NASH CRN

Nonalcoholic Steatohepatitis
Clinical Research Network

Nonalcoholic Fatty Liver Disease (NAFLD) Pediatric Database 2

Protocol

CONFIDENTIAL

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NAFLD Pediatric Database 2 Protocol

Contents

Design synopsis

Objectives

- To continue to investigate the etiology, pathogenesis, natural history, diagnosis, treatment, and prevention of nonalcoholic fatty liver disease (NAFLD)
- To provide a resource for clinical trials and ancillary studies of the pathogenesis, natural history, diagnosis or diagnostic biomarker development and treatment of NAFLD, NASH, or NASH-related cirrhosis
- To continue the development of histopathological methods for diagnosis and assessment of NAFLD, NASH, and NASH-related cirrhosis
- To maintain and expand the centralized histopathological NAFLD repository and reading center
- To develop imaging methods for non-invasive diagnosis and assessment of NAFLD, NASH, and NASH-related cirrhosis and to develop a NAFLD digital imaging repository and analysis center
- To add to and expand the specimen bank comprising liver tissue, serum, plasma, and DNA obtained from participants with biopsy confirmed NAFLD

Type of study

- Prospective follow-up

Population

- Participants at least 2 years of age and less than 18 years of age with known or suspected NAFLD or NASH-related cirrhosis

Inclusion criteria

- At least 2 years of age and not older than 17 years at time of initial screening
- Written informed parent consent and child assent to participate
- Willingness to be in the study for 1 or more years
- **For continuing participants:** Previously enrolled in the NAFLD Database study or TONIC trial
- **For new participants**:
  - Recent (≤120 days before enrollment) liver biopsy
  - Collection of serum and plasma within 90 days of enrollment and up to 90 days before or 4-90 days after standard of care liver biopsy
  - Absence of regular or excessive use of alcohol within 2 years prior to initial screening

Exclusion criteria

- **For continuing participants:** Any conditions or circumstances likely to interfere with follow-up visits and procedures (per investigator's opinion)

- **For new participants:**
  - Clinical or histologic evidence of alcoholic liver disease
  - Evidence of other causes of chronic liver disease
  - History of prolonged (> 1 month) total parenteral nutrition within a 6 month period before baseline liver biopsy
  - Short bowel syndrome
  - History of biliopancreatic diversion
  - History of bariatric surgery (Participants expecting to undergo bariatric surgery can be enrolled prior to the procedure)
  - Known HIV positive
  - Other condition that is likely to interfere with study follow-up

Recruitment

- **Continuing participants**: 200 (50 with clinically indicated liver biopsy within the target recruitment period, 150 continuing participants without liver biopsy at enrollment)
- **New participants**: 650 participants with liver biopsy within 90 days of specimen collection
- Target for new liver biopsies: 700 (50 from continuing participants and 650 from new participants)
- Total sample size for pediatric participants in NAFLD Database 2: 850

Duration of follow-up

- One or more years of follow-up

Outcome measures

- Liver histology scores (derived from central reading of liver biopsy at entry, standard of care biopsy done during screening or follow-up, or liver biopsy obtained for the TONIC trial)
- ALT, AST levels
- Glucose, insulin levels
- Lipid profile
- Body mass index and anthropometric data

Visit schedule

- Screening and enrollment into NAFLD Database 2: screening must be completed within 90 days of the signing of consent. Enrollment marks the successful completion of the screening process and initiates the yearly (with a target of every 48 weeks) study visits
- Follow-up visits every 48 weeks

NAFLD Pediatric Database 2 Protocol

1. Background and rationale

1.1. Historical background and goals

Nonalcoholic fatty liver disease (NAFLD) is a spectrum of liver conditions associated with fat accumulation that range from benign, non-progressive liver fat accumulation to severe liver injury, cirrhosis, and liver failure. NAFLD appears to be highly prevalent within the United States. The spectrum of NAFLD encompasses nonalcoholic steatosis (nonalcoholic fatty liver [NAFLD] and nonalcoholic steatohepatitis (NASH) in which there is ballooning degeneration (with or without Mallory bodies) and/or fibrosis. In severe cases, NASH may progress to cirrhosis, in which steatosis may be present or absent. In the latter circumstance, end-stage NASH may evolve into and contribute to NASH-related cirrhosis. The pathology of NASH closely resembles alcoholic liver disease but occurs in patients who drink little or no alcohol. NASH is most common in adults above the age of 40 who are overweight or have diabetes, insulin resistance, or hyperlipidemia. However, the disease also occurs in children and in persons who are not obese or diabetic. Currently, there is no established effective therapy for NASH, and its natural history and prognosis are not well understood. In 2002, the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK), through the mechanism of RFA-DK-01-025, established a Clinical Research Network (CRN), the goal of which was to facilitate and perform clinical, scientific, epidemiological and therapeutic research in NASH. The NASH CRN through the RFA-DK-08-505 entered its third term of funding on August 1st, 2014, with the mission of renewing the NAFLD Database (Database 2) with new specific goals as outlined below. There are an estimated 40-90 million individuals within the United States with NAFLD, 10-30% of whom have NASH and may develop NASH-related cirrhosis. Identifying through non-invasive means those individuals who are at risk for progressive liver disease is a major priority of the NASH CRN.

1.2. Clinical Research Network

The NASH Clinical Research Network (NASH CRN) is a cooperative network of eight clinical centers and one Data Coordinating Center (DCC). Clinical centers are responsible for proposing study protocols, participating in their overall development, recruiting patients, conducting the research, and disseminating research findings. The individual clinical centers participate in a cooperative and interactive manner with one another and with the DCC in all aspects of the NASH CRN. The DCC supports study protocol development; provides sample size calculations and statistical expertise; supports forms development, data analysis and manuscript preparation; and provides overall study coordination and quality assurance, including preparation of reports and coordination of the activities of the Data and Safety Monitoring Board, the Steering Committee, and other standing NASH CRN subcommittees. The DCC also maintains the Histology Repository including stained and unstained liver biopsy slides and collaborates with the NIDDK Biosample (plasma, serum, and liver tissue) and Genetics (DNA) Repositories.

The Steering Committee is the main governing body of the NASH CRN and is composed of the principal investigators of each adult clinical center and each associated pediatric clinical center in the Network, the principal investigator of the DCC, and the NIDDK Project Scientist. The Steering Committee's responsibilities include the general organization of the NASH CRN, finalizing common clinical protocols and facilitating the development of a standardized nomenclature, diagnostic criteria, histological definitions, and the necessary components to the common database on patients. The Steering Committee is responsible for the conduct and monitoring of the NASH CRN studies and reporting study results.

