NASH CRN

Nonalcoholic Steatohepatitis
Clinical Research Network

Nonalcoholic Fatty Liver Disease (NAFLD) Database

Protocol

CONFIDENTIAL

22 April 2005

NAFLD Database Protocol

Contents

NAFLD Database Protocol

Design synopsis

Objectives

- To investigate the etiology, natural history, diagnosis, treatment, and prevention of nonalcoholic fatty liver disease (NAFLD) as defined by steatosis, steatohepatitis, and/or fibrosis
- To develop a specimen bank comprising liver tissue, serum, plasma, and DNA obtained from NAFLD or cryptogenic cirrhosis patients
- To provide a resource for clinical trials and ancillary studies of the pathogenesis, natural history, and treatment of NAFLD or cryptogenic cirrhosis

Type of study

- Observational

Population

- Patients at least age 2 years with definite or suspected NAFLD or cryptogenic cirrhosis

Inclusion criteria

- Histologic diagnosis of NAFLD or histologic diagnosis of cryptogenic cirrhosis or suspected NAFLD on the basis of imaging studies suggestive of NAFLD or clinical evidence of cryptogenic cirrhosis
- Absence of regular or excessive use of alcohol within 2 years prior to initial screening
- At least age 2 years at time of initial screening
- Consent

Exclusion criteria

- Clinical or histologic evidence of alcoholic liver disease
- Evidence of other chronic liver disease
- History of total parenteral nutrition, biliopancreatic diversion, or bariatric surgery
- Short bowel syndrome
- Suspected or confirmed hepatocellular carcinoma
- Known HIV positive
- Other condition that is likely to interfere with study followup

Recruitment

- To sample size goal
- 36 month period

Duration of followup

- 48-192 weeks

NAFLD Database Protocol

Design synopsis

Outcome measures

- Liver histology scores (derived from historical liver biopsy at entry, standard of care biopsy done during screening or followup, or liver biopsy done for NASH CRN trials)
- ALT, AST levels
- Lipid profile
- Imaging studies of NAFLD
- Glucose, insulin levels
- Body mass index
- Health related quality of life
- Alcohol consumption
- Nutritional intake
- Physical activity
- Medication for NAFLD

Visit schedule

- Screening/entry into NAFLD Database: enrollment must occur within 120 days of initiation of screening
- Followup visits at 24 weeks, 48 weeks, 96 weeks, 144 weeks, and 192 weeks

Sample size

- Total of 1500 patients (~200/clinic)

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 one category of cryptogenic 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 is to facilitate and perform clinical, scientific, epidemiological and therapeutic research in NASH.

1.2. Clinical Research Network (CRN)

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 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 protocol development; provides sample size calculations, statistical expertise, forms, and data analysis; supports manuscript preparation; and provides overall study coordination and quality assurance, including coordination of the activities of the Data and Safety Monitoring Board, the Steering Committee and other standing committees. The DCC also collaborates with the NIDDK Biosample (plasma, serum, and liver tissue) and Genetics (DNA) Repositories.

A Steering Committee composed of the principal investigators of each clinical center in the Network, the principal investigator of the DCC, and the NIDDK Project Scientist is the main governing body of the NASH CRN. The Steering Committee has primary responsibility for 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 studies and reporting study results.

2. Objectives and hypotheses of the NASH CRN

2.1. Primary objectives of the NASH CRN

- To elucidate, through the co-operative 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 construct a database of a large cohort of patients with NAFLD (or a history of NAFLD) with a broad range of severity, recruited by the NASH CRN (NAFLD Database). Core data collection will include clinical, demographic, laboratory, imaging, and histological features
- To develop a specimen bank comprised of liver tissue, serum, plasma, and DNA obtained from recruited patients
- To undertake clinical trials of promising therapies for NAFLD or cryptogenic cirrhosis

2.2. Secondary objectives of the NASH CRN

- To provide a resource for developing clinical and pathological criteria for standardizing diagnostic and staging criteria for NAFLD or cryptogenic cirrhosis
- To provide a resource for developing clinical and pathological criteria and measures and endpoints for therapeutic studies of NAFLD or cryptogenic cirrhosis
- To provide a resource for ancillary studies of the pathogenesis, diagnosis, natural history and treatment of NAFLD or cryptogenic cirrhosis

2.3. Hypotheses to be tested using the NAFLD Database

The NAFLD Database will collect information that will enable critical hypotheses pertinent to the etiology, pathogenesis, and natural history of NAFLD to be tested. These include:

- The severity and rate of progression of NAFLD is related to alcohol consumption
- The severity and rate of progression of NAFLD is related to body mass index
- The severity and rate of progression of NAFLD is related to dietary calorie consumption
- The severity and rate of progression of NAFLD is related to physical activity
- The severity and rate of progression of NAFLD is related to the presence and severity of diabetes
- The natural history of NAFLD includes progression to cirrhosis without histological features of NAFLD (cryptogenic cirrhosis)
- The risk for the development of NAFLD and NASH in overweight/obese individuals is greater in Hispanic and Caucasian racial/ethnic groups than in African American or Asian groups
- A family history of liver disease is more frequently associated with more advanced or more rapidly progressive NAFLD

3. Scientific background

3.1. NAFLD and NASH

Nonalcoholic fatty liver disease (NAFLD), a term used to encompass the entire spectrum of hepatic fat accumulation disorders, is increasingly prevalent in the United States. Nonalcoholic steatohepatitis (NASH) represents a more severe end of the histopathologic spectrum of NAFLD and is broadly defined by the presence on liver biopsy of lipid-filled liver cells (steatosis) with added necroinflammation (hepatitis) and variable degrees of fibrosis (scarring), in the absence of significant alcohol ingestion.[1-4] The accumulation of fat, predominantly as triglyceride in the liver cell, is the essential first step in the development of NASH and the defining morphology of all forms of fatty liver disease.[5] NASH is often associated with hyperlipidemia, particularly hypertriglyceridemia.[6] Liver biopsy is the diagnostic gold standard and the only means of establishing a firm diagnosis of NAFLD and of determining the grade of fat accumulation, necroinflammation and stage of fibrosis in NASH, the critical factors that reflect disease severity and determine disease progression.[7] The importance of NASH is emphasized by its increasing prevalence and potential to progress to cirrhosis and end-stage liver disease that may require transplantation or result in disability or death.[8-11]

