by the NIDDK repository.

1.5.4 NIDDK Data and Safety Monitoring Board

The Data and Safety Monitoring Board (DSMB) is appointed by the NIDDK and serves in a consultative capacity to the NIDDK.

ChiLDReN is monitored by two separate DSMBs. The Boards meet twice a year to provide independent review of data safety and monitoring procedures for the observational ChiLDReN Studies and additionally, meet twice a year to review the interventional ChiLDReN Studies. The Boards may also convene to review the study protocols if significant safety concerns arise. All protocols are reviewed and approved by the DSMB prior to implementation. The Boards meet to examine endpoints, participant enrollment, protocol compliance, completion of samples and data, toxicity, and safety data from NIDDK-supported protocols. Since the ChiLDReN Studies (BASIC, FORCE, LOGIC, MITOHEP, and PROBE) are observational studies with no drug or other medical interventions, few adverse events related to study-mandated procedures are expected. Reference the DSMB Charter and DSMB Membership Lists (**Appendix B**) for additional information regarding the DSMB.

1.5.5 ChiLDReN Website

Publicly accessible information about ChiLDReN is available on the ChiLDReN website home page. Some portions of the website are password-controlled to limit access to study group members (clinical centers, the DCC, NIDDK, and the DSMB), protect the integrity, security, and confidentiality of sensitive project information and the information system, and allow auditing for appropriate use.

The website contains workgroup/subcommittee member lists, meeting agendas, materials, and minutes, slides, and presentations, master documents (including final protocols and consent templates), calendar of events, and a study directory. The secure ChiLDReNLink data entry system is also linked via the password-protected portion of the website, affording a double login/password for access to participant data.

1.5.6 Website URL and Access Instructions

The address for the ChiLDReN website is https://ChildrenNetwork.org/. Website management resides with the DCC. The DCC is responsible for login accounts, study directory updates, postings, and maintenance. Upon assigning a username and password, an automatic welcome email will be generated, informing the user that access has been granted to the restricted areas of the website. Users must change their system-assigned password within 72 hours of receipt of the welcome email t or website access will be denied.

Usernames and passwords should not be shared. New personnel requiring access to the ChiLDReN website should request a unique username and password. For new account requests or trouble with usernames and passwords, please contact ChiLDReN-Monitors@arborresearch.org.

2. IRB Submission and Regulatory Documents

Regulatory documents are those documents that individually and collectively permit evaluation of the conduct of a trial and the quality of the data produced. These documents serve to demonstrate the compliance of the investigator, sponsor, and the monitor with the standards of Good Clinical Practice (GCP) and with all applicable regulatory standards. The minimum list of essential documents that has been developed is outlined below.

2.1 Protocol Version Control

Protocol version control is extremely important to ensure that all participating sites and their respective Institutional Review Boards (IRBs)/Ethics Review Committees (ERCs) receive identical documents. Before a protocol is considered final and versioned (e.g., version 1.0), it must go through a formal review by the ChiLDReN Steering Committee. Once finalized, the protocol document, consent templates, and any supplemental materials will be distributed to the sites by the DCC. Sites should submit only materials distributed by the DCC to their IRBs. Finalized protocols must NOT be edited, changed, or altered.

All amendments (a written description of changes to or formal clarification of a protocol) must undergo a similar approval process. Sites should only submit protocols and amendments to IRBs/ERCs as instructed by the DCC or NIDDK.

2.2 Certificates of Confidentiality

Certificates of Confidentiality constitute an important tool to protect the privacy of research study participants. Certificates of Confidentiality are issued by the NIH and/or the Food and Drug Administration (FDA) to protect identifiable research information from forced disclosure. They allow the investigator and others who have access to research records to refuse to disclose identifying information on research participants in any civil, criminal, administrative, legislative, or other proceeding, whether at the federal, state, or local level. Certificates of Confidentiality may be granted for studies collecting information that, if disclosed, could have adverse consequences for participants or damage their financial standing, employability, insurability, or reputation. By protecting researchers and institutions from being compelled to disclose information that would identify research participants, Certificates of Confidentiality help achieve the research objectives and promote participation in studies by assuring confidentiality and privacy to participants. For more information, please see the NIH's Certificate of Confidentiality Kiosk: http://grants.nih.gov/grants/policy/coc/.

The DCC will obtain and maintain Certificates of Confidentiality for the study. These Certificates provide coverage to all clinical sites. Please refer to **Appendix C** to view the study's Certificates of Confidentiality.

2.3 Informed Consent Document, Finalization, and Approval Process

Protocol-specific consent document templates will be provided to all ChiLDReN sites. Site-specific language should be inserted into the template.

Each site-specific informed consent form will be reviewed by the DCC for inclusion of all essential elements and compliance with federal regulations. After DCC review, the sites' draft informed consent documents will be returned to the sites for correction and submission to the IRBs. Once approved the site will submit their informed consent documents to the DCC and the documents will be sent to the NIDDK for review and approval. If approved by the NIDDK, a letter stating approval and version number will be distributed to each site through the DCC.

Please note: this is the process for initial approval prior to site initiation and/or amendments that include revisions to the informed consent document (additional procedures, increased risk, etc.).

Sites should file the IRB/ERC-approved consent document(s) (memo, consent, and other documents) in the site regulatory binder. Next, scan all approved documents and send them electronically to the DCC. Throughout the course of the study, the DCC will request these documents when there is an amendment to the Core Protocol and at the time of each site's IRB annual renewal.

2.4 Regulatory Documents

Required regulatory documents are to be kept on file at the site. The regulatory binder must be kept current and available for review during site monitoring visits. The following section describes the required essential regulatory documents for the ChiLDReN Studies. Please refer to Appendix E for a check-list of the required regulatory documents for this study.

If the site maintains Master Files for CVs, lab normals, etc., then a note should be placed in the study-specific regulatory binder to reflect the location of the documents.

REMEMBER, WHEN THE STUDY IS FINISHED AND READY FOR ARCHIVING, ALL DOCUMENTS IN THE MASTER FILES MUST BE COPIED TO BE STUDY-SPECIFIC DURING THE CONDUCT OF THE TRIAL. THE DOCUMENTS WILL BE STORED FOR THE LENGTH OF TIME DESIGNATED BY THE SPONSOR.

The following documents must be maintained in the regulatory binder throughout the study:

- IRB/ ERC approval notice/letter
- Documentation of the provision of IRB/ERC review and approval of the protocol ensures that the study is conducted with the appropriate local regulatory oversight. IRB/ERC approval will be obtained prior to the initiation of the study, and maintained throughout the conduct of the study and data analysis phase. Sites should maintain current IRB approval until directed by the DCC to close the study.
- All IRB/ERC approval letters must be on file. They include, but are not limited to, the protocol, consent(s), study advertisement(s), training and educational materials, participant letters, questionnaires, or any other documents receiving IRB/ERC approval or opinion. All of these documents must be forwarded to the DCC. **NOTE:** If contingent approval is granted, evidence of final approval must be present before the study can be implemented.
- All annual or periodic renewals.
- Approval letter for any protocol amendments and modifications (the sponsor and the IRB/ERC must approve all protocol changes prior to implementation unless the change is intended to

eliminate an apparent immediate hazard to participants).

- Any local or country-specific regulatory authorization relating to the protocol.
- All approval letters from the IRB/ERC should be addressed to the PI and should include the following information:
  - Protocol title, number, and version
  - Actual date of IRB/ERC approval
  - Specifically state approval of the protocol
  - IRB/ERC chairperson's or designee's signature
  - Renewal date or statement indicating when the approval must be renewed
  - List of the documents approved
  - List of all sites covered by the IRB/ERC approval

The DCC must receive IRB/ERC approvals for all protocols in which the study site participates. Send to ChiLDReN-Monitors@arborresearch.org

2.4.1 IRB/ERC Membership List

- The IRB/ERC's composition is constituted in agreement with GCP.
- IRB/ERC information including membership list, chairperson, and general assurance number or a letter stating that the IRB/ERC is in compliance with GCP.
- IRB membership list must be current.
- If your IRB/ERC does not release its membership list, a Department of Health and Human Services (DHHS) Multiple Assurance Number must be submitted on the IRB/ERC letterhead.
- If the IRB/ERC does not allow access to their membership list, then an anecdotal note must be written to reflect the standard operating procedure of the IRB/ERC and the note must be filed in the regulatory binder.

2.4.2 Sample Copy of the IRB/ERC-Approved Informed Consent or Waiver of Consent

A copy with the IRB/ERC approval stamp must be sent to the DCC for all protocols in which the study site participates. Send to ChiLDReN-Monitors@arborresearch.org.

The DCC will be responsible for forwarding the documents to the NIDDK biorepository's S. Kay Mobley (mobleys@niddk.nih.gov).

2.4.3 Protocol Signature Sheet (PI Signature Sheet)

Required for each ChiLDReN Study protocol. An electronic copy is sent to the DCC at ChiLDReN-Monitors@arborresearch.org.

Maintain a copy in the regulatory binder.

2.4.4 Protocol Log

This document is optional for each ChiLDReN protocol. It's a useful tool to keep track of protocol

amendments and approval notifications by your local IRB. Each site is responsible for obtaining IRB approval of the most current protocol used for each study.

Maintain a copy in the regulatory binder. See **Appendix F**.

2.4.5 Screening Log

Required for each ChiLDReN protocol. Each log should meet local IRB/REB requirements

Maintain in the regulatory binder as paper or kept electronically. The screening logs are made available for review at the time of the monitoring visits. See **Appendix G**.

2.4.6 Enrollment Log

Required for each ChiLDReN protocol.

Each site should maintain a log in the regulatory binder that meets local IRB/REB requirements. See example **Appendix H**.

2.4.7 Monitor Log

This log is required for each monitor visit. The Clinical Monitor signs the log for each day of site monitoring and the site representative also signs the log.

Maintain in the regulatory binder. See **Appendix I**.

2.4.8 Curriculum Vitae (CV) of Investigators and Sub-Investigators

A signed and dated CV must be on file for the PI and each sub-investigator. This is to document qualifications and eligibility to conduct trials and/or provide medical supervision of participants. Ensure the CV is complete and contains the following information:

- Current appointments/positions/citations, etc.
- Start and end dates (or “to present”) for all appointments and positions (no date gaps)
- Signed and dated (on first page) by the Investigator (or sub-investigator) and all study personnel to verify document is current
- Updated CVs are to be filed bi-annually

CVs may be kept in a Master File during the conduct of the study, but all the CVs must be archived with the study at the end of the trial.

2.4.9 Medical Licenses

Maintain copies of all licenses for licensed personnel (e.g., MDs, nurses, nurse practitioners, physician assistants, etc.) for the duration of the study.

Licenses may be kept in a Master File during the conduct of the study, but all the licenses must be archived with the study at the end of the study.

2.4.10 Certification of Completion of Human Participants Protection Training

All Investigators, sub-investigators, and study personnel listed on the delegation of responsibilities log must complete research ethics training.

Note: Required for all study site personnel who will be involved with participants or data collection on any ChiLDReN protocol.

Online training may be obtained at http://cme.cancer.gov/c01/ or you may use your own institutional training.

Any course on the protection of human participants provided by your institution will meet this requirement. The course title, student's name, and dates of completion and expiration (if applicable) must be on the certificate. A brief description of the course must also be placed on file. If the site-specific course is one that does not expire, this should be outlined in the description provided.

Training and certification can also be obtained at http://ohsr.od.nih.gov (NIH: Protection of Human Research Subjects).

New study personnel must complete all of the required human participants training, and their addition must be approved by the IRB prior to their participating in the study.

Maintain copies of certificates in the regulatory binder.

2.4.11 Duality of Interest Disclosure Form

Required each year. Can be completed on the ChiLDReN website at https://childrennetwork.org All PIs and co-investigators, as well as staff members will need to complete this form each year.

2.4.12 Delegation of Authority Log and Site Signature Log

Contains the list of all study personnel who are involved in the primary conduct of the trial at the site. It documents responsibilities assigned to research team members and their dates of involvement in the project. It helps to ensure the appropriate delegation of study related tasks, and documents authenticity of the written signature of personnel involved in the conduct of the study. When you add staff for any protocol, they will need to be added to this log. Sets of initials must be unique; the same set of initials cannot be used by two members of the research staff.

Maintain a list of all study personnel on the appropriate form and include:

- Initials
- Printed name
- Legal signature, including first and last name
- List of delegated responsibilities
- Start and end date for delegated responsibilities

See **Appendix K.**

2.4.13 Laboratory Accreditation or Certification

- Documents that laboratory tests are performed with appropriate care and oversight throughout the trial period.
- Each site's laboratory current certification(s), Clinical Laboratory Improvement Amendment (CLIA), College of American Pathologists (CAP), and all previous certification(s).
- CLIA exemptions for certain laboratory tests should be documented.
- Place note-to-file in the regulatory binder if either the CLIA and/or CAP certifications have expired, and the site is waiting for the renewal certification.

2.4.14 Institution Clinical Lab Normals

- Documents normal values and ranges (including revised) that were used during the conduct of the clinical trial.
- Record of current laboratory normal ranges. All units of measurement, the laboratory name, and document date should be included.
- Provide updates as necessary and retain the original document.
- Place a note-to-file in the regulatory binder to indicate if laboratory normals are kept in a Master File to reference.
- Copies of laboratory normals used during the conduct of the trial must be taken out of the Master File and placed in the study's archival file at the end of the study.

2.4.15 Federalwide Assurance (FWA) Number

Maintain a record of your study site IRB's FWA number in the regulatory file. Be sure to have the expiration date for the FWA as well. This information can be obtained by searching: http://ohrp.cit.nih.gov/search/asearch.asp#ASUR.

2.4.16 Certificates of Confidentiality

Certificates of Confidentiality are issued by the NIH and/or the FDA to protect the privacy of research participants by protecting investigators and institutions from being compelled to release information that could be used to identify participants with a research project.

Certificates of Confidentiality are issued to institutions or universities where the research is conducted. They allow the investigator and others who have access to research records to refuse to disclose identifying information in any civil, criminal, administrative, legislative, or other proceeding, whether at the federal, state, or local level.

The lead institution must ensure that all participating institutions conform to the application assurances and inform participants appropriately about the Certificate, its protections, and the circumstances in which voluntary disclosures would be made. This information is built into the template consents for the study.

The Certificates of Confidentiality can be downloaded and printed from the study website in the Master Documents area.