2. Objectives and hypotheses

2.1. Primary objectives

- To elucidate, through the cooperative effort of a multidisciplinary and multicenter group of collaborators, the etiology, natural history, diagnosis, treatment, and prevention of NAFLD, and in particular its more severe form of NASH and its complications
- To add to the existing NAFLD Database an additional 250 pediatric participants with a diagnosis of NAFLD, supported by a recent liver biopsy, with a broad range of severity. Core data collection will include clinical, demographic, laboratory, imaging, and histological features
- To increase the population diversity of the NAFLD Database to provide greater representation of Native American, African American, and Asian patients among the new pediatric participants recruited into the NAFLD Database 2
- To expand the current specimen bank comprised of serum, plasma, and DNA obtained from new participants and continuing participants undergoing repeat liver biopsy with the specific goal of optimizing the collection of plasma or serum suitable for biomarker development studies by obtaining specimens in close temporal proximity to the performance of liver biopsy

2.2. Secondary objectives

- To provide a resource for developing clinical and pathological criteria for standardizing diagnostic and staging criteria for NAFLD or NASH-related cirrhosis
- To provide a resource for developing clinical and pathological criteria and measures and endpoints for therapeutic studies of NAFLD or NASH-related cirrhosis
- To develop Magnetic Resonance Imaging (MRI) or Magnetic Resonance Spectroscopy (MRS) protocols to evaluate the utility of these diagnostic modalities for the non-invasive staging and grading of NAFLD
- To provide a resource for ancillary studies of the pathogenesis, diagnosis or diagnostic biomarker development, genomic, proteomic and lipidomic characterization, natural history and treatment of NAFLD or NASH-related cirrhosis

3. Scientific background

3.1. NAFLD and NASH

Nonalcoholic fatty liver disease (NAFLD) is a disorder of lipid metabolism marked by excessive accumulation of triglycerides in hepatocytes, and refers to a spectrum of histopathology occurring in the absence of clinically significant alcohol ingestion. NAFLD is strongly associated with metabolic conditions, including insulin resistance, dyslipidemia, visceral adiposity and hypertension, and is considered the hepatic manifestation of the metabolic syndrome. The histological appearance of NAFLD on liver biopsy ranges from bland steatosis to nonalcoholic steatohepatitis (NASH), which is characterized by necroinflammation with or without fibrosis, and to NASH-related cirrhosis. NASH is considered the most clinically significant form of NAFLD due to its propensity for progression to NASH-related cirrhosis, which may be complicated by portal hypertension and hepatocellular carcinoma, necessitating liver transplantation.(1, 2) The accumulation of fat in hepatocytes may be the first step in the development of NASH and its presence is the defining morphology of all forms of fatty liver disease.

3.2. Epidemiology and natural history

NAFLD is the most prevalent of all liver disorders and is the most common cause of chronic aminotransferase elevations in the United States.(3-13) However, the true prevalence of NAFLD in the pediatric population is incompletely defined because liver biopsy remains the diagnostic gold standard and is the only means of establishing a firm diagnosis of NAFLD and of determining the grade of fat accumulation, necroinflammation, and stage of fibrosis. As there currently are no accurate, sensitive and noninvasive tests useful for population screening for NAFLD, much of the data regarding NAFLD prevalence are derived either from select patient populations that have undergone liver biopsy, from autopsy reports, or from studies using presumptive clinical diagnoses of NAFLD, which were based upon unexplained aminotransaminase levels or radiographic imaging consistent with hepatic fatty infiltration.(3, 5, 14-23) Extrapolation of these data to the adult U.S. population suggests an overall prevalence of NAFLD of approximately 20-25%, with the prevalence of NASH specifically, ranging from 3% to 6%.(3, 24) A children's autopsy study in San Diego estimated the prevalence of NAFLD to be 9.6% in children and adolescents, with NASH in approximately one-quarter of these cases.(25)

The potential of NAFLD to progress to more advanced liver disease such as NASH-related cirrhosis is a primary focus of concern. The natural history of NAFLD and factors associated with its progression are not yet fully elucidated. Patients found on initial liver biopsy to have bland steatosis seem to have the best prognosis within the spectrum of NAFLD, whereas features of NASH or advanced fibrosis are associated with a worse prognosis.(26) There is also evidence for a significant risk for the development of hepatocellular carcinoma in patients with cirrhosis arising

from NAFLD.(27) Notably, mortality among NAFLD patients is higher than in the general population, with liver-related death being the 3rd leading cause of mortality; whereas, chronic liver disease and cirrhosis rank 12th as causes of death in the general population.(28) In view of the estimated high prevalence of NAFLD in the general population, these figures hold important public health implications. Moreover, the prevalence of NAFLD is only likely to increase as the prevalence of being overweight or obese increases in the United States.(7)

Although NAFLD is best characterized in Caucasians, racial and ethnic variation in NAFLD has been studied in a handful of U.S. investigations.(3, 14, 29-32) Available data suggest that NAFLD may not be uniformly distributed among different racial and ethnic populations in the U.S. Specifically, NAFLD appears to be most prevalent among Latinos, with African Americans being relatively underrepresented.(33-35) These findings warrant further investigation.

3.3. Pathogenesis of NAFLD

The cause of NAFLD is not yet well-defined, but represents a complex, multifactorial disorder influenced by the interaction of genes and environmental factors.(36-38) Peripheral, hepatic and adipose tissue insulin resistance, with resultant hyperinsulinemia, are central in the development of NAFLD.(39-43) However, the common occurrence of obesity, insulin resistance, and diabetes without NASH strongly suggests that other unidentified etiopathogenic factors also determine the risk of developing hepatic steatosis and NASH. It is widely hypothesized that NAFLD evolves from multiple insults. The first insult is the development of hepatic steatosis, making the liver vulnerable to subsequent insults, such as oxidative stress, endotoxin exposure, and cytokine-mediated cellular damage.(44) Additionally, the variable severity of NAFLD among individuals with similar clinical risk profiles and familial clustering of cases of NAFLD, suggest that genetic factors are also involved in the pathogenesis of NAFLD.(45, 46) Understanding the pathogenesis of NAFLD is of central importance in ultimately finding a treatment, cure, or means of prevention of this disease.

3.4. Noninvasive diagnosis of NAFLD

Liver biopsy remains the gold standard tool for diagnosing and staging NAFLD by providing important information about the degree of steatosis and degree of tissue damage, including inflammation and fibrosis. However, liver biopsy is an imperfect standard that is limited by the invasiveness of the procedure, which is not suitable for use as a population screening tool, and is also limited by sampling error. Therefore, there is an urgent need for the development of valid, reliable and easily applied non-invasive tests (ie, biomarkers and imaging studies) for NAFLD and its histological subtypes. Specifically, desirable markers for NAFLD would be markers that reliably distinguish bland steatosis from NASH, and markers that are accurate for staging fibrosis. The development of such non-invasive markers would not only be useful for diagnostic purposes, but would also have use for ongoing monitoring of disease progression and response to treatment. To

date, biomarkers of interest have been identified based upon the current understanding of the pathogenesis of NAFLD and include markers of oxidative stress, inflammation, hepatocyte apoptosis and markers of hepatic fibrosis.(47, 48) Furthermore, newer technologies are now being applied to NAFLD biomarker development, including lipidomics, proteomics, metabolomic, and genomics, which should help lead to the identification of novel biomarkers of disease.(48) With respect to radiologic imaging tools for the diagnosis of NAFLD, ultrasound, computed tomography, and magnetic resonance imaging have all been studied and applied in clinical practice.(49) However, ultrasound is not quantitative and has limited sensitivity, only detecting moderate to severe steatosis. Computed tomography is widely used in the detection of fatty infiltration of the liver, which is characterized by decreased attenuation of the liver. However, other diseases may also result in decreased liver attenuation on computed tomography (namely, iron overload, copper deposition, glycogen storage disease, or amiodarone therapy), thus limiting the ability of computed tomography to diagnose hepatic steatosis in these settings. Magnetic resonance imaging and magnetic resonance spectroscopy have been shown to be safe and non-invasive methods for the quantitative assessment of hepatic steatosis, and comparisons of magnetic resonance imaging with computed tomography and ultrasound have demonstrated superiority of magnetic resonance imaging for detection of hepatic steatosis, including in children.(50, 51, 52) Further investigations into innovative imaging techniques, with magnetic resonance imaging technologies being particularly promising, are warranted.