3.2. Epidemiology

Selected autopsy series have found NASH in 3% of lean and up to 19% of obese subjects.[12] NASH is most common in middle-aged persons but is found in all age groups including children.[13] NAFLD or NASH typically occurs in persons who are overweight or diabetic, but it has recently been shown to occur in subjects with normal body weight and normal glucose tolerance.[14] NAFLD has been found to be the single most common cause of serum aminotransferase abnormalities identified during routine blood testing.[15] NAFLD is increasingly prevalent in the United States.[16-18] Past estimates of the prevalence of NAFLD in the adult U.S. population have ranged from 15-39%, because of varied methods of establishing the diagnosis. These estimates were supported by NHANES III survey data reported in 2001 which indicate the prevalence of NAFLD in the U.S. population to be 7.9%.[16] Studies of the natural history of NAFLD suggest that 10-15% of persons with simple hepatic steatosis (without inflammation and fibrosis) progress to NASH and of those, between 16-30% will have a further progressive course, resulting in fibrosis and cirrhosis.[11,19] In view of the estimated high prevalence of NAFLD, these figures hold important public health implications even with accepting lower rates of progression to end-stage liver disease. Moreover, these estimates are only likely to increase as the mean body mass of individuals in the U.S. increases.[20]

3.3. Natural history and progression of NAFLD

The potential of NAFLD to progress to more advanced liver disease, as summarized in Figure 1, is a primary focus of concern.

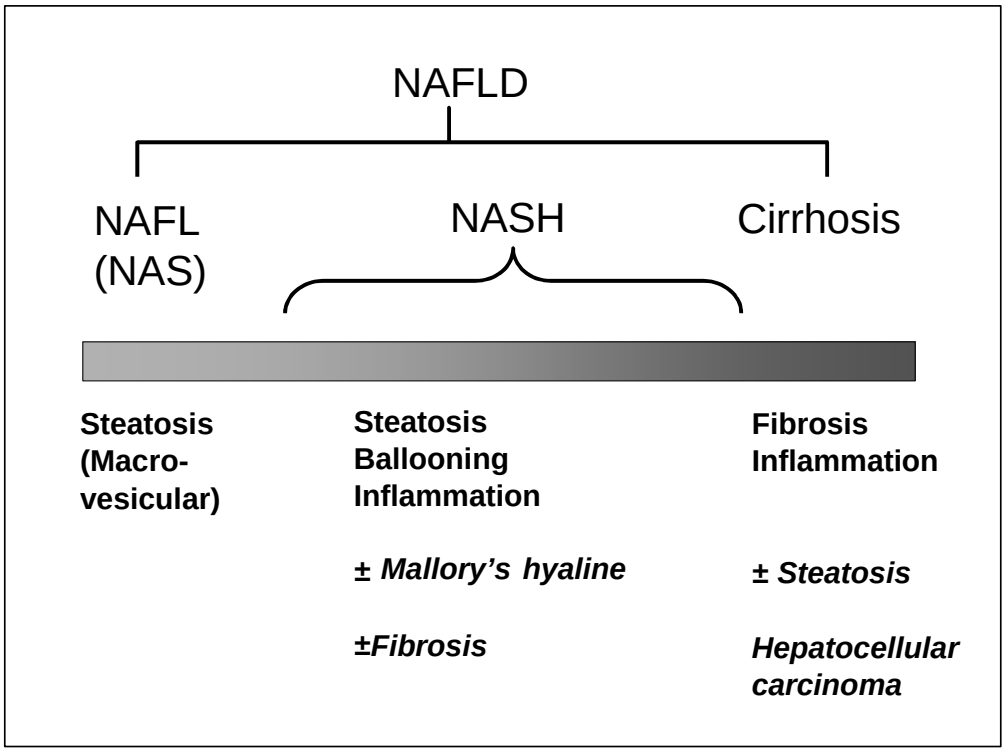

Figure 1: Concepts of the Natural History of NAFLD

With progressive injury, NASH may lose its defining histological feature of steatosis as well as other typical findings such as Mallory bodies, and simply appear as chronic hepatitis or cirrhosis.[5] This observation, as well as the finding of a significant association of metabolic and lipid abnormalities typical of NASH in patients with cryptogenic cirrhosis, suggests that a substantial proportion of cryptogenic cirrhosis may have evolved from NASH.[21-23] On the basis of UNOS 1987-1998 data, cryptogenic cirrhosis is the third most common indication for liver transplantation in the U.S. Most studies have been based on relatively small numbers of patients and suffer from selection bias. The natural history of NAFLD and factors associated with its progression are poorly understood. Patients found on initial liver biopsy to have pure steatosis seem to have the best prognosis within the spectrum of NAFLD, whereas features of steatohepatitis or advanced fibrosis are associated with a worse prognosis.[24] There is also increasing evidence for a significant risk for the development of hepatocellular carcinoma in patients with cirrhosis arising from NAFLD/NASH.[25]