2.4.17 Certification for Shipment of Biosamples

Each site must have at least one person certified to ship biosamples, and the certification (IATA/shipment of hazardous or dangerous goods) must be current.

Names of the research staff that are certified and a copy of the certificates should be maintained in your regulatory binder.

2.4.18 Advertisements/Educational Materials

After IRB approval, maintain copies of all advertisements (e.g., fliers, radio announcements, newspaper/internet advertisements) and educational materials (e.g., slide shows) utilized for the study.

All materials filed in this section and used in the study should be IRB approved and clearly listed on IRB approval letters/notices.

2.4.19 Major Sponsor, DCC, and IRB Correspondence

Maintain a copy of all correspondence (email, letters, faxes, memoranda, and phone contacts) between the Investigator or research staff, sponsor, and DCC relating to the clinical conduct of the study, especially correspondence pertaining to:

- Site activation letter
- Protocol decisions (by phone or email)
- Serious adverse events
- Deaths
- Protocol deviations
- Protocol modifications
- DSMB roster and letters from the Project Officer
- Site monitoring reports

Maintain a copy of all pertinent communications with the IRB/ERC relating to the study (e.g., study hold, safety report, removal of participant, protocol deviation, and notice of final study report).

CVs, medical licenses, IRB approvals, laboratory certifications/accreditations should be kept current. Current copies of required documents (IRB approvals) should be forwarded electronically to the DCC when available. The DCC will assist sites in monitoring IRB, CV, and license expirations.

Most of these essential (required) regulatory documents are shared across all studies. Rather than have several copies of the same documents, sites can use a central binder, as long as the study-specific regulatory binder contains place holders for those documents that are contained in the central binder and the place holder describes the location of the central binder. The central binder must be available to the Clinical Monitor for review during the monitoring visit. Storage of regulatory items electronically is acceptable, but the media must also be available to the Clinical Monitor during the monitoring visit.

3. Informed Consent

3.1 Informed Consent Document

The DCC will provide a protocol-specific informed consent template for all study sites for each study. Each study site will customize the template and receive approval from their study site's human participant protection committee.

The written informed consent should be brief and written in plain language so that a participant who has not graduated from high school can understand the contents. An Investigator, participant (in the case of assent), parent/guardian (in the case of a minor, as defined by the local IRB), and witness should each sign and date the informed consent documents. The participant should receive a copy of the signed and dated informed consent form. The study site must maintain a signed copy of the informed consent document for each participant in the study. GCP guidelines require that source documents should indicate that the informed consent form was signed, along with the date of signing.

3.2 Obtaining Informed Consent and Assent

All potential participants that are 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/assented by the PI or a designee, who has received appropriate training regarding human participant protection and HIPAA compliance, as established by the local institutional governing body requirements. Local IRB regulations regarding enrollment will be followed in all situations including, for example, if the child refuses.

NOTE: 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 local site IRB/ERC, they will also be asked to sign the Assent Form.

Screening of participants may occur without obtaining informed consent if only to review a potential participant's medical records to confirm eligibility for study participation. Assent will be sought from participants, if applicable, based on age and local IRB requirements. Each study site is responsible for having an appropriate consenting procedure in place.

Failure to give informed consent renders the participant ineligible for the study. No research testing/exams or study medication will occur before informed consent has been obtained.

3.3 Re-Consent

If there is a change in any of the study procedures that may affect the participant, the informed consent document must be revised and again approved by the IRB/ERC. Any participants enrolled in the study prior to such changes may be required to sign the amended consent form, dependent on your local IRB/ERC requirements.

3.4 Health Insurance Portability & Accountability Act (HIPAA) Compliance

The HIPAA provides guidelines for investigators pertaining to protection of participant confidentiality. Investigators should review information provided in *Impact of the HIPAA Privacy Rule on NIH Processes Involving the Review, Funding, and Progress Monitoring of Grants, Cooperative Agreements, and Research Contracts* (http://grants.nih.gov/grants/guide/notice-files/NOT-OD-03-025.html) and contact their appropriate institutional officials to learn how the Privacy Rule applies to them, their organization, and their specific research project. Another helpful source is *Protecting Personal Health Information in Research: Understanding the HIPAA Privacy Rule*, NIH Publication 03-5388, available online at http://privacyruleandresearch.nih.gov.

At most study sites, a HIPAA form is presented to a potential participant for signature, in addition to the Informed Consent Form, unless the necessary assurances are incorporated into the Informed Consent Form. The HIPAA form describes participant and data confidentiality associated with the study.

3.5 Non-English-Speaking Participants

Many IRBs/ERCs mandate whether a translated consent document is needed to obtain consent from non-English speaking participants or whether a translator can be used to obtain consent. Each study site must conform to their local requirements. With respect to completing Case Report Forms (CRFs), each study site should attempt to do their best to avoid errors as a result of translation.

3.5.1 Other Issues Related to Translators

A Human Protection certificate is not needed for the translator because the translator is only translating

what the health care professional is stating; they do not provide participant care or collect data.

Translation of any instructions is the responsibility of the study site and should be handled in the same manner as for non-research participants.

All expenses and budget issues related to using translators fall to the study site and should be discussed with the PI prior to any expenses being incurred.

3.6 Documentation of Informed Consent Process

3.6.1 Documentation

Site personnel must document in the participant's medical record that the participant has signed the informed consent, met enrollment criteria, and was enrolled into the LOGIC Protocol study. Other pertinent details of the consent process, including summaries of telephone conversations with participants, must also be carefully documented in the medical record.

The signed informed consent document should be maintained in the following locations:

- The original form is placed in the participant's research file.
- A copy is to be placed in the participant's medical chart.
- Participant or legal guardian will receive a copy.

Master Files of signed consents at the sites are not condoned. All of the participant's study-related documents are to be maintained in the participant's research file.

Per some local IRBs, phone consent has been approved to consent participants with the updated informed consent documents for obtaining saliva for the purposes of DNA extraction. The telephone consents (verbal consents), like the other site consents, should be sent to the DCC for review prior to submission to your IRB. The approved phone consent document should be maintained in the regulatory binder with the other study specific consent documents.

4. Training

Site staff will receive study training prior to implementation of the study. Training will include, but not be limited to, review of:

- Main protocol
- Informed consent process
- MOO
- Data collection electronic Case Report Forms (eCRFs)
- Schedule of events
- Study-specific procedures
- Collecting, processing, labeling, shipping, and tracking of biosamples
- Use of *ChiLDReNLink*
- Site initiations and monitoring plan

Please notify the DCC (ChiLDReN-Monitors@arborresearch.org) of new study team personnel so they can receive the appropriate training and web access.

4.1 New Study Site Personnel

When a study site has new personnel who will be working on the ChiLDReN study, please contact the DCC as soon as possible at ChiLDReN-monitors@arborresearch.org. See **Appendix L** for completion of the ChiLDReN Onboarding Form.

New study site personnel need to sign the site signature log and have their delegated study responsibilities listed as well.

Please provide us with the individual's name, mailing address, telephone number, fax number, and email address so that we may update the study directory.

The DCC will develop a MOO to assist Clinical Research Coordinators (CRCs) at each center in following the protocol, entering and transferring data, and collecting, processing, and shipping samples. The Clinical Monitors are available to answer questions regarding study protocol, the completion of source documents, the use of the web-based data entry system, and proper procedures for collecting, processing, and shipping samples that the study sites may have. Test runs of data entry into the web-based data entry system, as well as sample shipment will be organized prior to study site initiation. The Clinical Monitors will review the study protocol and data entry system, and check all governing body approvals prior to study site initiation. A meeting, or meetings, for all investigators and CRCs will be held in conjunction with the initiation of the study. Meetings for all CRCs will usually be held monthly via conference calls or face-to-face to review frequently encountered questions regarding the protocol, data entry, or sample processing.

5. Study Monitoring

Monitoring is the act of overseeing the progress of a clinical trial, and of ensuring that it is conducted, recorded, and reported in accorandance with the protocol, Standard Operating Procedures (SOPs), GCP, and the applicable regulatory requirement(s). Monitoring will include a combination of annual site visits and remote monitoring. Monitoring helps to catch problems and noncompliance before the actions become repetitive. It can identify systemic issues which can be corrected before a study is jeopardized.

Remote monitoring will occur at the DCC, and site-specific information in the form of reports reflecting data completion, integrity, and quality will be produced. These reports will be generated at least monthly and will be shared with the sites and NIDDK.

The DCC will produce reports showing:

- Overall data completion
- Data entry timeliness
- Form completeness
- Database queries comprised of logic checks
- Outstanding queries
- Biosample shipping
- Biosample collection
- Enrollment with consent status (including entire history of consent)
- Protocol deviations

- Visit completion
- Number (%) of queries resolved
- Number (%) of queries per study participant
- Regulatory review

The DCC will also request a sample of de-identified source documents from the site to check for transcription errors in the database. The DCC staff may conduct site management calls, if needed, to ensure data quality compliance and data query resolution.

The DCC will schedule a site visit with each site PI and study research staff on an annual basis. During the site monitoring visit, the site's performance on the metrics described above will be discussed. The coordinator(s) and PI must be available for the visit to be successful. The agenda for the visit will include such topics as:

- Essential elements of protocol adherence
- Regulatory document requirements
- Completeness or missingness of visits, forms, data, and samples (through DSMB reports)
- Responses to data queries
- eCRFs and source documents
- Site processes
    - Team Communication Plan
    - Site Training Plan
    - Recruitment Plan
    - Retention Plan

Other issues may be identified:

- Best practices
- Areas for improvement
- Strategies for improvement
- Barriers to success at site
- Regular attendance at study coordinator calls
- Additional monitoring activities, including more frequent on-site monitoring, may be scheduled at the request of NIDDK, the DCC, or the site PI.

As much as possible, data quality will be the responsibility of the study staff person entering the data. Data quality begins with the design of the CRFs and procedures and incorporates reasonable checks to minimize transcription and omission errors. Of the more important quality assurance measures are the internal validity checks for reasonableness and consistency. In addition to those described above,

these checks may be built into the initial tables and cross tabulations that should reveal any remaining data quality issues.

5.1 Monitoring of Source Documentation & CRFs

A number of procedures are established to ensure that the study data are of the highest quality possible. These include real-time data entry queries and data monitoring by the DCC. The DCC will also implement checks to identify forms that are due but have not been received.

The DCC will be capable of automatically generating compliance reports. These reports are posted monthly on the study consortia webpage. The study staff members are responsible for reviewing the compliance reports to monitor their site's compliance.

5.2 Goals of Monitoring

Proper monitoring helps to ensure adequate protection of the rights of human participants, the safety of participants involved in a clinical investigation, and the quality and integrity of the data submitted.

The ongoing monitoring will be conducted with the intent to:

- Verify that participant consent for study participation has been properly obtained and documented, ensuring compliance with standards for protection of human participants.
- Verify that research participants entered into the study meet inclusion and exclusion criteria.
- Verify that the study is conducted in compliance with the protocol.
- Verify the accuracy of the data collected.
- Verify that all essential documentation required by GCP guidelines is present, current, and appropriately filed.

5.3 Monitoring Visits

The Clinical Monitors will send the site a monitor visit confirmation letter detailing what will be reviewed during the monitoring visit at least 8 weeks prior to the proposed visit. Study sites will need to compile all supporting source documents (medical records, research shadow records, etc.) for those participants to be reviewed. If documentation is kept electronically (such as labs), all attempts should be made to have a paper version at the time of the study site visit or provide the Clinical Monitor with access to the electronic records. Study sites should also ensure that regulatory binders/folders are up-to-date and available.

5.4 Frequency and Content of Monitoring Visits

Routine monitoring will be scheduled at appropriate intervals. Additional visits can be scheduled at the request of NIDDK or the DCC. For each visit, the Clinical Monitor will sign the monitoring log provided in the regulatory binder/file. For multi-day visits at a study site, the Clinical Monitor will sign the log for each day spent at the site.

For observational studies, there will be review of 25% or 20 participants, whichever is greater, monitoring of informed consent documents, and inclusion/exclusion criteria.

For the most current version of the ChiLDReN Studies Monitoring Plan see **Appendix M**.

6. LOGIC Study Protocol

Refer to **Appendix A** for the latest version of the LOGIC Study Protocol (Amendment 6, March 14,

2017).

7. Screening and Recruitment

7.1 Population

The study population to be enrolled will consist of male and female patients from birth through 25 years of age. All racial and ethnic groups will be included.

7.2 Screening/Recruitment Plan

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

1. Current and future ChiLDReN Clinical Site patients. All patients currently followed at the ChiLDReN clinical sites (plus those with a1-AT deficiency followed at the Saint Louis University enrollment site), and those 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 (see section 7.4 for descriptions of the study groups). It is estimated that there are approximately 1675 patients followed at the ChiLDReN Clinical Sites with these four disorders. For Group 5, siblings (who are PIZZ or PISZ) of known a1-AT deficiency participants who are enrolled in this study will be offered enrollment if they have no evidence of liver disease. Affected siblings with a1-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), or 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. Printed information about the study and the consent form will be given to the family. The IRB/ERC 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 and case report forms will be completed. For potential participants 18 years of age and older, consent will be obtained from the participant. Participants who gain the age of majority (18 or emancipated minor) must provide written consent in order to continue in the study. Assent of minors will be obtained according to institutional requirements at each study site.

2. Advertising Strategy for Voluntary Enrollments. To increase the sample size of the longitudinal study, several steps will be taken to add to the recruitment of study participants. LOGIC will be listed and described on the ChiLDReN website. It will include a list of all participating clinical sites, along with the contact information of the study coordinators and the ChiLDReN Administrative Core. LOGIC and its objectives will be advertised on the Pediatric GI Bulletin Board, a listserve managed by the North American Society for Pediatric Gastroenterology, Hepatology and Nutrition and the society's website (www.naspghan.org). Over 1,000 pediatric gastroenterologists around the world participate on this listserve, including over 700 in North America. A periodic IRB/ERC-approved announcement of the objectives of LOGIC will be made on the Pediatric GI bulletin board, with a request for referral of patients to the ChiLDReN clinical sites. In addition, LOGIC will post IRB/ERC-approved notices on the websites of the patient support/advocacy organizations involved in ChiLDReN (and related to this study) to announce new studies and provide contact information for interested families or

patients. Notices will request that families and patients contact the ChiLDReN Administrative Core Office in Denver or a CRC to obtain further information. If the family or patient is interested in participation, the PI or CRC will, upon request, contact the patient's current physician to explain the nature of the study.