Given the current content within the NASH CRN and the anticipated NAFLD patient enrollment in the continuation phase starting in August 2014, the NAFLD Pediatric Database 2 affords outstanding opportunities to (i) continue to explore the natural history of NAFLD and its different histological subtypes; (ii) understand NAFLD disease variation occurring in different segments of the population by enrolling a racially and ethnically diverse group of patients; (iii) further characterize the pathogenesis of NAFLD and develop novel biomarkers of NASH, with the aid of cutting edge technologies such as genomics, metabolomics, lipidomics, and proteomics; and (iv) investigate innovative imaging technologies for the non-invasive diagnosis of NAFLD and fibrosis.

4. Definitions and target population

4.1. Categories of NAFLD

A key challenge is the need to capture a representative clinicopathological cohort of patients with NAFLD, while recognizing that there is significant heterogeneity in the etiology and major associations of this condition. NAFLD occurring in association with rare metabolic disorders, certain drugs, and iatrogenic gastrointestinal disorders may share important pathogenetic mechanisms with the more common variety of NAFLD encountered in the general population. Although such patients are not typical of the condition common in the general population, patients with these disorders offer a means to investigate and better understand the pathogenesis of NAFLD and its progression. The NAFLD Database 2 will also follow selected patients with NASH-related cirrhosis as this entity appears to represent an advanced manifestation of the natural history of NAFLD.

The following broad categories of NAFLD are recognized:

Primary (Insulin Resistant): NAFLD occurring in association with obesity, hyperlipidemia, insulin resistance or diabetes, or occurring without any other apparent associated metabolic abnormalities or other recognized etiologies associated with NAFLD (Secondary NAFLD).

Secondary (Non-Insulin Resistant): NAFLD occurring in association with major disorders of nutrition (gastrointestinal bypass or weight loss-inducing procedures, total parenteral nutrition, rapid weight loss associated with fasting, or gastrointestinal disease) or pharmacologic and toxic agents. Some etiologies of secondary NAFLD (e.g., gastrointestinal surgery, bypass) may lead to rapidly progressive disease.

Acute syndromes of microvesicular fatty liver disease: Acute fatty liver of pregnancy, Reye's syndrome, inborn errors of metabolism with known genetic defects (urea cycle, fatty acid β-oxidation pathway), hepatotoxicity from valproic acid, nucleoside analogues, and other drugs and toxins.

4.2. Definitions for the purposes of the NASH CRN studies

NAFLD

- Fat accumulation in the liver (steatosis) involving at least 5% of hepatocytes on routine stains
- No evidence of other acute or chronic liver disease

Cryptogenic or NASH-related cirrhosis

- Either histological or clinical cirrhosis without etiologic evidence of other chronic liver disease that is known to lead to cirrhosis
- Histological cirrhosis that fails to meet the histological definition of NAFLD and without histological evidence of other chronic liver disease that is known to lead to cirrhosis

4.3. Target composition of Database 2 pediatric population

The NAFLD Database 2 will recruit at least 650 new pediatric participants and will also invite pediatric participants from the prior NAFLD Database and TONIC trial to enroll in the NAFLD Pediatric Database 2 study.

All the new pediatric participants will have had a liver biopsy within 120 days prior to enrollment coupled with contemporaneous biosamples within 90 days prior to enrollment and up to 90 days before or 4-90 days after the biopsy. We estimate that at least 50 of the continuing pediatric participants will be due for a standard of care liver biopsy at the time of their enrollment into the pediatric Database 2 study, and will, as a result, also have a liver biopsy and contemporaneous biosamples. Combining the new and continuing participants leads to a recruitment goal for the Database 2 of 700 pediatric participants with liver biopsies and contemporaneous biosamples during the 18 month enrollment period.

An estimated additional 150 pediatric participants continuing from the NAFLD Database study and TONIC trial will also be enrolled, but without a contemporaneous liver biopsy at the time of enrollment. It is expected that many of these pediatric participants may receive clinically indicated liver biopsies during the period of follow-up for Database 2 which will be combined with contemporaneous biosamples.

To summarize, the target composition for the pediatric Database 2 study is:

New pediatric participants	650
Continuing pediatric participants, biopsied at enrollment	50
Continuing pediatric participants, not biopsied at enrollment	150
Total pediatric participants in the Database 2 study	850

The number of new enrollees with NASH-related cirrhosis will be limited to at most 40 pediatric participants from all clinical centers combined. Site specific enrollment of participants in each category will be monitored by the DCC during the enrollment period to ensure adherence with this limitation.

5. Selection and enrollment of participants

5.1. Recruitment

The NAFLD Pediatric Database 2 study will build upon the population recruited in NAFLD Database by enrolling approximately 650 new pediatric participants. Enrollment of participants was initiated in December 2009 and will continue to at least April 2019. The expectation is that recruitment will come from 11 participating clinical centers with rates of recruitment varying according to the resources and patient populations of each center.

5.2. Inclusion criteria

Continuing participants previously met inclusion (and exclusion) criteria for the NAFLD Database study or the TONIC trial; these criteria are not listed here, but are in the applicable protocols for these NASH CRN studies. Both continuing and new pediatric participants must meet all of the inclusion criteria below, which are listed separately for continuing and new participants.

Continuing participants:

- Previously enrolled in the NAFLD Database study or TONIC trial
- Age at least 2 years and not older than 17 years during the consent process
- Willingness to be in the study for 1 or more years
- Ability and willingness to give written, informed parental consent and child assent, per local IRB guidelines, to be enrolled into the pediatric Database 2 study

New participants:

- Age at least 2 years of age and not older than 17 years during the consent process
- Willingness to be in the study for 1 or more years
- Ability and willingness to give written, informed parent consent and child assent to be enrolled into the pediatric Database 2 study
- Minimal or no alcohol use history consistent with NAFLD
- Having undergone a liver biopsy that is obtained within 120 days of enrollment
- Collection of biosamples (serum, plasma, DNA, and, if available, liver tissue) within 90 days prior to enrollment and 0-90 days before or 4-90 days after the standard of care liver biopsy

5.3. Exclusion criteria

Continuing participants who meet the following criterion will not be eligible:

- Any condition or circumstances, which, in the opinion of the investigator, would interfere with completion of scheduled follow-up visits and procedures for the duration of the pediatric Database 2 study

New participants who meet any of the following criteria will not be eligible:

- Total parenteral nutrition for more than 1 month within a 6 month period before baseline liver biopsy
- Short bowel syndrome
- History of gastric or jejunoileal bypass preceding the diagnosis of NAFLD. Bariatric surgery performed following enrollment is not exclusionary. Liver biopsies obtained during bariatric surgery cannot be used for enrollment because of the associated surgical or anesthetic acute changes and the weight loss efforts that precede bariatric surgery
- History of biliopancreatic diversion
- Evidence of advanced liver disease defined as a Child-Pugh-Turcotte score equal to or greater than 10
- Evidence of chronic hepatitis B as marked by the presence of HBsAg in serum (participants with isolated antibody to hepatitis B core antigen, anti-HBc total, are not excluded)
- Evidence of chronic hepatitis C as marked by the presence of anti-HCV or HCV RNA in serum
- Low alpha-1-antitrypsin level and ZZ phenotype (both determined at the discretion of the investigator)
- Wilson's disease
- Known glycogen storage disease
- Known dysbetalipoproteinemia
- Known phenotypic hemochromatosis (HII greater than 1.9 or removal of more than 4 g of iron by phlebotomy)
- Prominent bile duct injury (florid duct lesions or periductal sclerosis) or bile duct paucity
- Chronic cholestasis
- Vascular lesions (vasculitis, cardiac sclerosis, acute or chronic Budd-Chiari, hepatoportal sclerosis, peliosis)
- Iron overload greater than 3+
- Zones of confluent necrosis, infarction, massive or sub-massive, pan-acinar necrosis
- Multiple epithelioid granulomas
- Congenital hepatic fibrosis