3.4. Risk factors and pathogenesis

Hyperlipidemia, usually hypertriglyceridemia, is evident in 20-81% of patients with NASH[26]. This feature has been poorly characterized and is without explanation either in terms of disease pathogenesis or disease consequence. Insulin resistance has emerged as a potential common thread linking obesity, diabetes, and hyperlipidemia in patients with NASH.[21-31] NASH is increasingly recognized in non-obese individuals, although insulin resistance appears common in these cases. The common occurrence of obesity, insulin resistance, and diabetes without NASH strongly suggests that other unidentified etiopathogenic factors determine the risk of developing simple hepatic steatosis and its subsequent progression to NASH.[32] Lipid and lipoprotein disorders of genetic origin are already of major public health importance because of their impact upon cardiovascular disease. Genetically acquired abnormalities and variant expressions of lipid homeostasis that result in the accumulation of fat, predominantly as triglyceride, may contribute to the pathogenesis of hepatic steatosis.[33] Other evidence indicates that there is a second factor that ultimately leads to cell injury and inflammation in fatty liver.[34] The suspected second "hit" may be intracellular oxidative stress that can be induced by multiple mechanisms, such as excess iron accumulation, endotoxin exposure, pro-inflammatory cytokines, or other unknown factors. Understanding the pathogenesis of NASH is of central importance in ultimately finding a treatment, cure, or means of prevention of this disease.[35]

3.5. Treatment

Therapy for NASH has focused on the management of associated conditions, and currently, there are no approved therapeutic agents.[36] Studies to date have involved a diverse group of interventions and agents reflecting limited knowledge of pathogenesis. Moreover, these studies have been ultimately inconclusive, limited by small sample size, lack of longer-term follow up, and failure to utilize liver histopathology rather than liver biochemistry as the treatment endpoint. Weight loss, exercise, and weight-reduction surgery have shown at least partial benefit, but have not been thoroughly evaluated. Among other agents, hypolipidemic drugs, ursodeoxycholic acid, betaine, and vitamin E have all been used to treat NASH, with variable improvements in biochemistry and histology. Recently, encouraging results have been reported with insulin-sensitizing agents including metformin and thiazolidinediones such as rosiglitazone and pioglitazone.[37-39] Several pilot studies of these agents have shown both biochemical and histological improvements in NASH. The growing evidence for a role for insulin resistance in the pathogenesis and progression of NASH provides a strong rationale for the systematic assessment of the therapeutic role of insulin-sensitizing drugs in the management of patients with NASH. The treatment component of the NASH CRN objectives will be fulfilled by the adult and pediatric treatment trials currently under development. The NAFLD Database will be a resource for identification and recruitment of suitable patients for such trials. The Database thus represents an important opportunity to evaluate both the potential therapeutic role for insulin sensitizing agents in preventing progression of NAFLD and the safety of these agents in this patient population.

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 will also recruit selected patients with cryptogenic 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 and/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, TPN, rapid weight loss associated with fasting, or gastrointestinal disease); 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

Operational definition of NAFLD

- Fat accumulation in the liver (steatosis) involving at least 5% of hepatocytes
- Absence of regular or excessive use of alcohol within 2 years prior to entry. Regular/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 definition for screening patients; it is recognized that there is substantial variability in alcohol content of these classes of beverages. More detailed estimates of alcohol consumption will be undertaken as part of the core data collection on all enrolled patients in the Database.

Operational definition of cryptogenic cirrhosis

- Histologic or clinical cirrhosis occurring in the absence of regular or excessive use of alcohol within 2 years prior to entry as described above and in the absence of a history of significant lifetime alcohol consumption defined as alcohol consumption which, in the opinion of the study investigator, could have contributed to the underlying liver disease
- Absence of etiologic evidence of chronic viral hepatitis (except hepatitis B core antibody), genetic metabolic disease, autoimmune or other accepted causes of liver disease
- Absence of primary or secondary biliary disease

Histologic definition of NAFLD

- Steatosis involving at least 5% of hepatocytes on routine stains
- No evidence of other acute or chronic liver disease

Histologic definition of cryptogenic cirrhosis

- Histologic cirrhosis that fails to meet the histologic definition of NAFLD (i.e., cirrhosis but with less than 5% fat) and without evidence of other chronic liver disease that is known to lead to cirrhosis

Clinical definition of cryptogenic cirrhosis

- Clinical cirrhosis without etiologic evidence of other chronic liver disease that is known to lead to cirrhosis

4.3. Diagnostic categories used in the Database

The following diagnostic levels of NAFLD will be used for screening and recruitment of patients for the Database (in each case, criteria relating to alcohol use as described in Section 4.2 must be met):

Definite NAFLD: Patients with histologically confirmed disease either on the most recent liver biopsy or on a prior biopsy if not on the most recent biopsy

Suspected NAFLD: Patients for whom liver histology is not available and who have:

- No exclusion criteria and
- An imaging study (ultrasound, MRI, or CT) suggestive of fatty liver

Definite cryptogenic cirrhosis: Patients with histologically confirmed disease

Suspected (clinical) cryptogenic cirrhosis: Patients for whom liver histology is not available and who have:

- No exclusion criteria and
- An imaging study (ultrasound, MRI, CT) compatible with cirrhosis (small liver, nodularity, heterogeneous echo pattern) and at least one of the following:
  - Imaging evidence of portal hypertension (splenomegaly, portosystemic collaterals)
  - Albumin less than 3.5 g/dL
  - INR greater than 1.3
  - Platelet count less than 140,000 cells/μL
  - Esophageal or gastric varices on endoscopy
  - Ascites

4.4. Target composition of Database population

Patients with primary NAFLD including selected patients with cryptogenic cirrhosis and patients with secondary NAFLD not directly associated with gastrointestinal surgery/bypass will be enrolled in the Database. Patients with acute syndromes of microvesicular fatty liver disease will not be enrolled. Total patient recruitment into the Database is estimated at approximately 1500 patients (about 200 patients per clinical center). Twenty percent of the patients are expected to have suspected NAFLD, 15% are expected to have cryptogenic cirrhosis, and 65% are expected to have definite NAFLD. Thirty to forty percent are expected to be diabetic (about 550 patients). At least 25% of patients are expected to be in the pediatric age group. Site specific enrollment of patients in the category of "suspected NAFLD" will be monitored by the DCC; there will be a cap on the total patients enrolled in this category set at 20% of the projected total enrollment. Of the patients with cryptogenic cirrhosis, the group enrolled with "suspected cryptogenic cirrhosis" will be similarly monitored to limit the total to 50% of all patients with cryptogenic cirrhosis.