In addition, IRB/ERC-approved notice of this study will be included in newsletters and educational material distributed by the patient advocacy/support groups to its members and to people requesting information about these disorders. Finally, the objectives of LOGIC and the study design will be posted at the annual scientific meetings of the North American Society for Pediatric Gastroenterology, Hepatology and Nutrition and the American Association for the Study of Liver Disease, in order to provide physicians across the United States with appropriate information concerning this study and the potential for recruiting additional patients. ChiLDReN will be registered on the ClinicalTrials.gov website. Interested families/patients will be given their choice of participation in any of the ChiLDReN clinical sites; however, participation at the clinical site most convenient for the family will be encouraged. It is anticipated that enrollments following these recruitment strategies will increase the number of patients referred to this study.

7.3 Eligibility/Exclusion Criteria

7.3.1 Inclusion Criteria

All current and newly diagnosed patients (based on diagnostic and enrollment criteria) listed as a1-AT, ALGS, PFIC (or BRIC), and BAD, both before and after liver transplant, followed at or referred to each LOGIC Clinical Site will be offered enrollment into this study. Siblings of participants with a1-AT 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. After informed consent is obtained, participants will be enrolled into this study through five study groups defined in Section 7.4. The inclusion criteria for the study are:

- Children and young adults diagnosed with one of the four cholestatic diseases from birth through 25 years old of age (only if enrolling into Groups 1-4)
- Siblings of participants with a1-AT who are affected with a1-AT deficiency but with no evidence of liver disease (only if enrolling into Group 5)
- Both sexes, all races and ethnic groups (all groups)
- Participant meets the enrollment criteria for one of the four cholestatic liver diseases outlined in Section 7.4

7.3.2 Exclusion Criteria

- Inability to comply with the longitudinal follow-up described below, or
- Failure of a family/patient to sign the informed consent/assent document or the HIPAA form

7.3.3 Rationale for the Inclusion/Exclusion Criteria

These criteria define the age group to be studied and assure that no patients/participants will be excluded because of race, sex, or ethnicity. These criteria also assure that only patients with the four defined cholestatic diseases will be enrolled, explicitly excluding patients with other cholestatic disorders (e.g., biliary atresia, cystic fibrosis, metabolic liver diseases, primary sclerosing cholangitis, infectious diseases including ascending cholangitis, immune deficiency including HIV/AIDS) from entering the study. Siblings or parents who are 25 years of age or less and are affected by these diseases will be offered enrollment, as well as affected siblings without liver disease who have a1-AT

deficiency. The data from these siblings will be instrumental in determining if there is a genetic predisposition to the development of liver disease that tracks in certain families. Lastly, these criteria will identify participants/families that are felt to be compliant and capable of completing the study.

7.3.4 Exceptions to the Inclusion/Exclusion Criteria

Infants and children highly suspected of having one of the four LOGIC diseases by the local 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 via protocol exemption. The PI will request permission for a protocol exception by completing the online Protocol Exemption CRF. The site study coordinator will notify the Clinical Monitor via email: ChiLDReN-Monitors@arborresearch.org and provide as much information as possible. Once the Clinical Monitor has sufficient information, an email will be sent to the Exemption Committee for a vote. Additional emails may be required to gather information or answer questions from the 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 participants at their own centers.

Whenever the answer to an inclusion criterion is “no” or to an exclusion criterion is “yes” (even if the “eligible diagnosis” for the exclusion is fulfilled), an exemption will be required.

The exemption request should be sent to the ChiLDReN DCC at the time of consent by the requesting site. Upon completion of the review by the Exemption Committee, the DCC will notify the site of the vote. A representative from the NIH will be copied on these emails. Instructions for completion of the Exemption Form 15 are found in **Appendix N (eCRF Completion and Definition).**

If the participant is allowed to enroll in the study through an exemption, answer the exemption question appropriately in Section G of the eligibility section of ChiLDReNLink. Following the completion of the exemption form, the study coordinator must also complete a Protocol Deviation Form 40. Once assigned, the research participant ID cannot be reused.

7.4 Enrollment Criteria for Specific LOGIC Diseases

7.4.1 Criteria for A1AT, ALGS, PFIC (or BRIC), and BAD

7.4.1.1 Alpha-1 antitrypsin deficiency

1. Participants

Participants must meet both criteria A and B.

A. A1-AT deficiency will be defined as:

For participants enrolled prior to liver transplantation:

- low serum alpha-1 antitrypsin concentrations (< lower limit of normal for laboratory)

  and

- the PIZZ or the PISZ phenotype or genotype, for participants prior to liver transplantation.

For participants enrolled after liver transplantation (Group 3):

- a history of the above criteria, or, alternatively, either low serum alpha-1 antitrypsin level or PIZZ or PISZ phenotype or genotype before transplantation

plus

- clear histologic evidence of alpha-1 antitrypsin deficiency liver disease on the explanted liver (PAS-positive diastase resistant smooth globules in hepatocytes).

and

B. Liver disease associated with serum a1-AT deficiency.

Liver disease will be considered present if there is:

- either evidence of neonatal cholestasis (conjugated hyperbilirubinemia and jaundice within the first three months of life),
- chronically elevated (for > 6 months) AST or ALT above 1.25 times the upper limit of normal,
- chronic hepatomegaly,
- clinically measured liver span at mid-clavicular line above the 95th percentile for age present for at least 3 months,
- clinical findings or complications of portal hypertension (consult PI) or cirrhosis,
- impaired liver synthetic function (consult PI), or evidence of inflammation, cholestasis, paucity of interlobular bile ducts, hepatic fibrosis or cirrhosis on liver biopsy,
- or having undergone liver transplantation for alpha-1 antitrypsin deficiency.

2. Siblings and parents (25 years old or less) of affected participants with Alpha-1 antitrypsin deficiency

Siblings of affected participants from birth through 25 years old who are 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 inclusion and exclusion criteria for these groups. If they have no evidence of liver disease they will be enrolled in Group 5. The purpose of including these siblings in the study is to determine if the natural history of the liver disease is consistent in a given family with multiple affected children.

PLEASE NOTE: Parents are eligible for Group 2 or 3 enrollment only. Asymptomatic parents are not eligible to participate in Group 5.

7.4.1.2 Alagille Syndrome

1. Participants

Probands (participants) must meet (1) the enrollment criteria for ALGS (Table 1) and (2) have evidence of liver disease (one of three from clinical, biochemical, or histological). The enrollment criteria for ALGS to be utilized are outlined in Table 1. These criteria include seven 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 JAGGED-1 or NOTCH2 mutation, and the following clinical criteria (symptoms or signs, a history, or presence of):

- Cardiac: Heart murmur (with further clinical studies to clarify), pulmonary valvular stenosis 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):
  - Fasting total serum bile acid > 3x upper limit of normal (ULN) for age, or
  - Direct bilirubin > 2 mg/dl, or
  - Fat soluble vitamin deficiency otherwise unexplainable, or
  - γGTP > 3x ULN for age, or
  - Intractable pruritus explainable only by liver disease, assessed at PI discretion

- Renal: functional defects (e.g. renal tubular acidosis), renal insufficiency, renal vascular hypertension, vesicoureteral reflux and/or structural defects (e.g. agenesis, small kidneys, renal cysts, renal artery stenosis, dysplastic kidneys)

2. Siblings or parents of affected participants

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 enrollment criteria for ALGS described in Table 1. If they have evidence of liver disease they will be enrolled into Group 1, 2, or 3, as appropriate.

Table 1. Enrollment Criteria for ALGS

ALGS Family History[a]	Paucity	*JAGGED-1*[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" faces of childhood or adulthood.

[a]Family history = ALGS present in a first degree relative.

[b]Identified = *JAGGED-1* mutation may have been identified in clinical or research laboratory. (Please note that research results ARE acceptable if they were conducted prior to enrollment [not via this study], provided to the participant, and a copy of the results is in the medical record).

[c]Not identified = Not identified on mutation screening, or not screened for.

[d]*JAGGED-1* mutation = mutation, whole gene deletion or deletion of chromosome 20p which includes *JAGGED-1* locus.

7.4.1.3 PFIC (or BRIC)

1. Participants

Inclusion criteria for enrollment of participants with PFIC (or BRIC) are described for "definite cases" and for "presumed cases". However, "definite" and "presumed" cases of PFIC (or BRIC) will be treated similarly in this study. For participants who have had partial biliary diversion or ileal exclusion, the criteria apply to the participant either before or after the procedure.

Definite PFIC (or BRIC):

Documentation of two mutant alleles in *ATP8B1, ABCB11,* or *ABCB4*, *TJP2* or other genes that may be described in the future that will be shown to be confirmed causes of PFIC (or BRIC).

or

Presumed PFIC (or BRIC):

Participants must meet both criteria 2 and criteria 3:

2. Evidence of chronic liver disease (one or more of the following):

- Duration of biochemical (laboratory tests) or clinical abnormalities of > 6 months,
- Clinical/pathologic stigmata of chronic liver disease, *or*
- Liver disease in a sibling of a known individual affected by PFIC (or BRIC) (predicted to be chronic)
- Recurrent and episodic cholestatic disease occurring on more than two occasions with episodes separated by at least 3 months and without other known cause

3. Evidence of cholestasis (a history or presence of one or more of the following):

- Fasting total serum bile acid > 3x ULN for age, or
- Direct bilirubin > 2 mg/dl, or
- Fat soluble vitamin deficiency otherwise unexplainable, or
- GTP > 3x ULN for age, or
- Intractable pruritus explainable only by liver disease, assessed by PI discretion

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

a) 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, BAD, A1AT, ALGS, mitochondrial defect, large duct primary sclerosing cholangitis (PSC), or PSC in the setting of inflammatory bowel disease or immunodeficiency. It should be noted that this does not exclude patients from being enrolled into LOGIC in one of the other three disease categories.

b) Short bowel syndrome/TPN related liver disease.

c) Chronic known infectious hepatitis (e.g. Hepatitis C, Hepatitis B, etc.)

d) Chronic known or strongly suspected drug toxicity (e.g. Augmentin-related cholestasis)

e) Acquired immunodeficiency syndrome

f) Acute liver failure

g) 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.

7.4.1.4 Bile Acid Synthesis Defects

Enrollment criteria for bile acid synthesis defects will be one or both of the following:

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

   or

2. Identification of 2 genetic mutations in one of the enzymes in the bile acid synthesis pathway

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.

7.5 Study Groups

Participants who meet the enrollment criteria for the LOGIC study and the enrollment criteria described above for a specific disease being studied will be enrolled into one of five study groups described in detail in the sections below:

7.5.1 Group 1: Infants < 6 Months of Age at Diagnosis and Enrolled in the PROBE Study

Group 1 applies to infants who were initially enrolled at <6 months of age into the PROBE study. For these PROBE participants, once the definitive diagnosis of a LOGIC disease is established, these participants will be offered enrollment into this LOGIC study, consented for 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. 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 CRC at the same time. For periodic reporting and for analyses, PROBE data will be downloaded from the PROBE database to the LOGIC database, and subsequent data collected for LOGIC will be shared with PROBE, and downloaded from the LOGIC database to the PROBE database. Any additional LOGIC data that are not collected on the PROBE CRFs will be collected on LOGIC CRFs. Consent forms and HIPAA forms for both studies will include a statement describing data sharing with the other study.

NOTE: If a potential participant is eligible for both the PROBE and LOGIC studies and a LOGIC disease is diagnosed before enrollment into PROBE, the participant should be enrolled in the LOGIC study.

Once transplanted, participant follow-up will be per the LOGIC study and the LOGIC CRFs for post-transplant Group 2 LOGIC.

7.5.2 Group 2: Participants from Birth Through 25 Years old at Enrollment and not Previously Enrolled in PROBE Study

Group 2 will apply to currently established patients at one of the ChiLDReN clinical sites who have one of the LOGIC diseases and are 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 the LOGIC study, consented, and followed in Group 2. Parents aged 25 and under who have children enrolled in this study may themselves be offered enrollment if they meet entry criteria for Group 2.

7.5.3 Group 3: Post-liver Transplant Participants

Group 3 will be for participants with LOGIC diseases, birth through 25 years of age, who have undergone liver transplantation prior to enrollment and are either followed at or referred to the LOGIC clinical sites. These patients will have a limited data set collected at a one-time visit, 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 for DNA for genetic studies. Sites have a time frame of 1 year from the time of consent to collect data and DNA for genetic studies.

Parents aged 25 years and under who have children enrolled in this study may themselves be offered enrollment if they meet entry criteria for Group 3.

7.5.4 Group 4: Screening Enrollment

Group 4 is a screening group of participants, birth through 25 years of age, suspected of having ALGS, PFIC (or BRIC), or BAD, who do not meet complete enrollment criteria for Group 1, 2, or 3. ChiLDReN Core Laboratories will perform bile acid analysis to detect BAD, or genotyping for ALGS or PFIC (or BRIC) on participants that the investigator believes may have one of these diseases but needs to confirm the diagnosis in order to make the participant eligible for enrollment in Group 1, 2, or 3. Consent will be obtained, a set of brief enrollment CRFs will be completed, and specimens will be collected from the participant for urine bile acid analysis or blood for genotyping, as described above. No specimens will be sent to the repository for Group 4participants.

7.5.5 Group 5*:* Affected Siblings (Without Evidence of Liver Disease) of a1-AT Participants who are Enrolled in LOGIC.

Group 5 is for enrollment of siblings of participants with a1-AT deficiency, birth through 25 years of age, 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 a1-AT deficiency is concordant in families, supporting a genetic modifier or environmental factor. If consented, affected siblings with evidence of liver disease should be enrolled in either Group 1, 2, or 3.

NOTE: It is possible for siblings within a single family to be enrolled in different groups or within the same group.

8. Study Visit Details

8.1 Study Visits Overview

This is an observational, longitudinal study that will involve collection of clinical information, family history, physical findings, laboratory test results, clinically indicated radiologic and imaging evaluations, and clinically indicated treatments and their outcomes. In addition to these "standard of care procedures," several special research procedures will be performed, including quality of life questionnaires, cognitive testing measures, neurodevelopmental evaluations, audiology testing, and collection of serum, plasma, one-time urine collection, tissue and blood /or saliva for DNA extraction, and storage for the NIDDK repository. Serum, plasma, blood/or saliva for DNA will also be collected from both biologic parents (when available) and from enrolled affected siblings of participants with a1-AT.