- Cystic fibrosis
- Polycystic liver disease
- Other metabolic or congenital liver disease
- Evidence of systemic infectious disease
- Known HIV positive
- Disseminated or advanced malignancy
- Concomitant severe underlying systemic illness that in the opinion of the investigator would interfere with completion of follow-up
- Active drug use or dependence that, in the opinion of the study investigator, would interfere with adherence to study requirements
- Any other condition, which in the opinion of the investigator would impede compliance or hinder completion of study
- Inability for parent to provide informed consent and child ∋ 8 years to give assent

5.4. Database 2 enrollment procedures

Clinical centers must be certified by the DCC to open enrollment in the NAFLD Pediatric Database 2 study. Prior to implementation of this protocol, the principal investigator must have the protocol, assent, and consent forms approved by his/her Institutional Review Board (IRB). Once a candidate for pediatric Database 2 study entry has been identified, study procedures will be carefully discussed with the participant and his/her family as required by the local IRB. The participant will be asked to read and sign the assent or consent form that was approved by the local IRB. There will be a separate consent for the collection, storage, and use of DNA for genetic research.

6. Schedule of visits and procedures

6.1. Screening, consent, and follow-up overview

While many of the new NAFLD Pediatric Database 2 participants may come from the current patient rosters of the NASH CRN investigators, children may be referred to the NASH CRN from physicians outside the Network and some patients may refer themselves. In addition, children may be recruited from established registries permitting patient contact for enrollment in clinical trials. Patients considered by the site investigator as likely to be eligible for enrollment as a new or continuing pediatric participant in Database 2 may be consented and screened at a visit that is part of the ongoing clinical care of the patient. Tests may be ordered and billed to insurance to appropriately complete the evaluation of liver disease and general medical condition according to a reasonable standard of care. Similarly, a standard of care liver biopsy billed to insurance will be obtained when clinically indicated to establish a diagnosis and stage the disease. Patients will be sought for participation in the pediatric Database 2 study who need or recently have had (≤120 days prior to enrollment) a liver biopsy to evaluate suspected NAFLD.

Patients who are thought to be candidates will be invited to be screened for enrollment in the pediatric Database 2 study. Screening will take place over two visits. At the initial screening visit, the study procedures of Database 2 participation will be introduced. If the participant is agreeable and is thought to have NAFLD or NASH-related cirrhosis, then he/she may enter the formal screening phase.

Screening and baseline data collection procedures will usually be conducted over two (or more) clinic visits completed on separate calendar days. The goal of the first screening visit is to obtain parent consent/child assent and start recording screening data regarding the study's inclusion and exclusion criteria on NAFLD Database 2 data forms. The goal of the second screening visit is to complete procedures and collection of baseline data on participants who appear to be eligible. This separation of procedures between two visits is provided as a practical guideline. Screening procedures and data collection can be organized as appropriate at each clinical center. The signing of the consent statement and the procedures during screening can occur on one day or separate calendar days and may occur over a period of up to 90 days. Baseline etiologic tests may not need to be repeated in continuing pediatric participants if there have been no substantial changes in risk factors to explain the etiology of liver disease.

Consent for screening and HIPAA authorization to disclose protected health information with the NAFLD Pediatric Database 2 study must be obtained from the participant and parent prior to initiating any data collection for the NAFLD Database 2 study; this consent and authorization must be obtained at the start of the initial screening visit. The consent will include a parent consent and a child assent for more general screening and enrollment in the pediatric Database 2 study, including follow-up for the duration of the study. Parent consent for the collection, storage, and use of blood

samples for current and future genetic research will be obtained separately. HIPAA authorization forms will be prepared according to clinical center IRB requirements and guidelines.

Minimum follow-up on a participant will be 48 weeks, and maximum follow-up on a participant will be 480 weeks. Data will be collected during screening (2 visits) and at yearly intervals thereafter (a maximum of 12 visits). Appendix 10.3 displays the data collection schedule for screening and follow-up.

6.2. Screening visit

Formal screening begins once the participant and his/her parent have signed the HIPAA authorization and consent or child assent for screening and enrollment in the NAFLD Pediatric Database 2 study. The participant is considered to be registered in the pediatric Database 2 study once the consent is signed and the Registration Form has been completed. Consent for enrollment may be conducted at standard of care clinic visits, or over the phone with eligible participants, who may receive the consent (at a clinic visit or mailed) to review prior to the registration visit. Recording of data on NAFLD Database 2 study forms may begin once the consent and authorization forms are obtained.

The screening visits may be conducted over two or more study visits. Clinical centers may alter the order of screening procedures done on a particular visit to meet the center's or participant's needs. The last screening visit may be combined with enrollment for the convenience of the participant. This visit schedule allows flexibility in completion of screening procedures, however, a participant will be enrolled only if the data system shows that the participant is eligible, has signed the consent statement, and has had all required screening forms keyed to the data system. Activities at initial screening visit include:

- Signature on the NAFLD Database 2 parent consent, pediatric assent, as needed, and genetic consent form
- Signature on NAFLD Database 2 pediatric study HIPAA authorization form
- Assignment of NASH CRN participant identification number (only for new pediatric participants, continuing participants retain their current ID)
- Medical and medication history
- Habitual beverage intake assessment (Beverage Questionnaire [BEVQ-15])
- Physical examination including vital signs, height, weight, anthropometric measurements, acanthosis nigricans, liver signs, and Tanner staging
- Alcohol use history (Alcohol Use Disorders Identification Test [AUDIT]) for participants 12 years or older, if any prior history of alcohol use is stated
- Review status of liver biopsy data
- Order etiologic tests if needed
- Instruct participant to bring to second screening visit his/her health history information or related materials
- Participant or the guardian to sign medical records release to obtain prior reports and

biopsy slides

- Participant to provide location and contact information
- Coordinator to register participant on clinic data system
- Coordinator to request prior reports and biopsy slides from health care provider
- Fasting blood draw for specimen banking (either screening visit-see below)
- Schedule liver biopsy, if needed
- Schedule second screening visit

The second screening visit should ideally be timed to coincide with the standard of care liver biopsy and is the encounter at which the participant has blood drawn for outstanding laboratory tests and specimen banking. The participant must attend this visit after a 12 hour fast. Blood for serum and plasma banking may be drawn immediately prior to the liver biopsy, but cannot be obtained in the 72 hour period after a liver biopsy. The goals for achieving optimum timing of blood draws for serum and plasma banking are that at least 50% of enrollees will have these biosamples obtained within ± 30 days of the enrollment liver biopsy, while the remaining 50% will have these drawn within ± 90 days of liver biopsy. Standard of care procedures are described in manuals of Standard Operating Procedures V: Standard of Care for Pediatric Participants with Fatty Liver Disorders and Standard Operating Procedures I: Pediatric Clinical Center Operations.