4.5. Sources of patients

Patients will be recruited from the following populations:

- HMO-based
- Primary care and internal medicine clinics
- Pediatric clinics

- Tertiary referral clinics:

- GI/Liver clinic
- Liver transplant clinic
- Obesity clinic
- Bariatric surgery clinic
- Diabetes clinic
- Lipid disorders clinic
- Pediatric weight disorder clinics

5. Selection and enrollment of subjects

5.1. Inclusion criteria

In order to be enrolled in the Database, patients must satisfy all of the following:

- Alcohol use history consistent with NAFLD
- Imaging study suggestive of NAFLD or biopsy materials meeting histologic definition of NAFLD or cryptogenic cirrhosis (histology must be confirmed by local NASH CRN pathologist) or imaging study and clinical findings meeting the criteria for suspected (clinical) cryptogenic cirrhosis (see section 4.3)
- Ability and willingness of patient or legal guardian/representative to give written, informed consent
- Age at least 2 years at initial screening visit
- Ability and willingness to participate in followup

5.2. Exclusion criteria

Patients who satisfy any of the following will not be eligible:

- Clinical 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 within 3 months of interview
- Short bowel syndrome
- History of gastric or jejunoileal bypass preceding the diagnosis of NAFLD. Bariatric surgery performed concomitant with or following the diagnosis of NAFLD does not exclude enrollment of patients.
- History of biliopancreatic diversion
- Evidence of advanced liver disease defined as a Child-Pugh-Turcotte score of equal to or greater than 10
- Evidence of chronic hepatitis B as marked by the presence of HBsAg in serum (patients with isolated antibody to hepatitis B core antigen, anti-HBc, 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 in an individual age 18 or older)
- 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/congenital liver disease
- Evidence of systemic infectious disease
- Known HIV positive
- Disseminated or advanced extrahepatic malignancy
- Concomitant severe underlying systemic illness that in the opinion of the investigator would interfere with completion of followup
- Active drug use or dependence that, in the opinion of the study investigator, would interfere with adherence to study requirements
- Inability to provide informed consent

5.3. Database enrollment procedures

Clinical centers must be certified by the DCC to open enrollment in the NAFLD Database. 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 entry has been identified, details will be carefully discussed with the subject. The subject (or parent or legal guardian if the subject is younger than 18 years of age or under guardianship) will be asked to read and sign the consent form that was approved by the IRB. Minors will be asked to provide assent. 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 followup overview

While many of the NAFLD Database patients likely will 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 in the Database 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.

Patients who are thought to be eligible will be invited to screen for enrollment in the Database. Screening will take place over two visits. At the initial screening visit, the details of Database participation will be introduced. If the patient is agreeable and is judged to have definite or suspected NAFLD, then he/she may enter the formal screening phase. Screening will include both prospective and retrospective data collection. Prospective data collection will be carried out by completion of forms and questionnaires by patients and by performance of various laboratory tests and clinical procedures on patients. Retrospective data collection will be carried out by review of the patient's medical chart and abstraction of various data elements. Abstracted data may include physical measurements and laboratory test results. Liver biopsies obtained previously will be reread by the local NASH CRN pathologist for inclusion of liver histologic findings in the Database. Liver imaging studies obtained elsewhere will be read for eligibility if a liver biopsy is not available or is inadequate for NASH CRN grading.

The two screening visits may occur on 2 separate calendar days and may occur over a period of up to 120 days. The data collected at these visits and during the retrospective chart review are used to determine eligibility and to establish baseline values.

Consent for screening and HIPAA authorization to disclose protected health information with the NASH CRN must be obtained from the patient prior to initiating any data collection for the NASH CRN; this consent and authorization must be obtained at the start of the initial screening visit. The consent will include consent for initial screening and consent to enroll in the Database, including followup.

A total of eight prototype consent and assent statements have been prepared for the NAFLD Database:

- Consent for screening and inclusion of data in the Database (adult patient)
- Consent for screening and inclusion of data in the Database (parent or legal guardian of prospective child patient)
- Assent for screening and inclusion of data in the Database (adolescent patient)

- Assent for screening and inclusion of data in the Database (child patient)
- Consent for the collection, storage, and use of blood samples for current and future genetic research (adult patient)
- Consent for the collection, storage, and use of blood samples for current and future genetic research (parent or legal guardian of prospective child patient)
- Assent for the collection, storage and use of blood samples for current and future genetic research (adolescent patient)
- Assent for the collection, storage and use of blood samples for current and future genetic research (child patient)

HIPAA authorization forms will be prepared according to local clinical center IRB requirements and guidelines.

Minimum followup on a patient will be 48 weeks, and maximum followup on a patient will be 192 weeks. Data will be collected during screening (2 visits), 24 weeks after enrollment, 48 weeks after enrollment, and at yearly intervals thereafter (a maximum of 7 visits). Appendix 10.3 displays the consent and data collection schedule for screening and followup.

6.2. Initial screening visit (visit s1)

Formal screening begins once the patient has signed the HIPAA authorization and consent for screening and enrollment in the NAFLD Database. The patient is considered to be registered in the Database once the consent is signed. Recording of data on NASH CRN forms may begin once this initial consent and authorization are obtained.

The initial screening visit is called visit s1. The purpose of the visit s1 data collection is to initiate collection of the data needed to determine eligibility. Activities at visit s1 include:

- Signature on the Database consent form
- Signature on NASH CRN HIPAA authorization form
- Assignment of NASH CRN patient identification number
- Medical/medication history
- Physical examination including vital signs, height, weight, anthropometric measurements, and Tanner stage (Tanner staging is done up to age 18 or full maturity)
- Alcohol use history (AUDIT, age 8 or older; Skinner, age 18 or older)
- Liver symptom questionnaire
- Review status of liver biopsy data
- Review status of liver imaging data
- Etiologic tests
- Instruct patient regarding retrospective health history information/materials to bring to visit s2

- Patient to sign medical records release to obtain prior reports and biopsy slides
- Patient to provide location/contact information
- Coordinator to register patient on clinic data system
- Coordinator to request prior reports/biopsy slides from health care provider

6.3. Second screening visit

The second screening visit is the encounter at which the patient completes the questionnaires needed for enrollment in the Database and has blood drawn for laboratory tests and specimen banking. The patient 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. Standards of Care procedures are described in NAFLD Database Standard Operating Procedures V: Standards of Care for Adult Patients with Fatty Liver Disorders, and NAFLD Database Standard Operating Procedures VI: Standards of Care for Pediatric Patients with Fatty Liver Disorders.

