Chronic Liver Disease in Urea Cycle Disorders NCT03721367
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1. Protocol Synopsis

Protocol Number:	5118
Protocol Title:	Prospective Cross-Sectional Non-invasive Assessment of Chronic Liver Disease in Urea Cycle Disorders
Study Chair:	
Statistician:	
Consortium:	Urea Cycle Disorders Consortium
Participating Sites:	Baylor College of Medicine, Houston, TX University of California San Francisco (UCSF), San Francisco CA Seattle Children's Hospital, Seattle, WA
Activation Date:	
Sample Size:	
Target Enrollment Period:	
Study Design:	Cross-Sectional, Pilot
Primary Study Objective:	To assess liver stiffness in individuals with CPS1D, NAGSD, ASS1D, ASLD, ARG1D, citrin deficiency, and OTCD.
Secondary Study Objective:	To assess markers of hepatocellular injury, function, and biomarkers for hepatic fibrosis in individuals with CPS1D, NAGSD, ASS1D, ASLD, ARG1D, citrin deficiency, and OTCD
Study Population and Main Eligibility/ Exclusion Criteria:	**Inclusion Criteria** 1. Age ≥ 5 years and ≤ 60 years 2. Weight ≥ 11 kg 3. Males or females with a diagnosis of OTCD based on molecular or enzymatic testing. Males or females with a diagnosis of CPS1D, citrin deficiency, NAGSD, ASS1D, ASLD or ARG1D based on biochemical OR molecular, OR enzymatic testing **Exclusion Criteria** 1. History of hyperammonemia (blood ammonia greater than 100 micromoles/L) documented in the medical record or reported by the patient in the 30 days preceding enrollment visit 2. History of Liver transplantation 3. Current pregnancy 4. Confirmed diagnosis of chronic viral hepatitis, autoimmune liver disease, or alcohol liver disease
Primary Outcome Measures:	Z-scores for liver stiffness as measured by • shear wave velocity in m/s • elasticity in kPA
Secondary Outcome Measures:	Plasma levels of • Aspartate transaminase • Alanine transaminase

	• Gamma glutamine transferase • Albumin • Total bilirubin • Conjugated bilirubin • Prothrombin time • Internationalized Normalized Ratio • AST-to-Platelet Ratio (APRI), • Fibrotest
Statistical Considerations (sample size and analysis plan):	This is a cross-sectional pilot study. The mean shear wave velocity and elasticity and the mean values of serum biomarkers will be compared among the individual disorders (ASS1D, ARG1D, ASLD) and OTCD using a one-way ANOVA.. Any correlation between the shear wave velocity and serum markers will be assessed by using appropriate correlation statistical methods. For the other disorders (CPS1D, NAGSD, citrin deficiency, and males with OTCD males,) we expect that sample size will be limited given that these disorders are ultra-rare or given that many patients receive early liver transplantation as part of routine management. Thus, data analysis for these other disorders will be descriptive only. Published data demonstrate that the mean (SD) shear wave velocities in population without liver disease to be 1.56 (0.4) m/s. We would expect that individuals with OTCD would have values comparable to these values. In individuals with ASLD and ARG1D, we would expect higher shear wave velocities comparable to individuals with grade 2-3 liver fibrosis. The mean shear wave velocities (SD) grade 3 fibrosis is 2.57 m/s (0.3). We hypothesize that the mean shear wave velocity in ASS1D will be intermediate between ASLD/ARG1D and controls. Assuming a normal distribution for, with a sample size of 8 individuals per group, we will be able to detect a minimum difference in means of 0.79 m/s, when the standard deviation is 0.4 m/s, at an alpha error of 0.05 and a power of 0.8.
Sponsors (federal, state, foundation and industry support):	National Institutes of Health (NIH)