Procedures at the second screening visit include:

- Hematology
- Clinical chemistry
- HbA1c
- Hepatic panel
- Fasting lipid profile and fasting glucose and insulin levels
- Fasting blood (serum, plasma) for specimen banking
- Liver biopsy (if needed)
- Provision of pediatric standard of care educational materials (delay providing these to participant until confirmed eligible for the pediatric Database 2 study)

Procedure for combining screening visits

Efficient compression of the enrollment visit schedule can be accomplished by centering initial data and serum and plasma sample collection around the performance of a scheduled standard of care liver biopsy. An overnight stay in a General Clinical Research Center (GCRC) or similar research unit may be used if a clinically indicated biopsy can be undertaken within these premises. Day 1: study questionnaires completed, physical examination, history data obtained; labs drawn including serum and plasma for banking (if fasting; can be done prior to the liver biopsy on day 2 if necessary). Day 2: Liver biopsy performed in the morning. If standard of care biopsy cannot be undertaken in the research unit, coordination with the outpatient procedure center will be necessary for the participant to be discharged from the research unit on the morning of Day 2 and transferred to the clinical procedure facility for the liver biopsy.

6.3. Liver histology requirements

For a new participant to be enrolled in the pediatric Database 2 study, the participant must have a recent liver biopsy that is available for review by the NASH CRN pathologist, and the NASH CRN pathologist must confirm that the biopsy is not exclusionary (i.e., evidence of liver disease not related to NAFLD – see SOP IV: Liver Biopsy and Histology Scoring System, section 4.2.8). A liver biopsy may be obtained as part of standard of care (e.g., for diagnosis or follow-up) as determined by the site investigator during screening. Ideally, this biopsy will be obtained within 30 days of the serum and plasma sample collection, but must be obtained no more than 120 days prior to enrollment and within 90 days of the serum and plasma collection. Liver biopsies will be performed primarily for histological purposes, with the additional goal of obtaining liver tissue, in excess of that needed for histology, from the same biopsy pass, for snap freezing in liquid nitrogen (expression library/cDNA production, proteomics, lipidomics). The procedures for collection and processing of liver biopsy materials are detailed in the NAFLD Database 2 Standard Operating Procedures IV: Liver Biopsy and Histology Scoring System.

6.4. Follow-up visits

The pediatric Database 2 study annual follow-up study visits will be scheduled at 48 week intervals after enrollment. The date of enrollment is when all screening procedures are completed and eligibility checks are fulfilled for study entry, and will be the date from which the follow-up visits are timed (i.e., time zero). Each follow-up visit will have a time window around the target date for the visit; the time window is an interval of months during which the study visit may be completed, and the data collected at the visit may be used to fulfill the data collection requirements for the visit. Data or serum and plasma samples collected outside the allowable time window for a visit are not useable as data for the visit. Each visit has an ideal date for the visit, a lower window date (opening date for the window) and an upper window date (closing date for the window). The dates for a specific participant are specified on the NAFLD Pediatric Database 2 study visit time windows sheet for the participant. This sheet is generated by the clinic data system when a participant is enrolled in the pediatric Database 2 study; it can also be obtained from the NASH CRN data system.

Procedures and forms to be completed at each of the follow-up visits are:

- Follow-up medical history (medication changes, key events or interventions, surgeries, hospital admissions, new diagnoses of co-morbidities, complications of liver disease [variceal bleeding, ascites, edema, hepatic encephalopathy]
- Physical examination
- Habitual beverage intake assessment (Beverage Questionnaire [BEVQ-15])
- Laboratory data (hematology, glucose and insulin, clinical chemistry, hepatic panel, HbA1c, lipid profile)
- Interim drinking history (Alcohol Use Disorders Identification Test - Consumption [AUDIT-C]), for patients 12 years or older
- Blood collection for plasma and serum banking
- Documentation of any additional liver biopsies performed as part of standard of care

6.5. Database 2 contents

Baseline medical history. The NAFLD Pediatric Database 2 study forms will capture whether the participant was ever diagnosed with NAFLD, dates of biopsies, abbreviated weight history, family history (siblings and parents; particularly of obesity and liver disease), demographic history, past medical or surgical events, illness history (including diabetes, gestational diabetes, hypertension, lipodystrophy, polycystic ovarian syndrome), other co-morbid conditions including previously diagnosed lipid and metabolic disease-related conditions (hypercholesterolemia, hypertriglyceridemia), and all diagnoses related to previous liver disease as well as other diagnoses of major organ systems.

Baseline medication history. Pediatric Database 2 case report study forms will capture selected medications taken within 3 months prior to entry. Specific medications queried will include those taken for the treatment of liver disease, diabetes, insulin resistance, hypertension, and hyperlipidemia. Any known medication allergies will be documented.

Follow-up medical history. Follow-up health history will include data on medication changes; key events or interventions, surgeries, hospital admissions; new diagnoses of comorbidities; complications of liver disease [variceal bleeding, ascites, edema, hepatic encephalopathy], liver cancer, other cancer.

Beverage intake. The objective of the beverage intake survey is to estimate the patient's habitual consumption of beverages and the amount of daily calories and grams of sugar consumed from these beverages. The Beverage Questionnaire (BEVQ-15) is administered at baseline and at yearly follow-up visits. The BEVQ-15 is a 15-item questionnaire asking how often and the amount each type of beverage is consumed during the past month. The estimated patient burden is 10 minutes to complete the survey.

Physical examination. Initial and subsequent exams will include: vital signs [temperature, pulse, respiratory rate, blood pressure], anthropometrics [height, weight (without shoes or heavy clothing), waist (at umbilicus), and hip circumference measurements]; general signs [lipodystrophic body habitus, muscle wasting, acanthosis nigricans]; liver signs [jaundice, spider angiomata, palmar erythema, hepatomegaly, splenomegaly, asterixis,]; and Tanner staging.

Alcohol consumption. The objective of the alcohol use data collection is to estimate recent quantity of alcohol ingestion. The AUDIT is a 10-item questionnaire with a simple scoring scale. The Alcohol Use Disorders Identification Test (AUDIT) questionnaire will be administered at baseline to patients 12 years or older; an interim drinking history (AUDIT-C) will be obtained at yearly follow-up visits as part of the follow-up medical history.

Laboratory tests. All laboratory items listed may be obtained from the participant's chart or should be collected as part of the standard of care. Acceptable time intervals between the collection of laboratory items and the pediatric Database 2 study registration will be specified on the data collection forms; for follow-up visits, the laboratory blood draws must be done within the visit window. The blood draw date of the laboratory test must be recorded on the data form.

Hematology: Hemoglobin, hematocrit, mean corpuscular volume (MCV), red blood cell count (RBC), white blood cell count (WBC), and platelet count.

Clinical chemistry and HbA1c: Creatinine, total protein, blood urea nitrogen (BUN), uric acid, and hemoglobin A1c (HbA1c).

Hepatic panel: Total bilirubin, direct bilirubin, aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase, gamma glutamyltransferase (GGT), albumin, prothrombin time (PT), and international normalized ratio (INR).

Fasting lipid profile and glucose-insulin levels: Fasting is defined as nothing by mouth except water and medications for at least 12 hours prior to blood draw. Lipid profile will include fasting triglyceride and cholesterol (total, low-density lipoprotein and high-density lipoprotein cholesterol). Please note whether on lipid medication when lipid profile blood was drawn. If in doubt about fasting status, the test should be repeated. The fasting blood glucose and insulin must be measured on the same blood draw and can be drawn at the same time as the lipid profile.

The tests listed below are required only during screening for new participants.

Screening etiologic tests: Hepatitis B surface antigen, hepatitis C antibody.

Ceruloplasmin and alpha-1-antitrypsin level (plus phenotype if below normal).