Procedures at visit s2 include:

- Hematology
- Clinical chemistry
- HbA1c
- Liver panel
- Fasting lipid profile and fasting glucose-insulin levels
- Fasting blood (serum, plasma) for specimen bank
- Block food questionnaire/Block brief food questionnaire
- Activity questionnaire
- Quality of life questionnaires (MOS SF-36 item short form health survey if adult, Pediatric Quality of Life forms if pediatric)
- Provision of standard of care educational materials (delay providing these to patient until patient is confirmed eligible for Database)

6.4. Liver histology requirements for the Database

To enter the Database with a diagnosis of definite NAFLD (see section 4.3 for definition), the patient must have a liver biopsy that is available for review by the NASH CRN pathologist and the NASH CRN pathologist must confirm the histologic diagnosis of definite NAFLD. A liver biopsy may be obtained as part of standard of care (e.g., for diagnosis or followup) as determined by the site investigator during screening. The biopsy may have been obtained at any time prior to the second screening visit.

To enter the Database with a diagnosis of definite cryptogenic cirrhosis (see section 4.3 for definition), the patient must have a liver biopsy that is available for review by the local NASH CRN

pathologist and the local NASH CRN pathologist must confirm the histologic diagnosis of cryptogenic cirrhosis. The biopsy may have been obtained at any time prior to the second screening visit. For patients with cirrhosis (either suspected or proven), needle core rather than surgical (intraoperative) wedge biopsies are preferred for histological review.

To enter the Database without liver histology that meets the NASH CRN requirements, the patient must meet the definition for suspected NAFLD or suspected (clinical) cryptogenic cirrhosis (see section 4.3).

The procedures for collection and processing of liver biopsy materials are detailed in the NAFLD Database Standard Operating Procedures IV: Liver Biopsy Procedure and NAFLD/NASH Histology Scoring System Manual.

6.5. Imaging data requirements for the Database

Patients who do not meet the histological requirements for entry into the Database must have a hepatic imaging examination (ultrasound, CT, or MRI) obtained within 12 months of the date of the first screening visit or following the first screening visit (as part of standard of care).

6.6. Followup visits

Database followup visits will be scheduled 24 weeks and 48 weeks after enrollment in the Database and at yearly intervals thereafter. The date of enrollment will be the date from which the followup visits are timed (ie, the zero time). Each followup visit will have a time window around the target date for the visit; the time window is an interval of days during which the visit may be completed, and the data collected at the visit may be used to fulfill the data collection requirements for the visit. Data 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 patient are specified on the visit time windows sheet for the patient. This sheet is generated by the clinic data system when a patient is enrolled in the Database.

Procedures and forms to be completed at each of the followup visits are:

- 24 weeks visit
  - Followup medical history (medication changes/additions, 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)
  - Focused physical examination
  - Interim drinking history (age 8 or older; included in Followup medical history)

- 48 weeks, 96 weeks, 144 weeks, and 192 weeks visits
  - Followup medical history (medication changes/additions, 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)
  - Liver symptom questionnaire
  - Physical examination
  - Laboratory data (hematology, glucose and insulin, clinical chemistry, liver panel, HbA1c, lipid profile)
  - Interim drinking history (age 8 or older; included in Followup medical history)
  - Food questionnaire
  - Activity questionnaire
  - Quality of life questionnaire
  - Serum specimen for banking and substudies
  - Documentation of any additional liver biopsies performed as part of standard of care

6.7. Database contents

Baseline medical history. The NAFLD Database forms will capture whether the patient was ever diagnosed with NAFLD, dates of biopsies, abbreviated weight history, family history (siblings and patients; particularly of obesity and liver disease), demographic history, past medical/surgical events and illness history (including diabetes, gestational diabetes, hypertension, lipodystrophy, polycystic ovarian syndrome) and other associated co-morbid conditions including previously diagnosed lipid and metabolic disease-related conditions (hypercholesterolemia, hypertriglyceridemia, diabetes mellitus), 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 forms will capture all prescription medications taken within 6 months prior to entry. These should include medications taken for the treatment of liver disease, diabetes, hypertension, and hyperlipidemia, hormone preparations, aspirin, acetaminophen, NSAIDs, thiazolidinediones, metformin, ursodiol, orlistat, vitamin E, corticosteroids, valproate, methotrexate, diuretics, statins, and fibrates. Database forms will also include information on use of alternative therapies, antioxidants, vitamins, and/or other dietary supplements including vitamins, milk thistle, N-acetyl-cysteine, zinc picolinate, α-lipoic acid, dichloroacetate, coenzyme Q, betaine and carnitine/acetyl carnitine. Any known medication allergies will be documented.

Followup medical history. Followup health history will include data on medication changes/additions; 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.

Liver symptom questionnaire. Current symptoms to be queried at screening and each annual followup visit include nausea, abdominal pain/distention, fatigue, insomnia, frequent waking at night, daytime somnolence, pruritus, and muscle cramps.

Physical examination. Initial exam (s1) will include: Vital Signs [temperature, pulse, blood pressure], Anthropometrics: [height, weight (without shoes or heavy clothing), waist (at umbilicus), triceps skinfold, middle upper arm circumference, and hip circumference measurements]; General Signs [lipodystrophic body habitus, muscle wasting, acanthosis nigricans, clubbing, strabismus, ophthalmoplegia, and nystagmus]; and Liver signs [jaundice, spider angiomata, palmar erythema, hepatomegaly, splenomegaly, asterixis,]; Tanner stage (patients age 17 or younger who have not reached full maturity in all Tanner assessments).