1.1 Overview

Urea cycle disorders (UCDs) are among the most common inborn errors of liver metabolism. With early diagnosis and improved treatments, the survival of individuals with UCDs has improved, and this improved survival has led to unmasking of some long-term complications such as hepatic dysfunction and progressive fibrosis in a subset of patients. Hepatic complications in UCDs are quite variable and dependent upon the specific metabolic defect. In ornithine transcarbamylase deficiency (OTCD) a preponderance of acute liver injury has been reported. In contrast, chronic hepatocellular injury that may progress to hepatic fibrosis, cirrhosis with portal hypertension, and even end-stage liver failure have been reported in patients with argininosuccinate lyase deficiency (ASLD) and arginase 1 deficiency (ARG1D). Similarly, liver failure has been observed in citrullinemia (ASS1D) and hepatic steatosis is a common feature in citrin deficiency. While serum aminotransferases and markers of synthetic function of the liver (e.g. albumin, coagulation parameters) are typically used to monitor hepatic disease, the sensitivity of such assays to detect progression of liver disease may be limited. Moreover, normal laboratory values may not necessarily indicate absence of hepatic disease. Currently, there are no guidelines for monitoring hepatic complications or extent of liver disease in UCDs. The only method for detecting liver fibrosis, a precursor for hepatic cirrhosis and potential chronic liver failure, is liver biopsy, which is an invasive procedure. Recently, new non-invasive ultrasonography techniques, such as shear wave elastography which assesses liver stiffness, a measure that correlates with the presence and extent of hepatic fibrosis, have been validated in both adult and pediatric populations with other forms of chronic liver disease. Likewise, numerous novel serum biomarker panels are being validated as complementary tools for the evaluation of fibrosis in patients at risk for progressive hepatic disease. Utilization of these techniques in individuals with UCDs could be invaluable in both the research and clinical arenas.

The purpose of this study is: 1) To determine whether liver stiffness is higher in individuals with ASS1D,ASLD, and ARG1D as compared to females with OTCD, and to assess liver stiffness in other UCDs (citrin deficiency, NAGSD, CPS1D, and males with OTCD), 2) To test whether markers of hepatocellular injury and function and novel serum biomarker panels for hepatic fibrosis provide evidence of chronic liver disease in individuals with ASS1D, ASLD, and ARG1D as compared to OTCD and to assess these sample markers of hepatocellular injury and function and novel serum biomarker panels for hepatic fibrosis in other UCDs (citrin deficiency, NAGSD, CPS1D, and males with OTCD).

2. Specific Aims (Hypothesis and Objectives)

Hypothesis:

We hypothesize that individuals with ASLD and ARG1D will have higher liver stiffness as assessed by non-invasive ultrasonographic techniques and greater serum biomarker evidence for fibrosis as compared to females with OTCD. We hypothesize that individuals with ASS1D will have liver stiffness and serum biomarkers that are intermediate between OTCD and the other two distal disorders (ASLD and ARG1D).

Study Objectives:

Specific Aim #1: To determine whether liver stiffness is higher in individuals with ASS1D, ASLD, and ARG1D as compared to females with OTCD, and to assess liver stiffness in other UCDs (citrin deficiency, NAGSD, CPS1D, and males with OTCD),

Specific Aim #2: To test whether markers of hepatocellular injury and function and novel serum biomarker panels for hepatic fibrosis provide evidence of chronic liver disease in individuals with ASS1D, ASLD, and ARG1D as compared to OTCD and to assess these sample markers of hepatocellular injury and function and novel serum biomarker panels for hepatic fibrosis in other UCDs (citrin deficiency, NAGSD, CPS1D, and males with OTCD).

We will conduct a pilot, cross-sectional study to assess liver stiffness and markers of hepatic injury, function, and fibrosis in patients with ASLD, ARG1D, ASS1D, and OTCD and in patients with other UCDs (citrin deficiency, NAGSD, CPS1D, and males with OTCD). This study will be conducted at 3 UCDC sites: Baylor College of Medicine in Houston, Texas, University of California San Francisco (UCSF), San Francisco, California and Seattle Children's Hospital, Seattle, Washington.