Autoantibody studies: anti-nuclear antibody (ANA), antismooth muscle antibody (ASMA), anti-liver/kidney microsomal antibody (Anti-LKM1)
Thyroid Stimulating Hormone (TSH)

6.6. Serum, plasma, DNA, and liver tissue for banking

Fasting serum and plasma, DNA, and any available snap frozen liver tissue will be banked in the NAFLD Pediatric Database 2 study. Blood will be drawn for serum and plasma banking during screening and yearly visits on all participants enrolled in the pediatric Database 2 study. Standardized methods for serum, plasma, DNA, and liver tissue processing that allow for maximal preservation of banked specimens and storage in designated -70 degrees C freezers will be applied across all clinical centers or at the NASH CRN central repository (see NAFLD Pediatric Database 2 Standard Operating Procedures I: Clinical Center Operations).

7. Statistical and design considerations

This is a clinicopathologic condition-based database designed primarily to allow the compilation of data and collection of specimens for the purpose of the study of the epidemiology and natural history of NAFLD in children. The study also aims to recruit patients for participation in randomized, controlled therapeutic trials. The recruitment goal is a database of 850 pediatric participants with NAFLD with at least 96 weeks of follow-up.

The primary and secondary objectives of the NAFLD Pediatric Database 2 study, as specified in Sections 2.1 and 2.2 of the protocol, are intentionally very broad. Since this is the largest and most comprehensive prospective study of NAFLD in children, it is expected that many, as yet unasked, questions related to the etiology, natural history, diagnosis, treatment, and prevention of NAFLD will be answered.

Addressing the questions that arise in connection with the NAFLD Database 2 will require the full gamut of statistical analysis procedures, from survival analysis to receiver operating characteristic (ROC) curves. Nearly all questions will require identification of appropriate subgroups of patients from all clinical centers contributing to the NAFLD Pediatric Database 2 study with analyses tailored to the hypothesis or question to be addressed.

This process will be accomplished through formal publication proposals submitted by investigators to the NASH CRN Steering Committee. Each of these proposals must include a justification for the sample size and plan for statistical analysis constructed with the help of the Data Coordinating Center. Each proposal must contain the following information:

- Specification of the primary and secondary outcome variables
- Specification of subgroups of special interest when making treatment comparisons (e.g., males vs. females or ethnic/racial subgroups)
- Specification of baseline covariates or methods to be used to select the covariates for adjustment of treatment comparisons
- Specification of methods for dealing with missing values or lost to follow-up
- Specification of primary and confirmatory analytic methods to be used

Since the NAFLD Pediatric Database 2 study will generate longitudinal data over time, analytic methods must account for, as applicable, time to events, repeated measurements, counts, or other discrete responses. For time to event data, we will use the Cox proportional hazards regression model with covariates tailored to the hypothesis under investigation. For hypotheses involving repeated measurements, events, counts or other discrete responses, we will use either of two approaches: (1) generalized linear models with generalized estimating equations (GEE) with robust variance estimation to account for the clustering; or, (2) multilevel generalized linear mixed models with random coefficients to account for within patient clustering as well as other sources of variations like clinic effects.

8. Human research participant issues

8.1. Overview

The study protocol, consent forms, and data collection forms will be submitted to each clinical center's IRB and to the DCC's IRB. Additionally, each clinical center will submit to their IRB any recruitment materials to be used at their site. A site may not initiate any participant contact about the NAFLD Pediatric Database 2 study until the site has IRB approval and the DCC has certified the site for initiation of screening activities. All study personnel will have completed training in the Protection of Human Participants per NIH guidelines. The Pediatric NAFLD Database 2 study anticipates recruiting a significant proportion of racial/ethnic minorities (African American, Hispanics, Asian, American Indian/Alaska Native, Native Hawaiian or other Pacific Islander) as well as non-Hispanic white participants. We anticipate that the participants recruited from diverse sources, including community and tertiary referral populations, will capture the entire spectrum of NAFLD in children.

Standard of care

All participants enrolled in the NAFLD Pediatric Database 2 will receive the standard of care for NAFLD and identified associated medical problems as defined by the NASH CRN Standards of Care Committee (see SOP V: Standard of Care for Pediatric Participants with Fatty Liver Disorders). This will include provision of health care counseling and educational materials at enrollment and on an ongoing basis during follow-up.

Institutional Review Board (IRB) approval

A site may not initiate screening activities until the site has IRB approval for the NAFLD Pediatric Database 2 study. Assent and Consent forms must have IRB approval. Sites must provide the DCC with copies of the IRB approval notice and subsequent renewals, as well as copies of the IRB approved consent statements.

Consent

Prototype pediatric assents and consents will be prepared for the study. Individual sites may add material but may not delete material thought to be necessary for informed consent. Sites may reword information to conform to their local IRB requirements. A signed consent form will be obtained from the participant. The consent form will describe the purpose of the study, the procedures to be followed, and the risks and benefits of participation. A copy of the consent form will be given to the participant and this fact will be documented in the participant's record.

Subject confidentiality

All laboratory specimens, evaluation forms, reports, and other records that are part of the study data collection and entry materials will be identified by coded number only to maintain subject confidentiality. All records will be kept in locked file cabinets with access limited to NASH CRN investigators. All computer entry and networking programs will identify subjects by participant identification number. Clinical information will not be released without written permission of the subject, except as necessary for monitoring by the IRB or DSMB. Clinical information may be reviewed during site visits, but use of personal identifiers will be avoided. Consent procedures and forms, and the communication, transmission and storage of participant data will comply with individual site IRB and NIH requirements for compliance with The Health Insurance Portability and Accountability Act (HIPAA).

8.2. Adverse event reporting

The NAFLD Pediatric Database 2 study will monitor and report unanticipated or adverse events to ensure participant safety in compliance with 45 CFR Part 46, Subpart A the "Common Rule". The Common Rule requires written procedures and policies for ensuring reporting of "unanticipated problems" involving risks to participants, IRBs, appropriate institutional officials, and the Department or Agency Head. Since the adverse event definitions and reporting requirements for unanticipated events may differ at each participating site, the Pediatric Database 2 study definitions and procedures for adverse event reporting are designed to satisfy a wide spectrum of interpretations of the Common Rule requirements. While the definitions and monitoring procedures apply most directly to clinical trials, all participants in the pediatric Database 2 will be monitored for occurrence of adverse events, and any adverse events that occur will be reported as appropriate. The FDA Guidance for Clinical Investigators on Adverse Event Reporting to IRBs - Improving Human Subject Protection document also provides recommendations for adverse event reporting, while specifically focusing on unanticipated event reporting.(53) The FDA recommends that careful review of whether an adverse event is an unanticipated event that must be reported to IRBs should be considered while adhering to local IRB guidelines.

Definitions

Adverse event. An adverse event is any untoward medical occurrence that may present itself during treatment or administration with a pharmaceutical product or clinical procedure and which may or may not have a causal relationship with the treatment. Adverse events include any unanticipated problems involving risks to participants, or breaches of protocol which might entail risk to participants. The term "unanticipated problem" includes both new risks and increased rates of anticipated problems.

Adverse events will be recorded on study data forms whether or not they are thought to be associated with the pediatric Database 2 study participation or prior participation in a NASH CRN study. Adverse events may be discovered during regularly scheduled visits or through unscheduled participant contacts between visits.

Serious adverse event (SAE). A serious adverse event is an adverse event occurring at any time during the study that results in death, life-threatening adverse drug experience, in-patient hospitalization or prolongation of existing hospitalization, a persistent or significant disability or incapacity, or a congenital anomaly orbirth defect. Other events may also be considered an SAE if, based on medical judgment, the event jeopardized the participant to the point of requiring medical or surgical intervention to prevent the occurrence of any of the conditions for an SAE listed above.

