NASH CRN

Nonalcoholic Steatohepatitis
Clinical Research Network

Nonalcoholic Fatty Liver Disease (NAFLD) Adult Database 2

Protocol

CONFIDENTIAL

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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 18 years of age with known or suspected NAFLD or NASH-related cirrhosis

Inclusion criteria

- At least 18 years of age at time of initial screening
- Written informed consent to participate
- Willingness to be in the study for 1 or more years
- **For continuing participants:** Previously enrolled in the NAFLD Database study, PIVENS, or TONIC trials
- **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**: 1000 (300 with standard of care liver biopsy within the target recruitment period, 700 continuing participants without liver biopsy at enrollment)
- **New participants**: 1500 participants with liver biopsy within 90 days of specimen collection
- Target for new liver biopsies: 1800 (300 from continuing participants and 1500 from new participants)
- Total sample size for adult participants in NAFLD Database 2: 2500

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 PIVENS or TONIC trials)
- ALT, AST levels
- Glucose, insulin levels
- Lipid profile
- Body mass index and anthropometric data
- Alcohol consumption
- Medication use

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 Adult 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 ranges 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 simple nonalcoholic steatosis (nonalcoholic fatty liver [NAFL]) 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 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 May 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 600 adult 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 Hispanic, Native American, African American, and Asian patients among the new adult participants recruited into the NAFLD Database 2
- To expand the current specimen bank comprised of liver tissue, 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 adult 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)

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.(25) There is also evidence for a significant risk for the development of hepatocellular carcinoma in patients with cirrhosis arising from NAFLD.(26) 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.(27) 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 overweight and obesity 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, 28-31) 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. (32-34) 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.(35-37) Peripheral, hepatic and adipose tissue insulin resistance, with resultant hyperinsulinemia, are central in the development of NAFLD.(38-42) 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.(43) 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.(44) Understanding the pathogenesis of NASH 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.(45, 46) 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.(46) 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.(47) 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.(48, 49) 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 May 2014, the NAFLD 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

- Absence of regular or excessive use of alcohol within 2 years prior to entry. Regular or excessive alcohol is defined as an average alcohol intake of more than 14 drinks of alcohol/week in a man or more than 7 drinks of alcohol/week in a woman. One drink "unit" or one standard drink is equivalent to a 12 ounce beer, a 4 ounce glass of wine, or a 1 ounce shot of hard liquor. This is a minimal, but practical alcohol use definition for screening participants; it is recognized that there is substantial variability in alcohol content in these classes of beverages. More detailed estimates of alcohol consumption will be undertaken as part of the core data collection on all enrolled participants in the Database 2.

Cryptogenic 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
- Absence of regular or excessive use of alcohol within 2 years prior to screening without a history of significant lifetime alcohol consumption which, in the opinion of the study investigator, contributed to the underlying liver disease
- Absence of etiologic evidence of chronic viral hepatitis (other than hepatitis B core antibody), genetic metabolic disease, autoimmune or other liver disease that is known to lead to cirrhosis
- Absence of primary or secondary biliary disease

NASH-related cirrhosis

- Cirrhosis in a participant who meets either one of the following criteria:
  - Satisfies the histological definition of NAFLD
  - Satisfies the definition of cryptogenic cirrhosis and meets both of the following criteria
    - BMI 30 or greater at any time in the past 10 years
    - History of at least one risk factor for the metabolic syndrome, defined using the Adult Treatment Panel III criteria (50): waist circumference > 40 inches (male) or 35 inches (female); fasting glucose ≥110mg/dL, systolic blood pressure > 130 mm Hg or diastolic blood pressure > 85 mm Hg, triglycerides > 150 mg/dL, HDL cholesterol < 40 mg/dL (male) or < 50 mg/dL (female)

4.3. Target composition of Database 2 population

The NAFLD Database 2 will recruit at least 1500 new adult participants suspected or known to have NAFLD or NASH-related cirrhosis and will also invite adult participants from the prior NAFLD Database and related studies (PIVENS trial and TONIC trial) to enroll in the NAFLD Database 2.

All the new adult 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 300 of the continuing participants will be due for a standard of care liver biopsy at the time of their enrollment into the 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 1800 adult participants with liver biopsies and contemporaneous biosamples during the enrollment period.