3. Background

With the introduction of newborn screening, early initiation of treatment to prevent hyperammonemia, and improved survival in urea cycle disorders (UCDs), it is now apparent that hepatic fibrosis and dysfunction are long-term complications observed in a subset of patients. The manifestations of liver disease in UCDs are variable and may range from hepatomegaly and hepatocellular injury to acute liver failure or chronic liver disease. For the most common UCD, ornithine transcarbamylase deficiency (OTCD), two recent retrospective chart reviews identified liver injury in roughly half of affected individuals at some point in the course of their disease[1,2]. In most cases, the presentation was acute liver injury, and in some, this occurred in association with hyperammonemic events[1,2]. These data suggest that there is acute, and perhaps recurrent acute, liver injury in OTCD, which could potentially lead to chronic liver disease in a subset of patients[1,2]. In contrast, our analyses of the data from the Longitudinal Study of Urea Cycle Disorders demonstrates that patients with argininosuccinate lyase deficiency (ASLD) and arginase 1 deficiency (ARG1D) have a higher prevalence of chronic hepatocellular injury as assessed by serum levels of aspartate and alanine aminotransferases (AST and ALT, respectively) as compared to patients with more proximal disorders[3]. In some individuals with ASLD and ARG1D, this chronic hepatocellular injury has been associated with hepatic fibrosis, cirrhosis with portal hypertension, and impaired liver function, which in some cases, has necessitated liver transplantation[4-8]. Common liver histopathologic findings in patients with ASLD and ARG1D include hepatocyte enlargement with pallor, increased nuclear or cytoplasmic glycogen deposition, steatosis, and fibrosis[4,5,9-12]. Similar histopathologic findings have also been reported in ASS1D, OTCD, and other UCDs [1,2]. . Moreover, hepatocellular carcinoma has been described in at least five individuals with OTCD, ARG1D, and ASLD with a background of fibrosis noted on histopathology in two of these patients[12,14]. This suggests that chronic hepatic abnormalities in UCDs could potentially contribute to increased risk for liver cancer.

Currently, there are no reliable predictors to determine which individuals with UCDs will develop chronic hepatic complications. Serum levels of aminotransferases and markers of synthetic liver function, which are routinely performed, may be insensitive to detect progression of liver disease, and thus, normal laboratory values are not necessarily reassuring. Hematologic features of hypersplenism (e.g. thrombocytopenia) are a late manifestation of chronic liver disease. The gold standard for detection of fibrosis or cirrhosis is liver biopsy, an invasive procedure with inherent risks. However, the patchy nature of many liver diseases calls into question the presumed accuracy of liver biopsy. Recently, ultrasonographic techniques, such as shear wave elastography (SWE), have been utilized for non-invasive assessment of liver stiffness, a measure that correlates with liver fibrosis. Hepatic tissue has an inherent elasticity that may be altered by pathologic processes such as inflammation and fibrosis. Elastography has the ability to assess small changes in pliability of liver tissue across the entire liver and has been used to detect hepatic fibrosis in pediatric patients with a variety of chronic liver diseases[15]. Given that SWE is non-invasive, safe, and has excellent intraobserver and interobserver reproducibility, it has been proposed as a tool for monitoring progression of liver disease in populations at risk for progressive disease[15]. Likewise, panels of novel serum biomarkers have been demonstrated to correlate with the presence and degree of fibrosis in some pediatric and adult populations[16-18]. The recent introduction of SWE in the pediatric population provides a unique opportunity to evaluate the utility of this technology for assessment of liver disease in patients with UCDs. Clinically, identification of patients with hepatic fibrosis during the early stages, would allow for closer monitoring, earlier referral to hepatology, and anticipatory guidance regarding lifestyle factors and avoidance of medications that may increase risk for progression of liver disease.

4. Study Design and Methods

This is a multi-center cross-sectional pilot study that will assess liver stiffness and serum biomarkers for liver fibrosis and dysfunction in patients with urea cycle disorders. The details of the trial schedule of events are listed in 4.7.

4.1 Inclusion Criteria

1. Age ≥ 5 years and ≤ 60 years
2. Weight ≥ 11 kg
3. Males or females with a diagnosis of OTCD based on molecular or enzymatic testing. Males or females with a diagnosis of CPS1D, citrin deficiency, NAGSD, ASS1D, ASLD or ARG1D based on biochemical OR molecular, OR enzymatic testing

    a. ASLD:
    - Presence of argininosuccinic acid in blood or urine and/or
    - Decreased AL enzyme activity in cultured skin fibroblasts or other appropriate tissue and/or
    - Identification of pathogenic mutation and/or
    - Hyperammonemia and first degree relative meets at least one of the criteria for AL Deficiency

b. ARG1D:
- >5 fold elevated arginine in blood and/or
- Decreased arginase enzyme level in red blood cells or other appropriate tissue and/or
- Identification of pathogenic mutation and/or
- Hyperammonemia and first degree relative meets at least one of the criteria for ARG Deficiency

c. OTCD:
- Identification of pathogenic mutation and/or
- <20% of control OTC activity in liver and/or
- Elevated urinary orotate (>20 uM/mM creatinine) in a random urine sample or after allopurinol challenge test and/or
- Hyperammonemia and first degree relative meets at least one of the criteria for OTC Deficiency