Focused physical examination. To include: Vital signs; Anthropometrics; Focused liver signs: [presence or absence of edema, ascites, 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 AUDIT questionnaire (age 8 and older) and Skinner Lifetime Drinking history (age 18 and older) will be administered at baseline; an interim drinking history will be obtained at each followup visit as part of the Followup medical history. The AUDIT is a 10-item questionnaire with a simple scoring scale (estimated time for completion: 2 minutes). The Skinner Lifetime Drinking history is a more detailed questionnaire and may take 15-30 minutes depending upon the level of alcohol consumption.

Nutrition. The objectives of the nutrition data collection are to aid in studies that examine the relationship between energy and macro- and micronutrient intake and hepatic histology in patients with NAFLD. Patients age 18 or older will complete the Block Food Questionnaire. This is a detailed questionnaire likely to take 30-40 minutes to complete. It estimates food frequency and quantity over the preceding 12-month period. Patients age 2 to 17 (or their parents) will complete the Block Brief Food Questionnaire. Food questionnaires will be completed at screening and at annual followup visits. Patients will complete the age appropriate questionnaire, regardless of which questionnaire was completed at previous visits.

Physical activity. The objective of the activity data collection component is to evaluate any correlations between level of activity and disease or mechanisms behind the disease. Patients age 18 or older will complete the Physical Activity Questionnaire; patients age 8 to 17 will complete the Modifiable Activity Questionnaire. Patients age 7 or younger will not complete an activity questionnaire.

Quality of life. The objective of the quality of life data collection component is to evaluate any correlations between self-reported quality of life and disease severity. Patients age 18 or older will complete the Modified SF-36, which is estimated to require 5 minutes to complete. Patients age 5-7 and 8 to 12 will complete the age-appropriate child version of the Pediatric Quality of Life questionnaire, patients age 13-17 will complete the adolescent version of the Pediatric Quality of Life questionnaire, and parents will complete a quality of life questionnaire on their children, including toddlers age 2-4. Patients age 4 or younger will not complete a quality of life questionnaire.

Laboratory items. All laboratory items listed may be obtained as archival material from the patient's chart or should be collected as part of the standard of care. Maximum acceptable intervals between the date of collection of laboratory items and Database registration (date of s1) will be specified on the data collection form. The draw date of the laboratory test must be recorded on the data form. In the case of tests marked **[‡]**, the lower and upper limits of the normal range will be entered into the data collection form.

Hematology: Hemoglobin, hematocrit, white blood cell count (WBC), platelet count.

Clinical chemistry and HbA1c: Creatinine, total protein, sodium, potassium, chloride, bicarbonate, calcium, phosphate, BUN, uric acid, and HbA1c.

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

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

Serum iron, total iron binding capacity, ferritin, and hepatic iron index.

Ceruloplasmin (obtain if age 40 or less at s1), alpha-1-antitrypsin level‡ (plus phenotype if abnormal). 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.

Autoantibody studies *(ANA, ASMA, AMA).*

Fasting lipid profile and glucose-insulin levels: Fasting is defined as nothing by mouth except water for at least 12 hours prior to blood draw. Lipid profile will include fasting triglyceride and cholesterol (total, LDL and HDL cholesterol). Note if on lipid medication when lipid profile taken. If in doubt about fasting status, test should be repeated.
The fasting blood glucose and insulin must be estimated from the same blood draw and can be drawn at the same time as the lipid profile.

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

Fasting serum, plasma, DNA, and stored liver tissue will be banked for NASH CRN substudies or ancillary studies. Blood will be drawn at the second screening visit and yearly thereafter on all patients enrolled in the Database. 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 Database 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 1500 patients with NAFLD with at least 48 weeks of follow-up.

The primary and secondary objectives of the NAFLD Database, as specified in Sections 2.1 and 2.2 of the protocol, are intentionally very broad. Since this is the largest and most comprehensive 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 will require the full gamut of statistical analysis procedures, from survival analysis to ROC curves. Nearly all questions will require identification of appropriate subgroups of patients from all clinical centers contributing to the NAFLD Database 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 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 subjects 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 patient contact about the NAFLD Database until the site has IRB approval for the Database and the DCC has certified the site for initiation of patient activities. All study personnel will have completed training in the Protection of Human Subjects per NIH guidelines. The proposed 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 subjects. We anticipate that the patients recruited from diverse sources, including community and tertiary referral populations, will capture the entire spectrum of NAFLD.

8.2. Standard of care

All subjects enrolled in the NAFLD Database will receive a standard of care for NAFLD and identified associated medical problems as defined by the NASH CRN Standards of Care Committee (see SOP V: Standards of Care for Adult Patients with Fatty Liver Disorders, and SOP VI: Standards of Care for Pediatric Patients with Fatty Liver Disorders). This will include provision of health care counseling and educational materials at enrollment and on an ongoing basis during followup.

8.3. Institutional Review Board (IRB) approval

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

8.4. Consent

Prototype consents and assents 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 reformat and reword information to conform to their local requirements. A signed consent form will be obtained from the subject (or parent, legal guardian, or person with power of attorney for subjects who cannot consent for themselves, such as those below the legal age). The subject's assent must also be obtained if he or she is able to understand the nature, significance, and risks associated with the study. 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 subject, parent, or legal guardian, and this fact will be documented in the subject's record.

8.5. 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 Database investigators. All computer entry and networking programs will identify patients by patient 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 patient data will comply with individual site IRB and NIH requirements for compliance with The Health Insurance Portability and Accountability Act (HIPAA).