Participants with each of the four cholestatic liver diseases will be enrolled at each LOGIC clinical site, including those who are currently followed at the clinical sites and new patients referred to the LOGIC clinical sites. Participants and siblings and parents of participants with a1-AT deficiency may also be enrolled at the a1-AT enrollment site at Saint Louis University. There are five enrollment groups in this study. Table 2 provides an overview of study visits. The information to be collected at each specific study visit for each group is outlined in Tables 2, 3, 4, 5 and 6.

Table 2: Summary Schedule of Study Visits

Group	Baseline	Annual Follow Up to 20 years (Pre- and Post-Transplant)	At Time of Transplant or Biopsy or Biliary Diversion Surgery*
1	X	X[a]	X
2	X	X	X
3	X*		
4	X*		
5	X	X[b]	X

*Surgical Procedures where tissue is excised for clinical purposes. Excised tissue that is not needed for clinical purposes can be utilized for the LOGIC study.
a. This study visit coincides with PROBE annual visits starting at 12 months of age**.** The PROBE 18-month visit is not included in this study.
b. Does not apply to Group 5 as these patients will not be followed post-transplant, even if they develop liver disease during the course of the study.

Participants in Groups 1 and 2 will be followed for up to 20 years, with annual visits scheduled through year 20, including after liver transplantation.

Participants enrolled in Group 3 will only be seen one time (baseline, with 12 months to complete all study data and specimen collection).

Participants in the Screening Group 4 will be seen for an abbreviated baseline visit for collection of blood for genetic testing. If found to have a LOGIC disease diagnosis and eligibility criteria are met for the main study, the participant should be approached for enrollment into the main study.

Participants in Group 5 will be followed for up to 20 years or until the time of liver transplantation.

8.2 Types of Visits by Group

8.2.1 *Group 1 Participants:* Infants or Children who were Originally Enrolled in the PROBE Study at < 6 Months of age

- **Recruitment/Baseline:** 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 CRC 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 the participant in the PROBE database will be transferred from the PROBE database to the LOGIC database.

- **Follow-up:** The participant will be followed at yearly intervals for up to 20 years including after transplantation or until death. Annual follow-up will begin at 12 months of age and be scheduled within six months of the participant's birthday as per PROBE study protocol. Participants that undergo a liver transplant while in the study will continue to be seen at annual follow-up visits per the LOGIC protocol (under Group 2 post-transplant follow-up). Data will be collected but no further serum or plasma samples will be obtained. **PLEASE NOTE:** If the PROBE and LOGIC visits do not coincide for a given participant, please continue to follow the PROBE visit schedule.

    - **Note:** in the ChiLDReNLink database, the PROBE and LOGIC visits should be scheduled on the same day for the data to be merged in each study. Bio-sample collection will occur in the PROBE study. In the LOGIC study the date of the visit needs to be entered (as the same visit date in the PROBE study) and the collection status for the biosamples should be selected as "not collected" and reason "other",enter the PROBE Study.

- **Transplantation, biopsy, or biliary diversion surgery procedure:** Pertinent data about interval history and liver function, as well as indication and type of liver transplantation or biopsy or biliary diversion surgery procedure, will be collected. Explant liver tissue, naïve liver tissue, gallbladder, lymph node, or bile will be obtained and stored, if available. **PLEASE NOTE:** When these specimens are collected, you will use the labels that are provided via the PROBE study since all specimens collected on Group 1 participants are tracked through PROBE.

- **Post Transplantation:** The participant will be exited from the PROBE study (per Amendment 8) and follow-up will continue in the LOGIC study per Group 2. The participant will be followed yearly post-transplant in a 6-month window around the date of transplant. Data will be collected but no further serum, plasma, or urine samples will be obtained, except to complete the DNA trios.

- **Death:** At time of death, the LOGIC Final Status eCRF should be completed.

Table 3: Group 1 Schedule of Events

Study Visit Procedures	Baseline Visit	Annual Visits to 20 years[a]	At Liver Transplant or Biopsy or Biliary Diversion Surgery*
Recommended windows for visits (different from other Groups to coincide with PROBE Study Design)		**± 6 mo**	
Informed Consent	X		
Eligibility	X		
Intake history/exam	X		
Diagnosis	X		
Surgical procedure (if performed)	X		X
Parents' Medical History	X		
Follow-up visits [b]		X	X
Audiology Testing (ALGS & PFIC (or BRIC only)[c]	X		
Bile Acid Biochemistry (urine)	X[d]		
Visit Procedures to be Performed as Part Of PROBE Study. The Data Will be Shared With LOGIC			
HRQOL with Cognitive Function Scale (CFS)[e]		X	
Developmental Assessments [f]		X	
Liver biopsy or operative samples (if available)	X		
Serum, Plasma, [g]	X	X	
Blood /or saliva for DNA		X[h]	
Blood from parents[i]	X		

Visit Procedures to be Performed on all Participants who Undergo Transplant During the Course of the Study

Study Visit Procedures	Baseline Visit	Annual Visits up to 20 years
Follow-up visits [j]		X
Functional status		X
HRQOL with Cognitive Function Scale (CFS)[k]		X

*Surgical procedures where tissue is excised for clinical purposes. Excised tissue that is not needed for

clinical purposes can be utilized for the LOGIC study.

a. This study coincides with PROBE annual visits starting at 12 months of age. The PROBE 18 month visit is not included in this ChiLDReN study.

b. Follow-up visits will include interval history, physical examination, and clinically indicated lab and imaging tests.

c. Performed once at 3 years of age or older, at baseline or follow-up visit, in ALGS AND PFIC (or BRIC) PARTICIPANTS ONLY. Participants with a documented hearing loss will not undergo Audiology testing.

d. Participant must refrain from taking Urso for 5-7 days prior to collection. Performed annually beginning at 2 years of age.

e. Performed annually beginning at 2 years of age.

f. Performed once between ages 6-16 per the PROBE protocol, the WISC is done at one time at any age 6, 8, 10, 12, 14, or 16 years

g. Serum and plasma samples will not be collected after transplantation.

h. Only if not collected at a previous PROBE visit. When not obtained at the time of transplantation, blood may be collected during a blood draw at a subsequent clinical visit. Prior to 12 months of age, obtain at least 1ml (up to 4.0) of whole blood for DNA while remaining within weight restrictions. If whole blood collection is not possible or contraindicated, saliva will be obtained for DNA extraction. 2ml saliva will be collected in a saliva collection kit. Saliva is not collected on participants <12 months of age.

i. Performed one time, at baseline or at follow-up visit

j. Follow-up visits will include interval history, physical examination.

k. Performed annually.

8.2.2 *Group 2 Participants:* Birth Through 25 Years of age at Enrollment and not Enrolled in PROBE

- **Recruitment/Baseline:** 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. If the child is over 7 years of age (or the age required by your local IRB/ERC), he/she will also be asked to sign the Assent Form. Participants $\geq$ 18 years will be asked to sign Informed Consent form. Once informed consent is obtained, the CRC 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 (date of consent) in this study.

- **Follow-up:** The participant will be followed yearly, from the date of consent for up to 20 years (including after liver transplantation) or until death.

- **Transplantation, biopsy, or biliary diversion surgery procedure:** Pertinent data about interval history and liver function, as well as indication and type of liver transplantation or abdominal surgery/procedure, will be collected. Explant liver tissue, gallbladder, small intestine or colon, lymph node, or bile will be obtained and stored, if available.

- **Post Transplantation**: The participant will be followed yearly post-transplant in a 6-month window around the date of transplant. Data will be collected but no further serum or plasma samples will be obtained. DNA sample will still be collected if not completed prior to transplant.

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

Table 4: Group 2 Schedule of Events

Study Visit Procedures	Baseline Visit	Annual Follow-Up to 20 years	At Liver Transplant, Biopsy or Biliary Diversion Surgery Procedure*
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
Developmental Assessment[b]	X		
HRQOL with Cognitive Function Scale (CFS)[c]	X	X	X
Audiology Testing (ALGS & PFIC (or BRIC only)[d]	X		
Liver biopsy or Operative samples	X[e]		X[e]
Serum, Plasma[f]	X	X	X
Blood /or Saliva for DNA	X[g]		X
Parents Medical History	X		
Blood and/or Saliva from parents	X[j]		
Bile Acid Biochemistry (urine)	X[k]		

Visit Procedures to be Performed on all Participants who Undergo Transplant During the Course of the Study

Study Visit Procedures	Baseline Visit	Annual Follow-Up to– 20 years
Follow-up visits [h,i]		X

Functional status	X
HRQOL with Cognitive Function Scale (CFS)[i]	X

*****Surgical procedure where tissue is excised for clinical purposes. Excised tissue that is not needed for clinical purposes can be utilized for the LOGIC study.

a. Follow-up visits will include interval history, physical examination and clinically indicated lab and imaging tests.
b. Performed once at 2 years of age or older, at baseline or at follow-up visit.
c. Performed annually starting at 2 years of age and at time of surgery or transplantation. Annual testing will also include the Cognitive Function Scale Questionnaire.
d. Performed one time at 3 years of age or older, ONLY IN ALGS AND PFIC (or BRIC). PARTICIPANTS, at baseline or follow-up visit. Participants with documented hearing loss will not undergo audiology testing. If testing is not done pre-transplant, testing can be done at a subsequent visit post-transplant.
e. If obtained for clinical reasons and extra tissue is available.
f. Serum and plasma samples will not be collected after transplantation.
g. Performed one time at 1 year of age or older, at baseline or at follow-up visit.
Prior to 12 months of age, obtain at least 1ml (up to 4.0) of whole blood for DNA while remaining within weight restrictions. If whole blood collection is not possible or contraindicated, saliva will be obtained for DNA extraction. 2 ml of saliva will be collected in a saliva kit.
h. Follow-up visits will include interval history, physical examination.
i. Performed annually.
j. Performed one time, at baseline or at follow-up visit.
k. Performed one time, at baseline or at follow-up visit. Participant must not be taking Urso at the time of collection.

8.2.3 *Group 3 Participants*: Post-Liver Transplant Participants

Recruitment/Baseline: Following diagnosis of one of the four LOGIC diseases in an infant or young adult from birth through 25 years of age who has undergone liver transplantation and is either followed at or referred to the LOGIC clinical sites. 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. If the child is over 7 years old (or the age required by your local IRB/ERC), he/she will also be asked to sign the Assent Form. Participants > 18 years old will be asked to sign informed consent. Once informed consent is obtained, the CRC 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 the baseline visit in this study.

Participants who have undergone liver transplant prior to study enrollment will be seen for a baseline visit only. Baseline visits may extend up to one year from date of consent in order to complete DNA and audiology testing.

Table 5: Group 3 Schedule of Events

Study Visit Procedures	Baseline Visit[a]
Informed Consent	X
Eligibility	X
Intake history	X
Diagnosis	X
HRQOL	X
Audiology Testing (ALGS & PFIC (or BRIC) only) Performed at 3 years of age or older*	X
Blood /or saliva for DNA	X[b]
Parents' Medical History	X

Blood /or saliva from parents for DNA	X

a. May take place at any age.
b. Prior to 12 months of age, obtain at least 1ml (up to 4.0) of whole blood for DNA while remaining within weight restrictions. If whole blood collection is not possible or contraindicated, saliva will be obtained for DNA extraction. 2ml of saliva will be collected in a saliva collection kit. Saliva can not be utilized for participants <12 months of age.
*Participants with documented hearing loss will not be required to undergo Audiology testing.

8.2.4 Group 4 Participants: Screening Enrollment

DO NOT enroll into Group 4 without contacting the Denver Admin Core First:

Joan Hines:
720-777-2598 (office)
303-913-5210 (mobile) ***This number is to be utilized in time-sensitive situations only.***
If above is unreachable, contact Terri Howell (DCC Clinical Monitor): 734-369-9683 (office)

Recruitment/Baseline: A Group 4 consent form will be signed, a brief enrollment data form 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), ALGS, or bile acid analysis for BAD.

After bile acid analysis results are known, and if the participant fulfills eligibility criteria, he/she will be offered full enrollment in Group 2. The bile acid biochemistry testing is being performed in a CLIA approved laboratory, so the results will be provided to the family.

If ChiLDReN Core Lab genotyping reveals disease-causing mutations, the family will be informed that the results may be positive and that they should seek clinical genotyping in a CLIA approved laboratory, at their own expense. Specific genotyping results from ChiLDReN Core Labs (which are not CLIA-approved) will be not shared with the families because this testing is done for research purposes only.

If the clinical genotyping results are positive for PFIC (or BRIC) or ALGS, the participant will be offered enrollment into Group 2. If the test results obtained for the participant are negative, the participant will have completed the study.

PLEASE NOTE: For PFIC (or BRIC) and ALGS, a participant for whom research results from a ChiLDReN Genetics Core Lab are positive, must obtain a positive clinical result from a CLIA-approved lab before that participant will be offered enrollment into another LOGIC study group.

These participants should meet study criteria within two years from date of consent or be removed from the study.

Table 6: Group 4 Schedule of Events

Study Visit **Procedures**	**Baseline Visit** **(any age)**
Informed Consent	X
Eligibility	X
Intake history/exam	X
Suspected Diagnosis	X
Diagnostic Study[a]	X
Blood to Genotyping Core Lab on parents[b]	X

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

8.2.5 *Group 5 Participants:* Affected Siblings (Without Evidence of Liver Disease) of Participants with Alpha-1 Antitrypsin Deficiency who are Enrolled in the Study.

- Recruitment/Baseline: Siblings of participants with a1-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 can be offered enrollment. At least one parent or guardian must sign written informed consent before data collection can begin for participants under 18 years of age. If the child is over 7 years old (or the age required by your local IRB/ERC), he/she 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 CRC will abstract information from the participant's medical chart and meet with the participant/parent(s)/guardian(s) to complete the enrollment eCRFs.

- Follow-up: The participant will be followed yearly for up to 20 years until the time of liver transplantation or until death.

- Transplantation or abdominal surgery/procedure: Pertinent data about interval history and liver function, as well as indication and type of liver transplantation or abdominal surgery/procedure, will be collected. Explant liver tissue, gallbladder, small intestine or colon, lymph node, or bile will be obtained and stored, if available.

- Post-Transplantation: These participants will NOT be followed post-transplant, even if they develop liver disease during the course of the study.

- Death: At time of death, the LOGIC Final Status eCRF should be completed.