An estimated additional 700 participants continuing from the NAFLD Database study and related studies will also be enrolled, but without a contemporaneous liver biopsy at the time of enrollment. It is expected that many of these participants will receive standard of care 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 Database 2 study is:

New adult participants	1500
Continuing adult participants, biopsied at enrollment	300
Continuing adult participants, not biopsied at enrollment	700
Total adult participants in the Database 2 study	2500

The number of new enrollees with NASH-related cirrhosis will be limited to at most 150 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 Database 2 will build upon the population recruited in NAFLD Database by enrolling approximately 2500 adult participants suspected or known to have NAFLD or NASH-related cirrhosis. Enrollment of participants was initiated in December 2009 and will continue to at least April 2019. The expectation is that recruitment will come from 8 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 PIVENS or TONIC trials, and these criteria are not listed here, but are in the applicable protocols for these NASH CRN studies. Both continuing and new 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, PIVENS or TONIC trials
- Age at least 18 years during the consent process
- Willingness to be in the study for 1 or more years
- Ability and willingness to give written, informed consent to be enrolled into the Adult Database 2 study

New participants:

- Age at least 18 years during the consent process
- Willingness to be in the study for 1 or more years
- Ability and willingness to give written, informed consent to be screened for and, if eligible, to be enrolled into the Database 2 study
- Minimal or no alcohol use history consistent with NAFLD (see exclusion criteria)
- Collection of a liver biopsy that is obtained within 120 days of enrollment as part of standard of care or for evaluation in FLINT trial
- 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 Database 2 study

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

- Clinical or histological evidence of alcoholic liver disease: Regular and excessive use of alcohol within the 2 years prior to interview defined as alcohol intake greater than 14 drinks per week in a man or greater than 7 drinks per week in a woman. Approximately 10 g of alcohol equals one 'drink' unit. One unit equals 1 ounce of distilled spirits, one 12-oz beer, or one 4-oz glass of wine
- 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
- 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 to provide informed consent

5.4. Database enrollment procedures

Clinical centers must be certified by the DCC to open enrollment in NAFLD Database 2. Prior to implementation of this protocol, the principal investigator must have the protocol and consent form approved by the Institutional Review Board for Human Research (IRB) at his/her institution. Once a candidate for Database 2 entry has been identified, details will be carefully discussed with the participant. The participant will be asked to read and sign the consent form that was approved by the 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 Database 2 participants will likely come from the current patient rosters of the NASH CRN investigators, patients may be referred to the NASH CRN from physicians outside the Network and some patients may refer themselves. Patients considered by the site investigator as likely to be eligible for enrollment as a new or continuing 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 Database 2 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 Database 2. Screening will take place over two visits. At the initial screening visit, the details 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 consent 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 participants if there has 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 Database 2 must be obtained from the participant 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 consent for screening and enrollment in Database 2, including follow-up for the duration of the study. 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 has signed the HIPAA authorization and consent for screening and enrollment in the NAFLD Database 2 study. The participant is considered to be registered in the 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 consent and genetic consent form
- Signature on NAFLD Database 2 HIPAA authorization form
- Assignment of NASH CRN participant identification number
- Medical and medication history
- Habitual beverage intake assessment (Beverage Questionnaire [BEVQ-15])
- Physical examination including vital signs, height, weight, anthropometric measurements, acanthosis nigricans, and liver signs
- Alcohol use history (Skinner Lifetime Drinking history, Alcohol Use Disorders Identification Test [AUDIT])
- 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 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 and should be instructed to bring a snack to be eaten after fasting blood is drawn. 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 ± 7 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 Adult Participants with Fatty Liver Disorders and Standard Operating Procedures I: 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 standard of care educational materials (delay providing these to participant until confirmed eligible for Database 2)

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 an indicated or a standard of care 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 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 or a baseline assessment for participation in FLINT trial. Ideally, this biopsy will be obtained within 7 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 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

Database 2 annual follow-up 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 Database 2 visit time windows sheet for the participant. This sheet is generated by the clinic data system when a participant is enrolled in the 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], liver cancer, other cancer, diabetes, angina, myocardial infarction, and stroke)
- Habitual beverage intake assessment (Beverage Questionnaire [BEVQ-15])
- Physical examination
- Laboratory data (hematology, glucose and insulin, clinical chemistry, hepatic panel, HbA1c, lipid profile)
- Interim drinking history (Alcohol Use Disorders Identification Test - Consumption [AUDIT-C])
- Blood collection for plasma and serum banking
- Documentation of any additional liver biopsies performed as part of standard of care or during participation in FLINT trial

6.5. Database 2 contents

Baseline medical history. The NAFLD Database 2 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 including cardiac disease, renal disease, endocrine disease, hypertension, gout or other arthropathies, disturbances of vision, peripheral neuropathy, myopathy, pancreatitis, and cholelithiasis.

Baseline medication history. Database 2 case report forms will capture selected prescription 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, diabetes, angina, myocardial infarction, and stroke.

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]; and liver signs [jaundice, spider angiomata, palmar erythema, hepatomegaly, splenomegaly, asterixis,].

Alcohol consumption. The objectives of the alcohol use data collection is to (a) estimate both recent and lifetime quantity of alcohol ingestion and (b) assess the effect of alcohol previously consumed on baseline histology. The Alcohol Use Disorders Identification Test (AUDIT) questionnaire and Skinner Lifetime Drinking history will be administered at baseline; an interim drinking history (AUDIT-C) will be obtained at yearly follow-up visits as part of the follow-up medical history. The AUDIT is a 10-item questionnaire with a simple scoring scale. The Skinner Lifetime Drinking history is a more detailed questionnaire and may take 15-30 minutes depending upon the level of alcohol consumption. Continuing participants do not need to complete the lifetime drinking history a 2nd time.