8.6. Adverse event reporting

The NAFLD Database will monitor and report unanticipated or adverse events to ensure patient safety in compliance with 45 CFR Part 46, Subpart A; the "Common Rule", shared by 17 Departments and Agencies. 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 definitions and reporting requirements for unanticipated events may differ at each participating site, the Database definitions and procedures for adverse events are designed to satisfy the Common Rule requirements. While the definitions and monitoring procedures apply most directly to clinical trials, all patients in the Database will be monitored for occurrence of adverse events, and any adverse events that occur will be reported as appropriate.

8.6.1. 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.

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, inpatient 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 judgement, the event jeopardized the patient 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.

8.6.2. Monitoring for adverse events

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

8.6.3. Reporting serious adverse events

Serious adverse events must be reported upon discovery at the clinical center. This will involve completing a data form describing the severity and details of the event. The SAE form, together with a memo summarizing the circumstances of event and the current status of the patient, must be faxed to the Data Coordinating Center and to the NIDDK project officer within one working day of the discovery of the SAE. Also within one day, the clinical center must notify the NIDDK and Data Coordinating Center of the SAE by telephone or confirmed e-mail. The NIDDK project officer will work with the Data Coordinating Center to transmit the SAE form and memo to all study centers and to the DSMB.

The DSMB will review each SAE report and provide comments to the NIDDK project officer 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 sponsor.

The clinical center must submit to the NIDDK project officer 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 officer will work with the Data Coordinating Center to distribute the follow-up memo to the clinical center and to the DSMB.

8.6.4. 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 [40].

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.

8.7. Participant withdrawal from Database

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

9. References

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10. Appendix

10.1. Participating centers

Clinical Centers

- Case Western Reserve University
- Duke University
- Indiana University
- Johns Hopkins University
- St. Louis University
- University of California, San Diego
- University of California, San Francisco
- University of Washington
- 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
- National Institute of Child Health and Human Development

NIDDK Central Repositories:

- Biosample repository: McKesson Bioservices Corporation
- Genetics repository: Rutgers, The State University of New Jersey
- Data repository: Research Triangle Institute (RTI)

10.2. Committees

- Steering Committee
- Executive Committee
- Database Committee
- Measures and Assessments Committee
- Pathology Committee
- Standards of Care Committee
- Pediatric Committee
- Ancillary Studies Committee
- Presentations and Publications Committee
- Pilot and Feasibility Studies Committee
- Treatment Trial Protocol Committee
- Data and Safety Monitoring Board

10.3. Data collection schedule

	Screening visits		Followup visits: Weeks from enrollment				
	s1	s2/enr	24	48	96	144	192
Consent and HIPAA authorization	X	.	.	.	.	.	.
Baseline medical history	X	.	.	.	.	.	.
Followup medical history (including interim drinking history)	.	.	X	X	X	X	X
Liver symptoms questionnaire	X	.	.	X	X	X	X
Detailed physical examination including Tanner stage (if < age 18)	X	.	.	X	X	X	X
Focused physical examination	.	.	X	.	.	.	.
Imaging studies of liver*							
CT scan†	.	A	A	A	A	A	A
Ultrasound†	.	A	A	A	A	A	A
MRI†	.	A	A	A	A	A	A
Liver biopsy†	.	A	A	A	A	A	A
Provision of standard of care materials	.	X	.	.	.	.	.
Database eligibility confirmation	.	X	.	.	.	.	.
Alcohol use questionnaires							
AUDIT (age ≥8)	X	.	.	.	.	.	.
Lifetime drinking history (Skinner; age ≥18)	X	.	.	.	.	.	.
Diet questionnaires							
Block Food Questionnaire (adult)	.	X	.	X	X	X	X
Brief Block Food Questionnaire (age 2-17)	.	X	.	X	X	X	X
Activity questionnaires							
Physical Activity Questionnaire (adult)	.	X	.	X	X	X	X
Modifiable Activity Questionnaire (age 8-17)	.	X	.	X	X	X	X
Quality of life questionnaires							
SF-36 (adult)	.	X	.	X	X	X	X
Pediatric Quality of Life							
Child's form (age 5-7, or age 8-12)	.	X	.	X	X	X	X
Adolescent form (age 13-17)	.	X	.	X	X	X	X
Parent's form (age less than 18)‡	.	X	.	X	X	X	X
Hematology	.	X	.	X	X	X	X
Liver panel	.	X	.	X	X	X	X
Clinical chemistry	.	X	.	X	X	X	X
HbAlc§	.	X	.	X	X	X	X
Lipid profile (fasting)	.	X	.	X	X	X	X
Glucose and insulin levels (fasting)	.	X	.	X	X	X	X
Etiologic tests	X	.	.	.	.	.	.
Specimens for banking¶	.	X	.	X	X	X	X

* At least one imaging study for suspected NAFLD or suspected cryptogenic cirrhosis.

† A = as available

‡ Parents complete the Parent form of the Pediatric Quality of Life questionnaire until the child is age 18 (separate parent versions are used for child or teen patients as appropriate)

§ In established or newly diagnosed diabetes.

¶ Specimens for banking and ancillary studies include: liver tissue, serum, plasma, and DNA.

10.4. Whole blood draw schedule: mL of blood to be drawn at screening and followup visits

	Study visit (wk)							
Procedure*	**s1**	**s2**	**24**	**48**	**96**	**144**	**192**	**Total**
Fasting glucose and insulin	.	5	.	5	5	5	5	**25**
Fasting lipid	.	5	.	5	5	5	5	**25**
CBC	.	5	.	5	5	5	5	**25**
Clinical chemistry	.	5	.	5	5	5	5	**25**
Hepatic panel	.	5	.	5	5	5	5	**25**
HbA1c	.	5	.	5	5	5	5	**25**
Plasma: TGF-β	.	5 *	.	5	5	5	5	**25**
Serum: fibrosis markers	.	10 *	.	10	10	10	10	**50**
Serum: banking	.	30 *	.	30	30	30	30	**150**
Genetics	.	20 *	.	.	.	.	.	**20**
Other screening	40	.	.	.	.	.	.	**40**
Total	**40**	**95**	**0**	**75**	**75**	**75**	**75**	**435**

All Database study visits except for first screening visit 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.