Table 7: Group 5 Schedule of Events

Study Visit Procedures	Baseline Visit	Annual Follow-up to 20 years	At Liver Transplant, Biopsy or Biliary Diversion 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
HRQOL	X	X	X
Liver Biopsy or intra-operative samples	X[b]		X[b]
Serum, Plasma,	X	X	X
Blood /or saliva for DNA	X[c]		X[d] (If < 12 mo)
Bile Acid Biochemistry (urine)	x[e]		

*****Surgical Procedure where tissue is excised for clinical purposes. Excised tissue that is not needed for clinical purposes can

be utilized for the LOGIC study.

a. Follow-up visits will include interval history, physical examination, and clinically indicated lab and imaging tests.
b. If obtained for clinical reasons and extra tissue is available
c. Performed one time at 1 year of age or older, at baseline or at follow-up visit
d. Prior to 12 months of age, obtain at least 1ml (up to 4.0) of whole blood for DNA while remaining within weight restrictions. If less than 4.0 ml is drawn prior to 12 months then obtain the full 4.0 ml at the Year 1 follow-up visit. Saliva can not be utilized for participants <12 months of age.
e. Performed one time, at any age, at baseline or at follow-up visit, participant must not be taking Urso at the time of collection.

8.3 Completing eCRFs at Each Visit

- eCRFs MUST be completed within three weeks of the study visit date.
- Peds QL adult instruments CAN be sent home with the parents or adult participants for self-administration, if necessary. These adults should be provided with a self-addressed, stamped envelope for return to the study coordinator. Please inform these adults that you will be calling to remind them to return the forms. All Peds QL documents must be completed within three months of the study visit date.
- Cognitive Function Survey – completed annually in conjunction with the Annual Peds QL.The survey comprises six questions, the survey is automatically populated to the participant's details page in ChiLDReNLink when a visit has been scheduled.
- The Cognitive Function Survey is not completed for those participants in Groups 3 and 5.

NOTE: Neurodevelopmental testing is not performed on LOGIC Post-transplant participants.

NOTE: Audiology testing is done post-transplant if not done while the participant is pre-transplant. Audiology testing is not expected on participants with a documented hearing loss or the presence of bilateral hearing aids.

See **Appendix O** for complete QoL Surveys and Developmental Instruments.

See **Appendix N** for a complete list of the eCRFs to complete for each group at each study visit.

8.4 Schedule of Evaluations

The following tables provide an overview of study visits and eCRFs to be completed at each of the scheduled visits for participants in Groups 1-5.

9. Specimen Collection

Blood, bile, and tissue specimens will be collected for research purposes as outlined below. Specimens from participants enrolled in Groups 1, 2, 3, and 5 will be shipped from the clinical sites to the Repository. Specimens from participants enrolled in Group 4 will be sent directly to a central laboratory (under contract with the NIDDK) for DNA extraction. Once DNA extraction has occurred, the DCC will coordinate the shipment of the sample to the central research core laboratory responsible for testing to determine eligibility. Note: this process (determining diagnosis for disease group eligibility) for Group 4 may take several weeks.

9.1 Schedule for Collection of Blood

Whenever possible, blood samples should be drawn at the same time as blood is taken for clinical testing or when there is IV access for a clinical procedure. Blood samples must be drawn in accordance

with local IRB/ERC regulations with respect to timing and amounts.

Study samples are to be obtained:

- At time of enrollment
- Annually thereafter for up to 20 years, up until the time of liver transplantation
- Abdominal surgery/procedure
- ***Transplantation

PLEASE NOTE: Blood specimens, except DNA, are not collected from participants following transplantation when the transplant occurs during the course of the study. DNA can be collected post-transplant but not until at least two weeks after the transplant procedure.

If the Rutgers Repository determines that a participant's blood sample is not adequate for DNA isolation, they will contact the DCC to request another blood draw. The DCC will contact the site with details.

If whole blood for DNA extraction is not possible or contraindicated, saliva may be collected for purposes of DNA extraction. 2 ml of saliva will be collected in a saliva kit obtained from Rutgers University. Saliva can not be utilized on participants <12 months of age.

9.1.1 Blood Collection for LOGIC Study Groups (1, 2, 3, 4, and 5)

Note: Please refer to the PROBE MOO, as all Group 1 participants in LOGIC should follow the PROBE protocol for blood collection.

Table 8: Summary of Biosample Collection for Participants in Groups 2, 3, 4, and 5

Group Type	Time Point/Sample Types			
	Baseline	**Annual Follow-up**	**S/P**	**TX**
Group 2	Plasma/Serum/Whole Blood or Saliva* Urine**	Plasma/Serum	Plasma/Serum	Plasma/Serum
Group 3	*Whole Blood or Saliva	NA	NA	NA
Group 4	*Whole Blood Urine**	NA	NA	NA
Group 5	Plasma/Serum/Whole Blood or Saliva* Urine**	Plasma/Serum	Plasma/Serum	Plasma/Serum

*Whole blood is drawn only once (EDTA tubes). If not possible to collect at baseline, collect at a future visit, transplant, or hospitalization within one year of consent date.
Prior to 12 months of age, obtain at least 1 ml (up to 4.0) of whole blood for DNA while remaining within weight restrictions. If less than 4.0 ml is drawn prior to 12 months then obtain the full 4.0 ml at the Year 1 follow-up visit. Saliva can not be utilized for participants <12 months of age.
**Urine at Baseline or at a subsequent visit is a one time collection for testing of Bile Acid Synthesis Defects.

For participants >50 kg,10 ml is drawn (1 [10 ml] tube), when participants are <50 kg, 4.0 ml are drawn (1 [4.0 ml] tube). In the case where whole blood is not possible or contraindicated, 2 ml of saliva will be obtained in a saliva kit for DNA extraction. Whole blood collected in SST tubes (2 ml (total of 4 ml can be collected per year)) for processing into serum. Whole blood collected in EDTA tubes (2 ml (total of 4ml can be collected per year)) for processing into plasma.

Table 9: Summary of Blood Collection for Each Biological Parent(s) of Participants in Groups 2, 3, 4, and 5

Baseline	Group 2	Group 3	Group 4	Group 5
Whole Blood	10 ml	10 ml	10 ml	NA
Serum	15 ml	NA	NA	NA
Plasma	15 ml	NA	NA	NA

9.1.2 Processing Blood Collection Tubes

See **Appendix P** for specific instructions on processing blood collection tubes.

9.1.3 Priority List for Blood Samples

Blood samples for tests that are needed for clinical care and for screening of adverse events.

- Complete Blood Count (CBC)
- Liver Function Tests (LFT), Prothrombin Time (PT)/International Normalized Ratio (INR)
- Electrolytes, creatinine, BUN, glucose
- Others (based on clinical care needs)
- Blood/or saliva for DNA extraction and storage for the repository
- Plasma for the repository
- Serum for the repository

Note: When insufficient blood is collected for both ***plasma and serum***, first collect the blood for plasma and collect any remaining blood for serum.

9.2 Collecting Alternate Genetics/DNA for RUCDR

9.2.1 Obtaining Saliva for DNA Extraction and Storage

Whole blood is the preferred source for DNA. When whole blood for DNA extraction is not possible or contraindicated, saliva will be obtained for purposes of DNA extraction and storage. 2 ml of saliva will be collected in saliva kits obtained from Rutgers University. These saliva kits will be distributed to participants in person during a clinic visit or shipped to participant's residence. For complete instructions, including instructions to be sent in the saliva kits to participants, see **Appendix T**.

9.2.2 Shipment

All samples should be shipped at ambient temperature in an insulated container provided by Rutgers, and shipped overnight by FedEx. Label all specimens with the linked labels (provided by the DCC) and be sure not to apply the DCC labels over the Rutgers label (apply DCC labels lengthwise to the cryovial). Complete the Rutgers collection form (provided in the shipping kit) per instructions. An email notification of shipment will be sent to Rutgers and the DCC from ChiLDReNLink once the shipping process has been completed. For detailed shipping instructions, see **Appendix Q**.

9.3 Summary Tissue Specimens to be Collected from the Participants in Groups 2, 3, and 5 (only if Available after Clinical needs)

Table 10: Tissue Specimens collected from the participants in Groups 2 and 5

Visit	Whole Blood	Tissue Specimens*
Group 2		
Baseline		Liver, gallbladder, bile, intestines, lymph node, bile duct - if available AND if collected PROSPECTIVELY*
Liver Transplant/Biopsy or Biliary Diversion Surgery Procedure	X	Liver, gallbladder, bile, intestines, lymph node, bile duct - if available AND if collected PROSPECTIVELY*
Group 5		
Baseline		Liver, gallbladder, bile, intestines, lymph node, bile duct - if available AND if collected
Liver Transplant/Biopsy or Biliary Diversion Surgery Procedure	X	Liver, gallbladder, bile, intestines, lymph node, bile duct- if available AND if collected PROSPECTIVELY*

*Surgical Procedure where tissue is excised for clinical purposes. Excised tissue that is not needed for clinical purposes can be utilized for the LOGIC study.

9.3.1 Collecting Tissue in Cryovials AND as Slides for Precision for Medicine

At the time of a liver biopsy, exploratory surgery, liver transplant, or biliary diversion surgery procedure, any biopsy material that is removed as part of the procedure, but is not needed for diagnostic purposes, will be collected for the repository. Hence, when removed as part of the clinical procedure and based on availability after samples needed for diagnosis, additional specimens may be obtained for the repository as follows:

- Tissue from the liver that will be frozen and stored at -70°C
- Tissue from the gallbladder
- Tissue from small intestine or colon
- Lymph node (mesenteric)
- Bile from gallbladder aspirate or biliary diversion

9.3.2 Percutaneous Biopsy

Tissue (when there is extra tissue): Each Investigator will consult with the pathologist at their facility to determine why there is extra tissue from the percutaneous biopsy that is not needed for the clinical diagnosis and how this extra tissue may be collected for the repository. When there is any tissue at the time of the biopsy that is not necessary for clinical care, snap freeze this remaining tissue as a section (2-5 mm) core in liquid nitrogen. Label the cryovials using the appropriate bar-coded labels (Procedure Cryovials, with the correct procedure type selected in scheduler window) supplied by the DCC and following the instructions in the ChiLDReNLink User's Guide (**Appendix S**) and sent to Precision for Medicine Repository monthly using the shipper provided by Precision for Medicine. Note the time in minutes from harvesting to snap-freezing on the Surgery Form 11.

Slides: From the specimen used for clinical care, the pathologist should cut as few as 5 to a maximum of 20 additional slides (depending on amount of tissue), which should be paraffin embedded and left

unstained. Charged slides should be used. These slides should be labeled using the appropriate bar-coded labels (Procedure Slides, with the correct procedure type selected in the scheduler window) supplied by the DCC and following the instructions in the ChiLDReNLink User's Guide (Appendix S) and sent to Precision for Medicine Repository monthly using the kit provided for slides.

9.3.3 Surgical Wedge Biopsy (e.g., liver biopsy, liver transplant, biliary diversion surgery procedure, or exploratory surgery)

Tissue: The wedge biopsy is to be divided in half, with one half going to pathology. The second half will be further divided into at least two equal portions within 10 minutes after it is removed from the participant and snap-frozen for the repository. Label the cryovials prior to use, using the appropriate bar-coded labels (Procedure Cryovials) supplied by the DCC and following the instructions in the ChiLDReNLink User's Guide (**Appendix S**). Note the time in minutes from harvesting to snap-freezing on the Surgery Form. The tissue cryovials are sent to the repository with the monthly shipment.

Slides: From the specimen used for clinical care, the pathologists should cut 20 additional slides, which should be paraffin embedded and left unstained. Charged slides should be labeled using the appropriate bar-coded labels (Procedure Slides) supplied by the DCC and following the instructions in the ChiLDReNLink User's Guide (**Appendix S**) and sent to the repository with the monthly shipment.

9.3.4 Bile (at the time of cholangiogram, Transplant, Gall Bladder Surgery, Biliary Diversion): Obtained ONLY when the Surgeon is Operating on the Biliary System

When there is bile in the gall bladder or any cystic structure at the time of any abdominal procedure, the surgeon should collect the fluid (up to 1 ml) and snap freeze it in a sterile cryovial. Label the vial prior to use using the appropriate bar-coded labels (Procedure Cryovials) supplied by the DCC and following the instructions in the ChiLDReNLink User's Guide (**Appendix S**).

9.3.5 Small **I**ntestine **T**issue **(jejunum or ileum obtained at time of partial biliary diversion)**

Tissue: Two pieces of intestinal tissue should be obtained for the repository if the surgeon is sending intestinal tissue to pathology. Each specimen should measure 15 mm x 5 mm x 5 mm to fit into the 1.5 ml cryovial. Each piece of tissue should be cut transversely, so that the full thickness of intestine is obtained. Samples should be snap-frozen as soon as possible in the operating suite if possible. Label the cryovials prior to use using the appropriate bar-coded labels (Procedure Cryovials) supplied by the DCC and following the instructions in the ChiLDReNLink User's Guide (**Appendix S)**. The tissue cryovials are sent to the repository with the monthly shipment.

Slides: From the specimen used for clinical care, the pathologists should cut 20 additional slides, which should be paraffin embedded and left unstained. Charged slides should be labeled using the appropriate bar-coded labels (Procedure Slides) supplied by the DCC and following the instructions in the ChiLDReNLink User's Guide (**Appendix S)** and sent to the repository with the monthly shipment.

9.3.6 Colon Tissue (obtained at time of ileal exclusion)

Tissue: Two pieces of colon tissue should be obtained for the repository from the sample sent for clinical care to pathology. Each specimen should measure 15 mm x 5 mm x 5 mm, to fit into the 1.5 ml cryovial. Each piece of tissue should be cut transversely, so that the full thickness of colon is obtained. Samples should be snap-frozen as soon as possible, in the operating suite, if possible. Label the cryovials prior to use, using the appropriate bar-coded labels (Procedure Cryovials) supplied by the DCC and following the instructions in the ChiLDReNLink User's Guide (**Appendix S)**. The tissue cryovials are sent to the repository with the monthly shipment.

Slides: From the specimen used for clinical care, the pathologists should cut 20 additional slides, which should be paraffin embedded and left unstained. Charged slides should be labeled using the appropriate bar-coded labels (Procedure Slides) supplied by the DCC and following the instructions in

the ChiLDReNLink User's Guide (**Appendix S**). The slides are sent to the repository with the monthly shipment. See section 9.3.9 for more details on preparing slides.