d. ASS1D:
- > or = to 10 fold elevation above mean of citrulline in plasma and/or
- Decreased AS enzyme activity in cultured fibroblasts or other appropriate tissue and/or
- Identification of pathogenic mutation and/or
- Hyperammonemia and first degree relative meets at least one criteria for ASS1 deficiency

e. CPS1D
- Decreased (<20% of control) CPS I enzyme activity in liver and/or
- Identified pathogenic mutation and/or
- Hyperammonemia and first degree relative meets at least one of the criteria for CPS I deficiency

f. Citrin (CITR) deficiency
- Elevated citrulline in blood and pathogenic mutation and/or
- Hyperammonemia and first degree relative meets criteria for CITR deficiency

g. NAGSD
- Detection of a pathogenic mutation and/or
- Decreased (< 20 % of control) NAGS enzyme activity in liver and/or
- Hyperammonemia and first degree relative meets at least one of the criteria for NAGS deficiency

4.2 Exclusion Criteria

1. History of hyperammonemia (blood ammonia greater than 100 micromoles/L) documented in the medical record or reported by the patient in the 30 days preceding enrollment visit
2. History of liver transplantation
3. Current pregnancy
4. Confirmed diagnosis of chronic viral hepatitis, autoimmune liver disease, or

alcohol liver disease

4.3 Recruitment of Participants

We will use a number of different mechanisms to recruit subjects in this research protocol. Individuals may learn about the study through the RDCRN website or contact registry, a medical care provider, UCDC investigators, or the National Urea Cycle Disease Foundation (NUCDF). The sources for recruitment are outlined below:

Metabolism Consultation Services. The UCDC investigators include prominent clinicians and investigators in the field of UCDs. As a group, investigators are consulted on frequently regarding diagnosis and treatment of individuals throughout the country. This will provide a mechanism for recruitment of both currently treated patients and of prospectively diagnosed patients into the clinical studies.

National Urea Cycle Disorders Foundation (NUCDF). The NUCDF serves patients and families of patients with UCDs. NUCDF will play an integral role in recruitment by disseminating information to potential participants and assisting in the development of UCDC website content. Through the NUCDF newsletter and website, families will receive updated information about the UCDC clinical studies.

Contact Registry: As is the case for all RDCRN members, the Urea Cycle Disorder Consortium has a contact registry. Patients can register on-line, over the phone or by faxing or mailing in the registration form expressing an interest in receiving information about research studies that they might qualify for. The contact registry is managed by the DMCC, which will receive registration information. DMCC will make the initial contact with patients providing information about study protocols and who to contact if they would like to learn more about a study and possibly enroll.

Recruitment will occur by physicians, study nurses, and research coordinators. Past and current patients of Drs. Lee, Nagamani, Burrage, Gallagher, and Merritt and subjects who have participated in previous studies may be contacted directly by study staff for possible recruitment. The study population may also include patients enrolled in the Urea Cycle Disorders Consortium Longitudinal Study or patients who have enrolled in other UCD studies. These patients may be recruited at routine study visits or through phone contact. The study will be posted on Clinicatrials.gov. In addition, subjects may be recruited through advertisement by the National Urea Cycle Disorders Foundation. Details of the goals of the research and the risk and benefits of the protocol will be reviewed with each potential study participant. Strict patient confidentiality will be observed throughout all aspects of the study. Potential subjects may be requested to provide medical records prior to consenting to the study to establish eligibility. In this case, a medical records release form will be completed to request relevant medical records and laboratory data. While medical records will be reviewed by members of the research team, no individually identifiable patient data will be distributed to non-research or care-giving team members.

We plan to enroll n=8 subjects (male or female) with ASLD, n=8 subjects (male or female) with ARG1D, n=8 subjects (female) with OTCD, n=8 subjects (male or female) with ASS1D and a total of n=8 subjects with other UCDs (CPS1D, NAGSD,

citrin deficiency, and males with OTCD) across three study sites.
All study subjects must be 5 years of age or older. The projected enrollment at each site based on current patient population is provided in Table 1. These projected enrollment at each site is an estimate and may need to be adjusted based on actual enrollment.

Table 1. Projected study enrollment at each study site.