*For children younger than 8 years of age, blood draw will be as much as possible per institutional guidelines.

10.5. Glossary

ALT	-	alanine aminotransferase
AMA	-	antimitochondrial antibody
ANA	-	anti-nuclear antibody
ASMA	-	antismooth muscle antibody
AST	-	aspartate aminotransferase
BMI	-	body mass index (kg/m^2)
CC	-	Clinical Center
CRN	-	Clinical Research Network
CT	-	computed tomography
DSMB	-	Data and Safety Monitoring Board
DCC	-	Data Coordinating 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
MRI	-	magnetic resonance imaging
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
SAE	-	serious adverse event
SOC	-	standard of care
UDCA	-	ursodeoxycholic acid
ULN	-	upper limit of normal
WBC	-	white blood cell count

10.6. Document history

NAFLD Database protocol (22 December 2003)

NAFLD Database protocol (10 June 2004)

Numerous editorial and wording changes were made to the following sections:

§5.2 Exclusion criteria:
- The 4th exclusion criterion "History of jejunoileal bypass preceding the diagnosis of NAFLD" was changed to "History of gastric or jejunoileal bypass preceding the diagnosis of NAFLD"
- In the 9th exclusion, the qualifier "(determined at the discretion of the investigator)" was moved to the end of the statement since it applies to both criteria
- The 26th exclusion criterion "prisoners" was deleted
- The 27th exclusion criterion "current pregnancy" was deleted

§6.4 Liver histology requirements for the Database:
"Data captured in the case of historical biopsies will include an estimate of the patient's weight at the time of the biopsy and limited laboratory data (ALT, AST, alkaline phosphatase) as available" was deleted

§6.6 Followup visits:
- Changed "Focused physical examination" to "Physical examination" at 48 weeks, 96 weeks, 144 weeks, and 192 weeks visits

§6.7 Database contents:
- Baseline medication history. Deleted "Dosage, actual or estimated start and stop dates, and frequency of intake will be recorded"
- Quality of life. Added completion of Pediatric Quality of Life: Child age 5-7
- Added completion of Pediatric Quality of Life: Parents of toddler age 2-4
- Focused physical examination. Deleted "Tanner Stage (patients age 17 or younger who have not reached full maturity in all Tanner assessments)"

§7 Statistical and design considerations section was revised

§10.1 Participating centers section was revised

§10.3 Data collection schedule was revised

§10.4 Whole blood draw schedule was revised

§10.6 Document history was added

10.6. Document history

NAFLD Database protocol (22 April 2005)

Numerous editorial and wording changes were made to the following sections:

§ Design synopsis
- Added “cryptogenic cirrhosis” to objectives
- Changed enrollment period to 120 days

§ 2.1 Primary objectives of the NASH CRN
- Added cryptogenic cirrhosis

§ 2.2 Secondary objectives of the NASH CRN
- Added cryptogenic cirrhosis

§ 4.3 Diagnostic categories used in the Database
- Changed category for Definite NAFLD from “Patients with histologically confirmed disease” to “Patients with histologically confirmed disease either on the most recent liver biopsy or on a prior biopsy if not on the most recent biopsy.”

§ 6.1 Screening, consent, and followup overview
- Removed requirement that visits s1 and s2 occur on two separate days and changed enrollment period to 120 days.

§ 6.2 Moved laboratory measures (except etiologic tests) to section 6.3

§ 6.3 Second screening visit
- Deleted sentences about running the eligibility check on the data system prior to the patient’s s2 visit; this is not suggested until the s2 visit when lab results are available
- Added reference to adult and pediatric Standards of Care manuals
- Added Block Brief Questionnaire as one of the procedures completed at the s2 visit
- Last sentence - deleted “archived” and corrected name of SOP IV

§ 6.5 Imaging data requirements for the Database
- Deleted the list of data items to be recorded from the ultrasound

10.6. Document history

§ 6.6 Followup visits
- Changed “date of s2" to “date of enrollment” for the date from which the followup visits are timed (ie, the zero time).
- Changed “Interim medical history” to “Followup medical history” to be consistent with the form name
- Specified that the Interim drinking history is included in the Followup medical history
- Added HbA1c and lipid profile to laboratory tests completed at annual followup visits

§ 6.7 Database contents
- Changed Interim medical history to Followup medical history to be consistent with the form name
- Added triceps skin fold and middle upper arm circumference to the anthropometrics section
- Specified that the Interim drinking history is included in the Followup medical history
- Clarified that parents may help patients age 2-17 complete the Block Brief Food Questionnaire
- Deleted “LCA and LKM1 in pediatric patients” from the Autoantibody studies
- Deleted “MCV” from list of hematology tests
- Added sodium, potassium, chloride, bicarbonate, calcium, phosphate, BUN, and uric acid to clinical chemistries

§ 6.8 Serum, plasma, DNA, and liver tissue for banking
- Added reference to NAFLD Database Standard Operating Procedures I, Clinical Center Operations

§ 10.1 Participating Centers
- Added NIDDK Central Repositories

§ 10.2 Committees
- Added Data and Safety Monitoring Board

§ 10.3 Data Collection Schedule
- Deleted “Interim drinking history (modified Skinner)” and changed “Followup medical history” to “Followup medical history (including interim drinking history)”
- Added age ranges for AUDIT and Lifetime drinking history (Skinner), Block food questionnaires, and Pediatric Quality of Life questionnaires
- Moved hematology, liver panel, clinical chemistry, and HbA1C from s1 to s2 visit

10.6. Document history

- Deleted glucose and insulin levels (fasting) from the f024 visit

§ 10.4 Whole blood draw schedules

- Moved blood collection amounts for CBC, clinical chemistry, hepatic panel, and HbA1c from s1 to s2

§ 10.5 Glossary

- Deleted definitions for LCA, LKM1, NAS, and PBMC
- Fixed spelling of “gamma glutamyltransferase”
- Deleted MCV