9.3.7 Gall Bladder Tissue

Tissue: Two pieces of gall bladder tissue should be obtained for the repository if the gall bladder is excised and sent to pathology. Each specimen should measure 15 mm x 5 mm x 5 mm to fit into the 1.5 ml cryovial. Each piece of tissue should be cut transversely, so that the full thickness of gall bladder is obtained. Samples should be snap-frozen as soon as possible, in the operating suite, if possible. Label the cryovials prior to use, using the appropriate bar-coded labels (Procedure Cryovials) supplied by the DCC and following the instructions in the ChilDReNLink User's Guide (**Appendix S**). The tissue cryovials are sent to the repository with the monthly shipment.

Slides: From the specimen used for clinical care, the pathologists should cut 20 additional slides, which should be paraffin embedded and left unstained. Charged slides should be labeled using the appropriate bar-coded labels (Procedure Slides) supplied by the DCC and following the instructions in the ChiLDReNLink User's Guide (**Appendix S**). The slides are sent to the repository with the monthly shipment. See section 9.3.9 for more details on preparing slides.

9.3.8 Lymph Node Tissue

Two pieces of lymph node tissue should be obtained for the repository if the surgeon excises the tissue for clinical care. Each specimen should measure 15 mm x 5 mm x 5 mm, to fit into the 1.5 ml cryovial. Samples should be snap-frozen as soon as possible, in the operating suite, if possible. Label the cryovials prior to use, using the appropriate bar-coded labels (procedure cryovials) supplied by the DCC and following the instructions in the ChilDReNLink User's Guide (**Appendix S**). The tissue cryovials are sent to the repository with the monthly shipment.

9.3.9 Transplant Liver Tissue and Slides

Collect:

- Tissue from the liver
- Unstained paraffin-embedded slides of the liver

At the time of transplant, the research specimens must be removed from the native liver while it is fresh (not in normal saline or formalin). Specimens should be taken as soon as possible once the hepatectomy is completed. Ideally the tissue should be sectioned within 10 minutes after being removed from the participant. The tissue should be taken from the right lobe, and be at least 1 cm deep to the capsule. One approach would be to bisect the liver or alternatively take a large wedge out of the right lobe. An approximately 2 cm x 2 cm x 2 cm piece of parenchyma should be isolated. The piece should be from as representative a section of parenchyma as possible. From this block of tissue, 5 sections should be taken and placed in 5 cryovials. Try to make the specimens as large as possible but can still fit into the cryovial. Each cryovial should contain one piece approximately 15 mm x 5 mm x 5 mm. Once placed in the cryovials, label the cryovials using the appropriate bar-coded labels (Procedure Cryovials) supplied by the DCC and following the instructions in the ChilDReNLink User's Guide (**Appendix S**). After labeling, the specimens should be placed in a -70°C freezer. Note the time in minutes from harvesting to snap-freezing on the appropriate eCRF.

The second specimen should be removed from the explanted liver adjacent to the first specimen and then placed in formaldehyde for processing by pathology. The second specimen should be approximately 2 cm x 2 cm x 2 cm to be used for cutting slides, including routine clinical histology performed at the clinical site. Twenty (20) extra unstained, paraffin-embedded slides from this specimen should be cut, labeled using the appropriate bar-coded labels (Procedure Slides) supplied by

the DCC, and following the instructions in the ChilDReNLink User's Guide (**Appendix S**). After labeling, the slides can be sent to the repository with the monthly shipment.

NOTE: Transplants may be performed at any time during a 24-hour day making it difficult to have a research member available at the time of the procedure. Please discuss with the transplant team the possibility of snap-freezing the specimen at the time of transplant. Check slides to be sure all institutional identifiers have been removed.

The slides should be stored at the site at room temperature in dry boxes between monthly shipments to the repository.

9.4 Snap-freezing Liver and Other Tissues

9.4.1 General Information

Liquid nitrogen is dangerous and must be handled appropriately. Follow institutional guidelines regarding use of liquid nitrogen. Do not let it make contact with bare skin. Use safety glasses whenever working with liquid nitrogen.

The goal is to freeze the liver sample and other tissues immediately and to keep them frozen at –70°C or below. Also, liquid nitrogen evaporates (boils off) quickly so it is necessary to check that there is sufficient liquid nitrogen in a container before using it to freeze a sample. Small pieces of tissue can thaw in seconds. Allowing the specimen to thaw or warm can degrade it for use in many research studies.

Because timeliness is critical to proper freezing, it is important to run through all the steps in your mind before proceeding with the actual specimen. A "dry run" is often worthwhile. Before beginning, make sure that you have all the necessary supplies and that the cryovials are appropriately labeled.

9.4.2 The Snap-freezing Process

Before starting, label the 1.5 ml cryovial with the repository bar code and remove any institutional identifiers prior to the following procedure. Labels placed post-freezing will not adhere to vials and will result in unusable samples. In order for the label to adhere to the vial, attach the label as early as possible before the vial is to be used (the previous day would be preferable).

Freeze the tissue samples as follows:

- Pour the liquid nitrogen into a large plastic container. A cryovial containing the tissue will be dropped into this container. The liquid nitrogen will boil off rapidly so check that the amount in the container is adequate at the time that you are ready to drop the specimen into the liquid nitrogen.

- Place each specimen promptly into a labeled 1.5 ml cryovial. This should be done in a manner so that if the specimen was to drop or spill, it would not fall onto the floor but could be instantly picked up. For example, working on a tray may be helpful. A pair of forceps may be needed. It is not necessary to wrap the specimen in foil or other material. Just slide the tissue into the vial and cap the cryovial.

- Drop the cryovial directly into the liquid nitrogen. The specimen will freeze within seconds. During this time, it is important that there is liquid nitrogen in the container, i.e., that it has not evaporated.

- Take the liquid nitrogen containing the cryovials to the -70°C freezer. Remove the cryovial with forceps and place it immediately in the freezer. Frozen tissue can be sent to the repository with batch shipments of serum/plasma/urine.

As an alternate strategy, pour 5-10 cc of liquid nitrogen into a 50 cc plastic conical test tube secured in a test tube rack. Drop the specimen into the liquid nitrogen in the test tube. Transfer the frozen specimen into a labeled 1.5 ml cryovial. Quickly cap the cryovial and drop the entire cryovial into a larger container of liquid nitrogen then remove the cryovial and place it into -70°C freezer.

Regardless of how the specimen is snap-frozen, once the cryovial is in liquid nitrogen, it should be transported in liquid nitrogen to the -70°C freezer. The cryovial should then be retrieved from the liquid nitrogen and quickly placed in the -70°C freezer. Do not let the specimen thaw or warm.

9.5 Core Lab Procedures

9.5.1 The Histopathology Core

(All information below relates to PROSPECTIVE sample collection.)

The ChiLDReN Histopathology Core does NOT receive slides of any kind. All slides go to Precision for Medicine Repository. This Core does collect Electron Microscope (EM) pathology images when they are available. EM materials, if available, should be prepared by the pathologist as follows:

- Surgical biopsy samples should be processed as promptly as possible to ensure optimal fixation. To accomplish this, prepare the sample for EM by mincing a small 3x2 mm piece of liver tissue from the undisturbed interior of a complete thin slice of the wedge that excludes the surgical margin and the capsule. (Explant specimens are not routinely processed for electron microscopy because tissue preservation is typically suboptimal due to delayed fixation and surgically induced artifacts. If exceptions are made, sample preparation should be similar to that for surgical liver biopsy).

- Digital files of EM images should be de-identified and copied to a CD by the pathologist. The CD will be placed in an envelope or plastic case and provided to the coordinator. The coordinator will attach a label from manifest Form 82.

- Complete CRF 16 EM/LM and send a copy of this form to the Histopathology Core in the box with the EM materials.

- Extra EM grids are placed in a Beam capsule that carries a Histopathology Core slide label attached by the coordinator.

- Ship EM materials to the ChiLDReN Histopathology Core as they are available. You are not expected to batch-ship these items.

Send the EM materials to: Kevin Bove, MD

Kevin Bove, MD
Department of Pathology, HT-4 Cincinnati Children's Hospital 3333 Burnet Ave.
Cincinnati, OH 45229
Phone: 513-636-4261
Fax: 513-636-3924
Email: boveke@ucmail.uc.edu

Shipments should be packaged as follows:

1. Place the CD containing the EM image file and the Beam capsule containing extra grids inside a specimen bag, squeeze out the air, and seal the bag.

2. Place the bag(s) inside a shipping box, and fill any open space with bubble wrap.

3. Place the styrofoam lid on the shipper.
4. Place shipping documentation on top of the styrofoam lid.
5. Close and tape the outer corrugated box.
6. Attach the "UN3373 Diagnostic Specimens" label to the top of the box in the upper right corner.
7. Attach the Histopathology Core address label to the top center of the box.
8. Follow the instructions below for completing the FedEx air bill and shipping to Cincinnati:

Use the FedEx account provided by Joan Hines to create a label and ship the package(s) to the Histopathology Core. Fill in the date, your name, phone number, and return address as indicated.

Where appropriate, check the "no" block indicating no dangerous goods are contained in the shipment.

Complete the remainder of the shipping label according to FedEx instructions and attach the label to the box.

Call Federal Express, 1-800-GO-FEDEX (1-800-463-3339) to schedule a pick-up.

Contact Kevin Bove, MD with any questions.

9.5.2 Genetics Core Laboratories

The ChiLDReN Genetics Core Labs will be performing genotyping on LOGIC participants for research purposes only. They will be testing for mutations in JAG1, NOTCH2, FIC-1, BSEP, MDR3,and TJP2. Testing will be performed on participants' and some parents' DNA samples. All DNA samples will be sent from the repository to the central core research laboratory via quarterly requests by the DCC. The genotyping testing will be performed at a central core research laboratory under contract with the NIH This process does not require any additional input from coordinators once specimens have been sent to the repository.

9.5.2.1 Testing Processes

All LOGIC participants with a confirmed ALGS diagnosis will have their DNA samples tested for genes related to ALGS Participant samples are tested first for JAG1. If no mutations are found, those samples are tested for NOTCH2. Standard procedure dictates that testing stops once a disease-causing mutation is identified, even if they have not tested the entire gene. If no mutations are identified, the case is sent to the ALGS adjudication committee. This group will review testing results as well as other data collected via eCRFs to determine what, if any, additional testing should occur.

9.5.3 The Bile Acid Core

(This information does not apply to Group 4 participants.)

On all participants enrolled in the LOGIC study, qualitative bile acid metabolic profiling will be performed by fast atom bombardment ionization mass spectrometry to screen for potential defects in bile acid synthesis. Samples that yield an unusual or atypical mass spectrum will then be analyzed in more detail by gas chromatography-mass spectrometry (GC-MS) after solid-phase extraction of bile acids from 5-10 ml of urine and a process of solvolysis, ion-exchange chromatography, derivatization, and gas chromatography-mass spectrometry. Bile acid concentrations in urine are quantified by gas chromatography by comparing the peak height response with the response obtained for a known amount of the added internal standard, nor-deoxycholic acid. Identification of specific bile acids is made on the basis of the GC retention index relative to a homologous series of n-alkanes, referred to

as the methylene unit (MU) value, and the mass spectrum compared with authentic standards.

9.5.3.1 Collection Instructions

At enrollment into groups 1, 2, and 5 (or at a subsequent visit, if need be), urine **(**preferably 5-10 ml, but at least 1 ml is required) will be collected for bile salt analysis into a sterile collection cup, cotton balls, or bag depending (clean catch is preferred) on the age of the child. Samples should be placed in clean vials appropriately labeled with appropriate bar-coded labels provided by the DCC and following instructions as outlined in the ChiLDReNLink User's Guide, **Appendix S**, and frozen at -20°C or colder. Clear 15 ml vials should be used for this purpose. It is not necessary to aliquot these samples. Stored samples should be batch-shipped to the Bile Acid Core every month or when you have collected enough for a shipment.

The shipping manifest created in ChiLDReNLink provides the information needed by the Core Laboratory and replaces the former Bile Acid Core shipping form that accompanied specimen shipments. ChiLDReNLink provides an electronic copy of the manifest during the shipping process. A printed manifest is required for the shipping box and a copy made for study records.

IMPORTANT NOTE: It is required that the patient be off ursodeoxycholic acid (URSO) for at least seven days prior to collection of the urine sample to be used in bile acid testing. The following script has been provided for your use in explaining this to parents. Please edit this text as needed:

"Your child is enrolled in a research study of rare childhood liver diseases. We want to make certain that children in the study do not have inherited problems of making bile acids in the liver. In these diseases, the child cannot make bile acids (the body's natural detergents produced by the liver), which leads to build-up of materials that can damage the liver. To test for this problem, a urine sample needs to be collected. To be sure that there will be no interference with the urine test from medications that you give your child, you will need to stop ursodeoxycholic acid (URSO or Actigall) for a period of 7 days before the urine collection. Stopping the ursodeoxycholic acid for this short period of time should not have any harmful effect on your child and this medication may be restarted as soon as the urine is collected."

9.5.3.2 Shipping Instructions for Bile Acid Core Samples

1. Label samples with the appropriate bar-coded labels supplied by the DCC and follow instructions in the ChiLDReNLink User's Guide (**Appendix S).**

2. Pack the frozen vials that have been collected from recent participants in a packing box. Shipping materials are NOT provided for this purpose. Please use materials from your own hospital.

3. Ship the sample to the LOGIC Bile Acid Core via Dr. Kenneth Setchell, address information is provided in Section 9.5.3.3. Sites are responsible for the cost of this shipping and were required to submit the cost as part of their annual budget.

4. Packages should be picked-up by FedEx on Monday through Thursday (for arrival to Dr. Setchell on Tuesday through Friday). Please be sure the shipping label is marked for priority overnight delivery.

5. For routine shipments, be sure the outside of the box is labeled "Diagnostic Specimen Packed in Compliance with IATA Packing Instruction 650."

6. Call Federal Express, 1-800-GO-FEDEX (1-800-463-3339), and a courier will be dispatched to pick up the samples.

9.5.3.3 Results of Bile Acid Testing

The results of the urine bile acid analysis will be entered on the online eCRF Form 18 by the Bile Acid Core lab. These results are from a CLIA-approved laboratory but are conducted via study ID number only (not name, DOB, etc.) and therefore CANNOT be included in the medical record as an official, CLIA result. The local PI may choose to discuss the results of this testing with the family, but if an official result is desired, testing will have to be conducted clinically on a new sample at the expense of the patient. The cost of the LOGIC test, which is completed for research purposes only, will be paid for by this study.

Once the testing is complete, the participating site will receive an email notification that Bile Acid Testing results are available for review, including the participant's study ID.