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 Database 2 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.

Serum iron, total iron binding capacity, ferritin, and hepatic iron index (if liver iron measurement available). An *HFE* gene mutation analysis is indicated if there is an abnormality in an iron overload screening test, a family history of iron overload or hemochromatosis, or histological iron of 3+ or greater.

Ceruloplasmin (obtain if age 18-40 at screening) and alpha-1-antitrypsin level (plus phenotype if below normal).

Autoantibody studies: anti-nuclear antibody (ANA), antismooth muscle antibody (ASMA), antimitochondrial antibody (AMA)

Thyroid Stimulating Hormone (TSH)

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

Fasting serum and plasma, DNA, and snap frozen liver tissue will be banked in NAFLD Database 2. Blood will be drawn for serum and plasma banking during screening and yearly visits on all participants enrolled in Database 2. 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 Adult 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. The study also aims to recruit patients for participation in randomized, controlled therapeutic trials. The recruitment goal is a database of 2500 participants with NAFLD with at least 96 weeks of follow-up.

The primary and secondary objectives of the NAFLD Database 2, 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, 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 Database 2 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 Database 2 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 Database 2 until the site has IRB approval for the Database 2 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 NAFLD Database 2 study anticipates recruiting a significant proportion of racial/ethnic minorities (Black or African American, Hispanics or Latino, 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.

Standard of care

All participants enrolled in the NAFLD 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 Adult 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 Database 2 study. 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 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 Database 2 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 Database 2 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 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. (51) 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 Database 2 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 participant hospitalization or prolongation of existing hospitalization, a persistent or significant disability/incapacity, or a congenital anomaly/birth 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. (52)

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 Database 2, 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
- Duke University
- Indiana University
- Saint Louis University
- University of California, San Diego
- University of California, San Francisco
- Swedish Medical Center
- Virginia Commonwealth University

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)

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. Serum iron, total iron binding capacity, ferritin, and hepatic iron index (if liver iron measurement available). Ceruloplasmin (obtain if age 18-40), alpha-1-antitrypsin level.

Autoantibody studies (ANA, ASMA, AMA). TSH

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

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
PPARã	-	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 Adult Database 2 protocol (10 September 2009)

NAFLD Adult Database 2 protocol (14 February 2011)

Editorial and wording changes were made to the following sections:

Design synopsis

- Recruitment: the 1st bullet titled Target recruitment period: "October 2009 to March 2011" was changed to "December 2009 to December 2012"
- Duration of follow-up: "2-4 years (until December 31, 2013" was changed to "Minimum of one year of follow-up (1-4 years until June 2014)

§5.1 **Recruitment**

- 2nd sentence: "Enrollment of participants may be initiated, pending IRB approval, on October 1st, 2009 and continue until March 2011" was changed to: "Enrollment of participants may be initiated, pending IRB approval on December 2009 and continue until 31 December 2012."

- 3rd to 6th sentences:

  "For new adult participants (n=600), this will translate into 4.17 participants per month per center. For all adult participants, new biopsies from new and continuing participants (n=900) will average 6.25 participants per month per center. An additional 700 continuing participants who do not currently need a biopsy will also be enrolled. Thus, a total of 1,600 participants will be enrolled in Adult Database 2 study over 18 months, an average of 11.1 participants per month per center."

  Was changed to:
  "For new adult participants (n=600), this will translate into 2.03 participants per month per center. For all adult participants, new biopsies from new and continuing participants (n=900) will average 3.04 participants per month per center. An additional 700 continuing participants who do not currently need a biopsy will also be enrolled. Thus, a total of 1,600 participants will be enrolled in Adult Database 2 study over 37 months, an average of 5.41 participants per month per center."

§5.2 **New participants**

- 5th bullet "Collection of a liver biopsy that is obtained within 120 days of enrollment" was changed to "Collection of liver biopsy that is obtained within 120 days of enrollment as part of standard of care or for evaluation in FLINT trial"

10.5. Document history

§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"

§6.3 **Liver histology requirements**

- 1st paragraph, 2nd sentence: "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" was changed to "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 or a baseline assessment for participation in FLINT trial"

§6.4 **Follow-up**

- 1st bullet, 6th subitem; "Documentation of any additional liver biopsies performed as part of standard of care" was changed to "Documentation of any additional liver biopsies performed as part of standard of care or during participation in FLINT trial.

NAFLD Adult 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 600 to 1500 participants, increased target for new liver biopsies from 900 to 1800, and increased total sample size from 1600 to 2500 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 600 to 1500 participants, increased total sample size from 1600 to 2500 participants, and increased target for NASH-related cirrhosis from 75 to 150 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 1600 to 2500 participants

§10.1 Participating centers

- Updated data repository name

§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 2 contents

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

§10.1 Participating centers

- Replaced "Case Western Reserve University" with "Cleveland Clinic Foundation"
- Replaced "University of Washington" with "Swedish Medical Center"
- 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)