Study Site	OTCD (Female)	ASLD	ARG1D	ASS1D	Other UCDs
Baylor College of Medicine	3-5	5-6	4-6	6-7	6-7
UCSF	1-3	0-1	2-3	0-1	0-1
Seattle Children's Hospital	1-3	1-2	0-1	0-1	0-1
Target Enrollment Across Sites	**8**	**8**	**8**	**8**	**8**

Our study population will include children, cognitively impaired individuals, and women of child-bearing potential. Because of the rarity of these disorders, this study could not be completed if children and cognitively impaired individuals were not included in the study. Subjects may also include affected patients between 12 and 17 years of age who are capable of completing study procedures. Assent will be obtained if it is determined that the subject understands all aspects of the study. A participating investigator or his designee will obtain informed consent and sign the consent form along with the parent/legal representative. The subject will also sign the form if he/she is capable of giving assent.

4.4 Retention Strategies

Retention of subjects is not applicable since study procedures will be completed in a single visit.

4.5 Study Procedures

Primary Objective

To determine whether liver stiffness as assessed by shear wave elastography (SWE) is higher in individuals with ASS1D, ASLD and ARG1D as compared to females with OTCD and to assess liver stiffness in other UCDs (citrin deficiency, NAGSD, CPS1D, and males with OTCD),

Secondary Objective

To test whether markers of hepatocellular injury and function and novel serum biomarker panels for hepatic fibrosis provide evidence of chronic liver disease in individuals with ASS1D, ASLD and ARG1D as compared to OTCD, and to assess these sample markers

of hepatocellular injury and function and novel serum biomarker panels for hepatic fibrosis in other UCDs (citrin deficiency, NAGSD, CPS1D, and males with OTCD).

Study Procedures

This study will entail a single visit to the UCDC site. Subjects will come to the hospital or research center to sign the informed consent prior to conducting any procedures. Subjects will be asked to not eat or drink for at least four hours prior to arrival for the study visit. Vital signs including height, weight, heart rate, blood pressure, respiration rate and temperature will be measured. A blood sample of approximately 4 teaspoons or 20 mL will be collected to assess complete blood count (CBC), blood ammonia, aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma glutamyl transferase (GGT), total bilirubin, conjugated bilirubin, albumin, Prothrombin time (PT), Internationalized Normalized Ratio (INR), AST-to-Platelet Ratio (APRI), and Fibrotest. These tests will be ordered individually or as part of a panel test (e.g , hepatic function panel) if such a panel is available at the study site. A storage sample for future biochemical studies will also be collected. A urine sample for pregnancy testing will be collected if a female is of childbearing age.

If anything clinically significant were to be found on the ultrasound or the blood work, it will be reported to the subject and their clinical care provider.

A medical history will be reviewed and recorded. We will collect information from medical records including results from the most recent test and reports from a previous liver biopsy results if it is available.

A single ultrasound exam that includes shear-wave elastography (SWE) will be used to measure liver stiffness. A radiology technician or radiologist will perform the exam. The study should be completed in one outpatient visit. However, based on patient preferences, the ultrasound and blood draw may have to be schedules on two separate days. In such circumstances, both the blood draw and the ultrasound will be performed within a time period of two weeks.

4.6 Data Elements

Once referred for screening, the participant's diagnostic testing will be reviewed to assure that the eligibility requirements are met based on study inclusion and exclusion criteria. Data will be collected at a single study visit per the Schedule of Events. We will use a variety of methods to obtain these data. Certain information will be obtained from a historical review of existing medical records including laboratory and treatment data. Other data will be obtained from patients or their families through a standard interview, examination or laboratory/functional testing.

The study will maintain appropriate medical and research records. As part of conducting the study, sites will maintain all relevant source data. Source data are all information and original records of clinical findings, observations, or other activities necessary for the study. Examples of original source documentation include original documents and data records, electronic medical records, laboratory reports, memoranda, recorded data from automated instruments, copies and transcriptions after the copy has been verified as being accurate and complete, photographic negatives, x-rays, etc.

Shear Wave Elastography will be performed by experienced radiology personnel using standard equipment and protocols. The laboratory assessments will be performed in CLIA-certified laboratories or the research laboratory facilities at Baylor College of Medicine.

4.7 Schedule of Events

Sign Informed Consent	X
Confirm eligibility	X
Vital Signs: Blood pressure, temperature, heart rate, respirations measured	X
Blood draw for complete blood count (CBC), blood ammonia, aspartate aminotransferase (AST), alanine aminotransferase (ALT), albumin, gamma glutamyltransferase (GGT), total bilirubin, conjugated bilirubin, Prothrombin time (PT), Internationalized Normalized Ratio (INR), AST-to-Platelet Ratio (APRI), and Fibrotest	X
Urine pregnancy test for females of child bearing Age	X
Review medical history	X
Ultrasound SWE imaging	X