The study coordinator can view the results in Form 18 by clicking on the "reports" tab in ChiLDReNLink. Select Form 18 as the report to view, once the report page opens, you will be asked to enter a participant ID. Be sure to enter the correct participant ID (from the email notification) for viewing results from the laboratory.

The Bile Acid Biochemistry Core is located in the Clinical Mass Spectrometry Center at the Cincinnati Children's Hospital, directed by Kenneth Setchell, Ph.D. The address and contact information is:

Kenneth Setchell, PhD
Mass Spectrometry Lab- MLC 7019 Rm R-034
Cincinnati Children's Hospital Medical Center
3333 Burnet Avenue
Cincinnati, OH 45229
Phone: 513-636-4548
Fax: 513-803-5014
Email: Kenneth.setchell@cchmc.org

9.5.4 Specimen Collection for Group 4 ONLY

DO NOT enroll into Group 4 without contacting the Denver Admin Core first. Joan Hines: 720-777-2598 (office) or 303-913-5210 (mobile, this number is to be utilized in time-sensitive situations only).

9.5.5 Specimen Collection (Blood) for Group 4 ALGS or PFIC (or BRIC)

Participants enrolled in Group 4 with suspected PFIC (or BRIC) and Alagille Syndrome will (and their parents may) have blood collected for LOGIC research genotyping. **The blood volume required for genotyping is 5 ml in an EDTA (purple top) tube, regardless of weight/size of participants.** Samples are sent to Rutgers University Cell and DNA Repository for DNA extraction. The DCC is responsible for notifying the repository and identifying those samples to be sent to the appropriate Core facility for testing once DNA extraction has been performed. Please see detailed instructions below.

9.5.6 Specimen Collection (Urine) for Group 4 BAD

Bile Acid Testing of Group 4 Participants with Presumed Bile Acid Synthesis Defects

For all LOGIC participants enrolled into Group 4 because of presumed Bile Acid Synthesis Defects, testing will be conducted by the ChiLDReN Bile Acid Core.

Procedures for managing this study group are described in the following steps and should not be confused with any previous descriptions of bile acid testing for other LOGIC study groups.

1. Gather all urine collection materials that are typically used for collection and shipment of urine

samples to the Bile Acid Core lab. Clear 15 ml vials should be used for this purpose. It is not necessary to aliquot these samples.

2. Collect a total of 1-10 ml of urine for bile salt analysis (clean catch is preferred) into a sterile collection cup, cotton balls, or bag depending on the age of the child.

3. Label the participant's vial with the appropriate bar code label, "Bile Acid Urine" supplied by the DCC and follow the instructions in the ChiLDReNLink User's Guide (**Appendix S**). This specimen should not be frozen and batch-shipped. The Setchell Lab has its own shipping tab in ChiLDReNLink to be utilized to ship specimens to the Bile Acid Core laboratory.

4. Immediately pack the participant specimen in a packing box.

5. Ship the sample to the ChiLDReN Bile Acid Core via Dr. Kenneth Setchell, address information is provided below. Sites are responsible for the cost of this shipping and were required to submit the cost as part of their annual budget.

6. If the sample was collected and is being picked-up by FedEx on Monday through Thursday (for arrival to Dr. Setchell on Tuesday through Friday), be sure the shipping label is marked for priority overnight delivery.

7. If the sample was collected and is being picked-up by FedEx on Friday, please be sure to mark the package for Monday delivery as there are no deliveries to Dr. Setchell's lab on the weekend.

8. For routine shipments, be sure the outside of the box is labeled "Diagnostic Specimen Packed in Compliance with IATA Packing Instruction 650."

9. Call Federal Express, 1-800-GO-FEDEX (1-800-463-3339), and a courier will be dispatched to pick up the samples.

The Bile Acid Biochemistry Core is located in the Clinical Mass Spectrometry Center at the Cincinnati Children's Hospital, directed by Kenneth Setchell, Ph.D. The address and contact information is:

Kenneth Setchell, PhD
Mass Spectrometry Lab- MLC 7019 Rm R-034
Cincinnati Children's Hospital Medical Center
3333 Burnet Avenue
Cincinnati, OH 45229
Phone: 513-636-4548
Fax: 513-803-5014
Email: Kenneth.setchell@cchmc.org

10. Bio-Repositories and Shipping

Central repositories have been established by the NIDDK for long-term storage (bio-banking) of blood, urine, and tissue specimens. This bio-banking is a critical aspect of this longitudinal study in order to create a resource of tissues, DNA, and other specimens from a meaningful number of patients 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.

One repository has been established at Rutgers University for DNA extraction. Whole blood for DNA isolation will be shipped immediately to the facility at Rutgers University. Rutgers University will also be extracting DNA from saliva from those participants who have utilized saliva kits for collection of saliva for extracting DNA.

As of January 2016 the ChiLDReN Network agreed to cease the creation of cell lines from DNA.

NIDDK has also contracted with Precision for Medicine to establish a biosample repository for the long-term storage of blood, urine, and tissue specimens (i.e., all samples except the whole blood or saliva for DNA that are sent to Rutgers Repository). Specimens will be batched-shipped to this repository every month. At the time of percutaneous liver biopsy, open liver biopsy, liver transplant, biliary diversion surgery or autopsy, any liver tissue that is removed as part of the procedure, but is not needed for clinical purposes, will be collected. Specimens (in cryovials) will be sent to the repository and 20 unstained slides of paraffin-embedded liver tissue will be shipped to the Repository for storage.

All specimens sent to the two repositories will include a research study identifier but otherwise will be de-identified prior to shipment. A computer log will record all incoming samples at the repository, the storage location, the date, and the type of sample. Receipt of samples will be acknowledged to the originating clinical site and the DCC. The research study identifier will be sent to the DCC and linked to the participant.

10.1 Rutgers University Cell & DNA Repository (RUCDR)

10.1.1 RUCDR: Specimen Supply Kits

RUCDR will ship all the supplies for each study site in a kit, including pre-labeled vacutainers that the study site needs for collecting and shipping whole blood to RUCDR. The DCC supplies each site with specific bar-coded labels to be used for whole blood collection and for tracking purposes in ChiLDReNLink.

Whole blood/or saliva for DNA extraction will be collected from the biological parents. Please be sure to collect a full 10 ml of blood from each biological parent.

Please fill each tube completely before filling another tube. Be sure to invert each tube gently 8 to 10 times **(DO NOT SHAKE)** to mix blood with additives and keep them at room temperature.

If an inadequate volume was obtained for DNA extraction, a re-draw request may be made.

The whole blood must be shipped <u>on the day of collection</u> to RUCDR.

Each RUCDR “Domestic Collection Kit” whole blood kit consists of:

- Vacutainers (see note below)
    - Child: One 4 ml purple EDTA vacutainer
    - Parents: Two (10 ml purple EDTA vacutainers)
- One Model 470 Safety Mailer (body & lid)
- One 2-1/2” x 9” pre-cut section of absorbent material
- One corrugated shipping carton with locking tabs
- Red water-proof tape
- Press-lock plastic bag
- Collection form (one form is required for each participant collection (parents and child))
- FedEx Air bill
- Instructions for assembling the shipment

NOTE: Each kit will contain vacutainers for two adults and one child. The age appropriate vacutainers must be used, since they differ in type and size, depending on the weight of the person on whom blood is being collected.

- For a child ≤50 kg, use one (1) x 4.0 ml EDTA vacutainer.
- For anyone (child or adult) weighing ≥50 kg, use one (1) purple-top EDTA x 10 ml vacutainer.

Extra supplies can be requested from RUCDR by contacting commstaff@dls.rutgers.edu

Please include the name of the project (ChiLDReN – LOGIC) and the respective study site number in all communications.

10.1.2 RUCDR: Specimen Packaging

Process the phlebotomy collection form, see **Appendix Q.**

- The participant ID is entered on the DCC bar-coded label
- Double check the participant ID, verifying that the ID information on the tube matches that on the phlebotomy collection form.

- **Make a copy of the phlebotomy collection form and keep in the research file.** Send originals to RUCDR with the specimens.
- Include one (1) RUCDR phlebotomy collection form for each participant in the mailer box, outside of the plastic bag.
- You can ship more than one participant in the mailer box as long as each participant has a separate collection form.
- DO NOT place more than 8 tubes in each mailer box.
- Print two copies of the manifest created in ChiLDReNLink, place one copy of the manifest in the shipping box, keep the other copy for your records.

Package the blood tubes in the safety mailer following the instructions included with the kit. Be sure to seal the styrofoam container with the red water-resistant tape. For specimen shipments, be sure the outside of the box is labeled “Diagnostic Specimen Packed in Compliance with IATA Packing Instruction 650.”

Information on assembling the RUCDR DNA shipper can be found in **Appendix Q**.

10.1.3 RUCDR: Specimen Shipping

FedEx: Complete the FedEx air bill. Be sure the shipping label is marked for priority overnight delivery.

Whole blood should be shipped on the day of collection to RUCDR. Do **NOT** keep the sample overnight. If barriers (weather delays, late clinic appointments, holidays, and weekends) prevent same day shipping, sample should be stored at room temperature until time of shipment (up to 5 days).

The account number is already on the air bill. Call FedEx (1-800-GO- FEDEX (1-800-463-3339)) for sample pickup.

The address of the RUCDR contact is:

ATTN: CommStaff
RUCDR-Infinite Biologics
Nelson Laboratories
604 Allison Road, Room C125
Piscataway, NJ 08854
PH: 848-445-1498

Please Note: If the whole blood is collected on Friday, permission is needed from Rutgers for shipment with arrival on Saturday. Permission is obtained by calling this number 848-445-1498 and speaking with Kristina Carle or Dana Witt. If permission is granted for Saturday delivery, be sure to check the "Saturday Delivery" option box on the FedEx air bill.

An email can be sent to the following address: witt@dls.rutgers.edu (be sure to address it to Dana) with the same request, the response may not be as quick as a direct phone call.

NOTE: If whole blood collection is not possible or contraindicated, saliva will be collected for DNA extraction. 2 ml of saliva will be collected in a saliva kit. The saliva kits can be given to parents in clinic or mailed to their residences.

RUCDR Saliva Kits: With approval of LOGIC Amendment 6, each site will be sent 5 saliva kits for collection of saliva to be used for extraction of DNA when whole blood collection is not possible or contraindicated. When sites send to participants at their residences or give to participants to take home, it should be understood, the participant is responsible for shipping the kit(s) to Rutgers for processing.

Sites will be responsible for ordering additional saliva kits as needed. Sites may order up to 10 saliva kits per month, if more than 10 are needed, prior permission from the DCC is required.

Each RUCDR Saliva Kit contains the following items:

- Saliva collection kit
- Saliva collection form
- Collection and shipping instructions
- Bio-Hazard bag
- Bubble Wrap pouch
- Air bill for shipping to Rutgers
- Shipping Envelope

Complete instructions for prepping the saliva kit, shipping and tracking the saliva kit in ChiLDReNLink can be found in **Appendix T.**

To request whole blood and saliva shipping kits from Rutgers, utilize the following email address:

commstaff@dls.rutgers.edu. Be sure to indicate the number of shipping kits you are requesting in your email.

Sites may also request supplies and pre-register their samples through the following RUCDR LIMS portal website https://rucdrlims.rutgers.edu/starlims10.rucdrlims/limsportal/

10.2 Precision for Medicine Repository

10.2.1 Precision for Medicine: Specimen Supply Kits

Precision for Medicine will provide shipping containers for cryovials. Each shipping kit for mailing cryovials on dry ice includes:

- STP 320 Insulated shipper
- Three (3) absorbent sheets
- Three (3) specimen boxes with 9 x 9 grids (holds 81 cryovials)
- Three (3) biohazard bags
- Three (3) tyvek envelopes
- FedEx air bill
- "To" address label
- UN3373 Category B label
- Class 9 miscellaneous dangerous goods label (for dry ice)
- Mailing flap (to cover the dangerous goods markings on kits shipped to sites from the repository)

Precision for Medicine will provide the shipping kits for mailing slides. The shipping kit for mailing slides includes:

- UPS90 insulated shipper
- Three (3) biohazard bags
- Three (3) plastic slide storage boxes
- Bubble wrap
- FedEx air bill
- "To" address label
- Exempt Human Specimen label

Note: Precision for Medicine does not provide slides to the sites.

If additional containers are needed, notify the Precision for Medicine via email Niddk.mailbox@precisionformedicine.com and copy the following email: eduard.chani@precisionformedicine.org

Participating study sites may also call Eduard Chani, PhD, Senior Project Manager Office: (240) 415-6052; Mobile: (301) 318-8218; Fax: (301) 668-3416

Email correspondence is preferred.

Precision for Medicine
8425 Precision Way, Suite M
Frederick, MD 21701

Information on assembling the Repository shipper can be found in **Appendix R**.

10.2.2 Precision for Medicine: Specimen Labeling

The DCC supplies cryovials and bar-coded labels for each type of sample to be collected and/or aliquotted. Apply the labels lengthwise to the cryovials. Remove all other participant identifiers from the vials.

Please contact ChiLDReN-admin@arborresearch.org with a completed study request supply form for cryovials and/or bar-coded labels. The study supply request form can be found on the ChiLDReNLink website or the ChiLDReN Study website. Please keep supply ordering to once a month.

NOTE: The labels adhere better when placed on the cryovials a substantial time prior to freezing (the evening before when possible). This 'wait time' enables the temperature of the labels to equilibrate to the cryovial and form a solid bond. When preparing your cryovials for a visit or procedure, it's best to utilize the "prepped" collection status in ChilDReNLink. Using the "prepped" status makes the labels easier to unlink if the participant misses a visit, refuses a blood draw, or the procedure is canceled.

10.3 Shipping Schedule

Below is the shipping schedule for monthly shipments to Precision. Sites should be shipping based on the following schedule:

Chicago/University of Utah/Houston	First Mon.-Wed. of each month
Cincinnati/Philadelphia/Indianapolis/Los Angeles	Second Mon.-Wed. of each month
Denver/Pittsburgh/Toronto/Seattle/Atlanta	Third Mon.-Wed. of each month
UCSF/St. Louis	Fourth Mon.-Wed. of each month

Shipments are accompanied by a printed manifest to be used by the repository to confirm the presence of all specimens in the shipment. An electronic copy of the manifest is also sent to the repository. Any discrepancies noted by the repository will be sent to the DCC for follow-up with each site.