Unexpected adverse event. An unexpected adverse event is any adverse event with specificity or severity that is not consistent with the risk information in the study protocol, current investigator brochure, or current package insert.

Reporting serious adverse events

Serious adverse events must be reported upon discovery at the clinical center. This will involve describing the severity and details of the event on the case report form. The case report form, together with a memo summarizing the circumstances of event and the current status of the participant, must be faxed to the Data Coordinating Center and to the NIDDK Project Scientist within 3 working days of the discovery of the SAE. Also within 3 days, the clinical center must notify the NIDDK and Data Coordinating Center of the SAE by telephone or confirmed e-mail. The NIDDK Project Scientist will work with the Data Coordinating Center to transmit the case report form and memo to all study centers and to the DSMB if needed.

The DSMB will review each SAE report and provide comments to the NIDDK Project Scientist within one week of receipt of the report. If requested by any member of the DSMB, a teleconference will be scheduled to discuss the SAE and recommend any actions to the NIDDK.

The clinical center must submit to the NIDDK Project Scientist and to the Data Coordinating Center a follow-up memo within one month of the SAE (and periodic updates if needed) to report the details of the disposition of the SAE. The NIDDK Project Scientist will work with the Data Coordinating Center to distribute the follow-up memo to the clinical center and to the DSMB.

Review of adverse events by the DSMB

Summary data on adverse events will be monitored by the DSMB at its semi-annual meetings or more frequently, as needed. These summaries will include analyses comparing rates of adverse events by clinic, or in other subgroups requested by the DSMB. Where applicable, signs and

symptoms associated with the adverse event will be graded as to severity by the clinical site staff as mild, moderate, or severe using the Common Terminology Criteria for Adverse Events.(54)

After each meeting, the DSMB will issue a written summary of its review of the study data, including adverse events, for transmission to the IRBs at each of the study centers. Analyses or listings of adverse events will not be provided to the IRBs; however, adverse events involving unanticipated problems involving risks to participants, or breaches of protocol which might entail risk to participants must be reported to local IRBs as soon as possible after they are discovered. Each participating center is responsible for ensuring that all local IRB requirements for reporting adverse events are met.

8.3. Participant withdrawal

If a participant chooses to withdraw from the NAFLD Pediatric Database 2 study, all data collected up to the point of withdrawal will remain in the Database 2, but no further data may be collected. This is consistent with HIPAA guidelines and regulations.

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10. Appendices

10.1. Participating centers

Clinical Centers*

- Cleveland Clinic Foundation, PI: Arthur McCullough, MD
  - **University of Cincinnati Medical Center**, PI: Stavra Xanthakos, MD
- **Columbia University**, PI: Joel Lavine, MD, PhD
- Duke University, PI: Anna Mae Diehl, MD
  - **Johns Hopkins University**, PI: Ann Scheimann, MD, MBA
  - **Northwestern University**, PI: Peter Whitington, MD
- Indiana University, PI: Naga Chalasani, MD
  - **Riley Hospital for Children**, PI: Jean Molleston, MD
- Saint Louis University, PI: Brent Tetri, MD (Adult component); Ajay Jain, MD (Pediatric component)
  - **Baylor Medical College**, PI: Sarah Barlow, MD
- University of California, San Diego, PI: Jeffrey Schwimmer, MD (Pediatric component); Rohit Loomba, MD (Adult component)
- University of California, San Francisco, PI: Philip Rosenthal, MD (Pediatric component); Norah Terrault, MD (Adult component)
- Swedish Medical Center, PI: Kris Kowdley, MD
  - **Seattle Children's Hospital**, PI: Karen Murray, MD
  - **University of Buffalo**, PI: Susan Baker, MD, PhD
- Virginia Commonwealth University, PI: Arun Sanyal, MD
  - **Emory University**, PI: Saul Karpen, MD, PhD

Data Coordinating Center:

- Johns Hopkins University

National Institutes of Health:

- National Institute of Diabetes and Digestive and Kidney Diseases
- National Cancer Institute

NIDDK Central Repositories:

- Biosample repository: Fisher Bioservices Corporation
- Genetics repository: Rutgers University Cell and DNA Repository (RUCDR) Infinite Biologics
- Data repository: Information Management Services, Inc (IMS)

*NASH CRN clinical centers, which are bolded, participate in the pediatric component of the NAFLD Database 2 only.

10.2. Data collection schedule

			Follow-up visits: weeks from enrollment									
	Screening	Enrollment	t048	t096	t144	t192	t240	t288	t336	t384	t432	t480
Consent and HIPAA authorization	X	-	-	-	-	-	-	-	-	-	-	-
Baseline medical history	X	-	-	-	-	-	-	-	-	-	-	-
Follow-up medical history (including interim drinking history, medication)	-	-	X	X	X	X	X	X	X	X	X	X
Beverage Intake	X	-	X	X	X	X	X	X	X	X	X	X
Physical examination	X	-	X	X	X	X	X	X	X	X	X	X
Liver biopsy review†	A	-	A	A	A	A	A	A	A	A	A	A
Provision of standard of care materials	X	-	-	-	-	-	-	-	-	-	-	-
Database eligibility confirmation	-	X	-	-	-	-	-	-	-	-	-	-
Alcohol use questionnaires												
AUDIT	X	-	-	-	-	-	-	-	-	-	-	-
Lifetime drinking history (Skinner)	X	-	-	-	-	-	-	-	-	-	-	-
Hematology	X	-	X	X	X	X	X	X	X	X	X	X
Hepatic panel	X	-	X	X	X	X	X	X	X	X	X	X
Clinical chemistry	X	-	X	X	X	X	X	X	X	X	X	X
HbAlc	X	-	X	X	X	X	X	X	X	X	X	X
Lipid profile (fasting)	X	-	X	X	X	X	X	X	X	X	X	X
Glucose and insulin levels (fasting)	X	-	X	X	X	X	X	X	X	X	X	X
Etiologic tests‡	X	-	-	-	-	-	-	-	-	-	-	-
Specimens for banking§	X	-	X	X	X	X	X	X	X	X	X	X

A = as available

† Liver biopsy required for new patients; as available for continuing patients

Hepatic panel: Total bilirubin, direct bilirubin, aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase, gamma glutamyltransferase (GGT), albumin, prothrombin time (PT), INR.

Hematology: Hemoglobin, hematocrit, mean corpuscular volume (MCV), white blood cell count (WBC), red blood cell count (RBC), platelet count.

Clinical chemistries: Creatinine, total protein, BUN, uric acid, and HbA1c

Lipid profile: triglycerides, total cholesterol, LDL and HDL

‡ Etiologic tests: Hepatitis B surface antigen, hepatitis C antibody. Ceruloplasmin, alpha-1-antitrypsin level. Autoantibody studies (ANA, ASMA, anti-LKM1), thyroid stimulating hormone (TSH)

§ Specimens for banking include: liver tissue, serum, plasma, DNA

¶ Only have participants ≥ age 12 complete, if any prior history of alcohol use is stated

10.3. Whole blood draw schedule

	Study visit (week)											
Procedure	**Screening**	**t048**	**t096**	**t144**	**t192**	**t240**	**t288**	**t336**	**t384**	**t432**	**t480**	**Total**
Fasting glucose and insulin	5	5	5	5	5	5	5	5	5	5	5	55
Fasting lipid	5	5	5	5	5	5	5	5	5	5	5	55
Complete blood count	5	5	5	5	5	5	5	5	5	5	5	55
Clinical chemistry	5	5	5	5	5	5	5	5	5	5	5	55
Hepatic panel	5	5	5	5	5	5	5	5	5	5	5	55
HbA1c	5	5	5	5	5	5	5	5	5	5	5	55
Plasma	10	10	10	10	10	10	10	10	10	10	10	110
Serum	30	20	20	20	20	20	20	20	20	20	20	230
Genetics	10	-	-	-	-	-	-	-	-	-	-	10
Other screening*	20	-	-	-	-	-	-	-	-	-	-	20
Total	**100**	**60**	**60**	**60**	**60**	**60**	**60**	**60**	**60**	**60**	**60**	**700**

All Database 2 study visits are fasting visits and need to be scheduled for early morning. Fasting is defined as nothing by mouth except water in the 12 hours prior to blood draw.