Sites should adhere to the above schedule. If a holiday falls on the Monday, when the site is to ship, then the site should send the shipment the following day. Do not send shipments to the repository on a Thursday or Friday, unless arrangements have been made with the repository. Sites should notify the DCC monitors (prior to shipping) if they have a situation where they need to send a shipment a week earlier or later. Sites will resume their shipment schedule with the next shipment.

10.4 Samples from Ineligible Participants at the Repository (Precision for Medicine and Rutgers)

If samples from ineligible participants have been collected and sent to the repository, they cannot be used and will need to be destroyed. Therefore, when an exception/exemption is requested, please do not send samples to the repository until the decision is made about eligibility.

10.5 Utilizing ChiLDReNLink to ship biosamples

See **Appendix S,** the ChiLDReNLink User's Guide, for complete instructions on linking, identifying biosamples to create a shipping manifest, notification to the repositories and the DCC on the day of shipment, and the shipping of the linked biosamples.

11. AE/SAE/Regulatory Bodies Reporting

11.1 Definitions

AE: An adverse event (AE) is any unfavorable, harmful, or pathological change in a research participant as indicated by symptoms, psychological or 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 PIs to be clinically significant (that is, if it institutes a diagnostic evaluation or indicates additional therapy is necessary).

This is an observational, not an interventional, study and therefore few AEs are anticipated. AEs associated with venipuncture are the only expected AEs in this study. However, any serious AEs must be reported.

SAE: The term serious adverse event (SAE) is based on patient outcomes associated with events that could threaten a patient's life or functioning.

If a medical problem occurs during a procedure that is both clinical and research-related, it is not considered a study SAE unless it can be solely tied to the research component of the procedure (i.e., phlebotomy for clinical labs and biosamples during which the patient faints and hits his head).

For an event to be considered a SAE, one or all of the following must apply:

- Death
- Life threatening
- Persistent or significant disability/incapacity
- Required in-patient hospitalization or prolonged hospitalization
- Congenital anomaly or birth defect
- Important medical events requiring medical or surgical intervention to prevent one of the outcomes listed above

Expected AE: An expected AE is any AE, the specificity and severity of which is consistent with the current standard of care, or is consistent with the risk information described in the informed consent document. The list of expected AEs is compiled by the steering committee and is included in the protocol and in the informed consent documents.

Unexpected AE: An unexpected AE is defined as any AE, the specificity and severity of which is not consistent with the current standard of care; or the specificity and severity of which is not consistent with the risk information described in the informed consent document or elsewhere in the current application.

Any expected or unexpected AE that also qualifies as a SAE, based on the criteria above, is

considered a SAE by definition.

Related to study: The phrase ‘related to study’ implies causality or attribution to the study procedures. For purposes of defining a 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.

The SAE reporting window for each participant begins with the first study procedure, and ends 30 days after last study procedure.

SAEs must be reported to the DCC within 24 hours of the site’s awareness of the occurrence. The site should complete the SAE report form in *ChiLDReNLink* within this timeframe. Once you save the form, notification will immediately be sent to the DCC, DSMB, and NIDDK personnel.

12. Data Management

12.1 Gathering Data

Data should derive from source documents. Source documents are original documents (the first place the information was recorded) that serve as the “raw data” for a study. Source documents include patient progress notes, laboratory reports, electrocardiograms (EKGs), medication records, x-rays, hospital records, research clinic records, participant diaries, and recorded data from automated instruments.

Data on race/ethnicity can be collected by asking the participant directly for the information. Write an anecdotal note to file of the conversation to use as a source document, and file in the participant’s research file.

Keep in mind: “If it is not written down, it did not happen.”

If you have questions about the meaning of a question or data element, you should contact the DCC monitors for the definition. The goal is to keep interpretation of data elements consistent so that data collected can be properly analyzed and interpreted.

If you have questions about what a notation means on a chart, then you should contact your site PI for a definition and interpretation.

12.2 Data Discrepancies

The ChiLDReNLink electronic data entry system will have built-in data checks as part of study quality assurance. Protocol compliance will be assessed by monitoring the submission of data at required intervals. Data inconsistencies and discrepancy reports will be reviewed by a Clinical Monitor so that necessary queries can be generated, and sent to the transplant center study sites for verification and resolution.

Periodic requests may be generated for the submission of random source documents to assess the quality of data acquisition and data entry at each site. A Clinical Monitor will visit each site annually to review source documents, monitor regulatory compliance, and assess protocol adherence.

In addition to source document verification, the Clinical Monitors and Program Analysts will produce reports from the ChiLDReNLink system to look for inconsistencies in submitted data.

The DCC will develop data edit checks to clean the data. Edit checks are quality control checks on the entered data in an effort to identify incomplete or inconsistent data and to catch errors.

12.3 Data Timeliness

Confirmation that a scheduled visit (visit status) has occurred, and samples (sample status) were collected is required within 48 hours of the visit.

All participant data should be entered into the database within three weeks of study assessments.

Information on the number and types of samples collected (used or discarded on the sample label page) is required to be entered within one week from the time of the assessment.

SAE information should be entered into the database within 24 hours of the site being informed of the event. Reports should be updated as soon as information becomes available.

When queries are generated by the DCC on entered data and sent to the sites, a specific timeframe for resolution of the queries is included in the email with the attachment of the query spreadsheet.

NOTE: These are the measurements for overall protocol adherence as reported on the DSMB site report cards.

12.4 Data Sources

Participant medical records – Laboratory results will be collected. Exam, lab, and procedure data will be collected.

Participant survey responses will be collected.

12.5 Electronic Case Report Forms (eCRFs)

The DCC is responsible for data management for the ChiLDReN study. The DCC maintains a password-protected website for the ChiLDReN study. All transmissions to and from the website are encrypted using SSL. In addition to providing access to the study database, the website contains the protocol, MOO, current versions of the eCRFs that are used at the time of participant enrollment and at follow-up visits, and other information related to the study. The eCRFs do not contain any personal participant identifiers, except dates, such as date of birth (DOB), which are necessary for research purposes. The DCC will provide the CRFs electronically and the study site will print blank CRFs from the website. An investigator and/or CRC can only view data in the study database from his (or her) study site.

12.6 ChiLDReNLink

Sites will utilize the web-based ChiLDReNLink program as the data entry nucleus for the ChiLDReN studies. Briefly, ChiLDReNLink is a highly flexible database application that allows investigators to organize their research operations and perform common actions on research data within a single database.

ChiLDReNLink can be accessed through the ChiLDReN website at: https://childrennetwork.org/. A separate user ID and password is required to log into ChiLDReNLink. Note that passwords are case sensitive. In accordance with GCP guidelines, ChiLDReNLink user IDs and passwords must not be shared. New personnel requiring access to the study database should complete appropriate training with the DCC and request a unique username and password from their site's primary coordinator.

12.7 Logging in

The ChiLDReNLink database may be accessed from the following websites:

- The main ChiLDReN study page https://childrennetwork.org/
- Or https://childrennetwork.org/arborLink/

12.8 Use of PHI

The ChiLDReNLink study database will only utilize protected health information (PHI) on one page, and will use unique study identification numbers on all other data entry pages. Available PHI from ChiLDReNLink will be pre-populated into the ChiLDReNLink database. The PHI will be encrypted, and visible via a de-encryption key called the "*Patient Name Key"* available only to the site's authorized personnel. The DCC will not be able to view the encrypted data and will not have the key. Sites will only have access to their own data, and PHI will not be shared between sites. Data analysis files will be de-identified. At the earliest time possible, consistent with the completion of the project, the DCC will destroy data linkages that contain PHI.

12.9 Unencrypted Participant List

To view your study participant list with PHI enter the *"Patient Name Key"* when prompted to do so.

13. Protocol Compliance

13.1 Adherence to Protocol

Every effort will be made by study personnel to ensure adherence to the protocol by study participants. This includes proper enrollment of all eligible participants and initiation of data collection in a timely manner. All interactions with the study participants will be performed by the same personnel utilizing supportive and positive reinforcement communication skills.

13.2 Protocol Deviations

A protocol deviation is a departure from the expected conduct of an approved study that is not consistent with the current research protocol, consent document, or study addenda that had not been anticipated. All protocol deviations must be reported in writing to the DCC immediately upon discovery, using a protocol deviation report eCRF form, and must be reported to local IRBs/ ERCs, if required by your local IRB/ERC.

A protocol deviation may be a divergence in a procedure from that indicated in the protocol (such as drawing more blood than indicated in the protocol). Specific categories are provided on the eCRF.

Enrolling a participant who does not meet eligibility criteria, but for whom enrollment is authorized by the Exemptions Committee, also constitutes as protocol deviation. NIDDK and the DSMB will track the enrollment of participants who require exemptions to the inclusion/exclusion criteria.

13.2.1 When to Complete a Deviation

Complete a protocol deviation form when a deviation impacts one of the following:

- The inclusion and/or exclusion criteria
- Impacts the ability of the sponsor to evaluate the endpoints of the study
- A consent violation
- Protocol deviations also includes situations that result in noncompliance with the study protocol, GCP.

Below is a list of some of the protocol deviations (major and minor) the DCC will be tracking:

- Participant enrolled, but does not meet eligibility criteria.
- Non-adherence to study design.

- Loss of samples or data as per protocol schedule of events.
- Failure to obtain informed consent prior to initiation of study-related procedures.
- Falsifying research or medical records.
- Performing tests beyond professional scope.
- Working under an expired professional license/certificate.
- Breach of confidentiality.
- Improper or inadequate informed consent procedure.

Study Coordinators will print out the Protocol Deviation Form 40 and complete the form with all available information. Once completed, the deviation form is scanned and sent to the DCC via ChiLDReN-Monitors@arborresearch.org. The DCC Clinical Monitors and clinical study process manager will review the deviation and assess a major or minor deviation, sign and return to the site for IRB submission.

NOTE: Further follow-up in reference to the action plan outlined in the protocol deviation may be requested from the DCC.

Protocol deviations are submitted to site's IRBs per their reporting procedures. The response from your IRB to the deviation reports are to be filed in the regulatory binder under major correspondence.

14. Study Completion and Closeout

14.1 Study Closeout Activities

Study closeout activities are performed to confirm that the PI's study obligations have been met and post-study obligations are understood.

Closeout activities include, but are not limited to, the following:

- Verification that study procedures have been completed, data collected, and study supplies are returned to the responsible party or prepared for destruction.
- Review of completed and de-identified Screening and Enrollment Log.
- Review of PI's correspondence and study files against the DCC's records for completeness.
- Assurance that all data queries have been completed.
- Assurance that correspondence and study files are accessible for external audit.
- Reminder to PIs of the ongoing responsibility to maintain study records and to report any relevant study information to the sponsor or IRB.
- Meeting with PIs to ensure that they are aware of governing body obligations and requirements for record retention.
- Assurance that the PI will notify the IRB of study completion and obtaining a copy of the notification.
- Preparation of a report summarizing study conduct.

APPENDIX A:
Amendment 6 (Protocol version: 14 March 2017)

Appendix A LOGIC Amendment 6_v4 07

APPENDIX B: Data and Safety Monitoring Board

- **Data and Safety Monitoring Board (DSMB) Charter Childhood Liver Disease Research Network (ChiLDReN)**
- **Childhood Liver Disease Research Network (ChiLDReN) Data and Safety Monitoring Board Roster and Mailing List**


ChiLDReN DSMB Charter June 2014.p


ChiLDReN DSMB Roster 9-2016.pdf

APPENDIX C: LOGIC Confidentiality Certificate
DK-09-016, Amendment #1

Appendix C LOGIC CoC.pdf

APPENDIX D: Informed Consent Templates

Assent Template for LOGIC Study:

Assent Template for LOGIC Study.pdf

LOGIC Alpha1 Sibling Consent Adult:

LOGIC Alpha1 Sibling Consent Adu

LOGIC Consent Adult Alpha1 and BAD:

LOGIC Consent Adult Alpha1 and B/

LOGIC Consent Adult Screening:

LOGIC Consent Adult Screening.pdf

LOGIC Consent for Adult PFIC and ALGS:

LOGIC Consent for Adult PFIC and ALGS

LOGIC Post-transplant for Adult:

LOGIC Post-transplant for /

LOGIC Alpha1 Sibling Consent Parent for Child:

LOGIC Consent Adult Alpha1 and B/

LOGIC Consent Parent and Child Alpha1 and BAD:

LOGIC Consent Parent and Child Alp

LOGIC Consent Parent for Child Screening:

LOGIC Consent Parent for Child Scre

LOGIC Consent Parent and Child PFIC and ALGS:

LOGIC Consent Parent and Child PFI

LOGIC Post-transplant Parent for Child:

LOGIC Post-transplant Pare

APPENDIX E: Essential Regulatory Document Checklist

Appendix E
Essential Regulatory

APPENDIX F: Protocol log

Appendix F
protocol_log update

Appendix G: Screening log updated 01182018

Appendix G screening_log upda

Appendix H: Site Enrollment Log updated 01182018

Appendix H Site Enrollment Log upd

Appendix I: Site Monitoring Log v2

Appendix I Site Monitoring Log v2.p

Appendix J: Roles and Responsibilities 01172018

Appendix J Roles and Responsibilities

Appendix K: Delegation of Authority Site Signature Log updated

Appendix K
Delegation of Authc

Appendix L: ChiLDReN Onboarding-Offboarding-Change Form 20171103

Appendix L
ChiLDReN Onboardi

Appendix M: ChiLDReN Studies Monitoring Plan

Appendix M
ChiLDReN Studies M

Appendix N: eCRF Completion and Definition

Appendix N LOGIC
Study CRF Definitior

Appendix O: QoL Surveys and Developmental Instruments

Appendix O QoL Surveys and Develop

Appendix P: Processing Bio-samples 01182018

Appendix P
Processing Bio-samp

Appendix Q: Rutgers

Childrens' Network Blood Sample Collection & Shipping Instructions:

Childrens Network_2 Adult 1 C

RUDCR Collection Form for Whole Blood Genetics

RUCDR Collection Form for Whole Blo

Rutgers Collection Form Annotated

Rutgers Collection Form Annotated.pdf

Saliva Kit Instructions for Participants

Saliva Kit Instructions for Part

Saliva Kit Process

Saliva Kit Process_201707_v5.p

Appendix R: ChiLDRen Precision Shipping Instructions

Appendix R
ChiLDReN Precision

Appendix S: ChiLDReN-Link User's Guide

Appendix S
ChiLDReN-Link User

Appendix T: Saliva Kit

Saliva Kit Process:

Saliva Kit Process_201707_v5.p

Instructions for Participants:

Instructions for Participants.pdf