* Etiologic tests as needed

10.4. Glossary

ALT	-	alanine aminotransferase
AMA	-	antimitochondrial antibody
ANA	-	anti-nuclear antibody
ASMA	-	antismooth muscle antibody
AST	-	aspartate aminotransferase
AUDIT	-	Alcohol Use Disorders Identification Test
BMI	-	body mass index (kg/m^2)
BUN	-	blood urea nitrogen
CC	-	Clinical Center
CRN	-	Clinical Research Network
CT	-	computed tomography
DSMB	-	Data and Safety Monitoring Board
DCC	-	Data Coordinating Center
GCRC	-	General Clinical Research Center
GGT	-	gamma glutamyltransferase
HbA1c	-	hemoglobin A1c
HBc	-	hepatitis B core antigen
HBsAg	-	hepatitis B surface antigen
HCV	-	hepatitis C virus
HIPAA	-	Health Insurance Portability and Accountability Act
INR	-	international normalized ratio
IRB	-	institutional review board
MCV	-	mean corpuscular volume
MRI	-	magnetic resonance imaging
MRS	-	magnetic resonance spectroscopy
NIH	-	National Institutes of Health
NAFL	-	nonalcoholic fatty liver
NAFLD	-	nonalcoholic fatty liver disease
NASH	-	nonalcoholic steatohepatitis
NSAIDs	-	nonsteroidal anti-inflammatory drugs
PPARγ	-	peroxisome proliferator-activated receptor-gamma
PT	-	prothrombin time
RBC	-	red blood cell count
SAE	-	serious adverse event
SOC	-	standard of care
UDCA	-	ursodeoxycholic acid
ULN	-	upper limit of normal
WBC	-	white blood cell count

10.5. Document History

NAFLD Pediatric Database 2 protocol (25 September 2009)

Minor wording changes were made to the following section:

§10.1 Participating centers:

- Added **Columbia University**, PI: Joel Lavine, MD PhD
- Changed "University of California, San Diego, PI: Joel Lavine, MD (Pediatric component" to "University of California, San Diego, PI: Jeffrey Schwimmer, MD (Pediatric component)"

NAFLD Pediatric Database 2 protocol (16 February 2011)

Design Synopsis

- **Recruitment**: the 1st bullet titled Target recruitment period: "October 2009 to March 2011" was changed to "December 2009 to 31 December 2012"
- **Duration of follow-up**: "1-4 years (until December 31, 2013)" was changed to "Minimum of one year of follow-up (1-4 years until 31 December 2013)"

§5.1 **Recruitment**

- Second sentence was revised to extend recruitment: "Enrollment of participants may be initiated, pending IRB approval, on December 2009 and continue through December 31, 2012." Note: the parenthetical clause at the end of sentence: (18 month recruitment period)." was deleted.

- Assumptions revised in sentences 3-6 of paragraph: "For new pediatric participants (n=250), this will translate into 0.85 participants per month per center. For all pediatric participants, new biopsies from new and continuing participants (n=300) will average 1.01 participants per month per center. An additional 150 continuing pediatric participants who do not currently need a biopsy will also be enrolled. Thus, a total of 450 pediatric participants will be enrolled in the NAFLD Pediatric Database 2 over 37 months, an average of 1.52 pediatric participants per month per center."

§6.1 **Screening, consent, and follow-up overview**

- Last paragraph, 1st sentence: "Minimum follow-up on a participant will be 96 weeks, and maximum follow-up on a participant will be 192 weeks" was changed to "Minimum follow-up on a participant will be 48 weeks, and maximum follow-up on a participant will be 192 weeks."

NAFLD Pediatric Database 2 protocol (15 April 2014)

Design Synopsis

- Changed inclusion criteria from "Willingness to be followed for up to 4 years" to "Willingness to be in the study for 1 or more years"
- Deleted recruitment period
- Increased number of new participants from 250 to 650 participants, increased target for new liver biopsies from 300 to 700, and increased total sample size from 450 to 850 participants
- Changed duration of follow-up from "Minimum of one year of follow-up (1-4 years until 31 December 2013) to "One or more years of follow-up"
- Clarified visit schedule for follow-up visits every 48 weeks

§4.3 Target composition of Database 2 pediatric population

- Increased number of new participants from 250 to 650 participants, increased total sample size from 450 to 850 participants, and increased target for NASH-related cirrhosis from 20 to 40 participants

§5.1 Recruitment

- Clarified that recruitment began in December 2009 and will continue to at least April 2019
- Clarified that monthly center-specific recruitment goals will depend on resources and patient population at each center

§5.2 Inclusion criteria

- Changed inclusion criteria from "Willingness to be followed for up to 4 years" to "Willingness to be in the study for 1 or more years"

§6.1 Screening, consent, and follow-up overview

- Changed maximum follow-up from 192 weeks to 480 weeks and changed maximum number of visits from 6 to 12

§7. Statistical and design considerations

- Increased sample size from 450 to 850 participants

§10.1 Participating centers

- Updated principal investigators and data repository names

§10.2 Data collection schedule

- Added additional visits every 48 weeks through 480 weeks

§10.3 Whole blood draw schedule

- Added additional visits every 48 weeks through 480 weeks

NAFLD Adult Database 2 protocol (6 January 2016)

Editorial and wording changes were made to the following sections:

§6.2 Screening visit

- Second paragraph, 5th bullet: added the item "Habitual beverage intake assessment (Beverage Questionnaire [BEVQ-15])"

§6.4 Follow-up

- Second paragraph, 2nd bullet; added the item "Habitual beverage intake assessment (Beverage Questionnaire [BEVQ-15])".

§6.5 Database contents

- Fourth paragraph: added the paragraph labeled "Beverage intake" describing the Beverage Questionnaire.
- Seventh paragraph, Laboratory tests: added "red blood cell count (RBC)" to Hematology tests.

§10.1 Participating centers

- Replaced "Case Western Reserve University" with "Cleveland Clinic Foundation"
- Replaced "Virginia Mason" with "Swedish Medical Center"
- Added "University of Buffalo" center
- Replaced "Mt. Sinai Medical Center" with "Emory University"
- Under National Institutes of Health, deleted "The Eunice Kennedy Shriver NICHHD"
- Under NIDDK Central Repositories, replaced "The State University of New Jersey" with "University Cell and DNA Repository (RUCDR) Infinite Biologics"

§10.2 Data collection schedule

- Added the Beverage intake questionnaire at screening and every 48 weeks through 480 weeks
- Addition of red blood cell count (RBC) to hematology measures collected

§10.3 Whole blood draw schedule

- Reduced the total amount of blood collected for Genetics at screening from 20 mL to "10" mL, the total blood collected for Genetics from "20" mL to "10" mL, the total blood collected at screening from "110" mL to "100" mL, and the total blood collected from "710" mL to "700" mL.

§10.4 Glossary

- Added red blood cell count (RBC)