Rare Diseases Clinical Research Network (RDCRN)

Smith-Lemli-Opitz Syndrome: A Pilot Study of Cholic Acid Supplementation

Sterol and Isoprenoid Research Consortium (STAIR)

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List of Abbreviations

AE, adverse event

ALT, alanine aminotransferase

AST, aspartate aminotransferase

BID, twice daily

BUN, blood urea nitrogen

CBC, complete blood count

CMP, comprehensive metabolic panel

DHCR7, 7-dehydrocholesterol reductase

7-DHC, 7-dehydrocholesterol

8-DHC, 8-dehydrocholesterol

DMCC, Data Management Coordinating Center

DSMP, Data Safety Monitoring Plan

FDA, Federal Drug Enforcement Agency

GGT, gamma-glutamyltransferase

G-tube, gastrostomy tube

HMG-CoA, hydroxymethylglutaryl-Coenzyme A

J-tube, jejunostomy tube

MOO, Manual of Operations

MRO, Medical Review Officer

NIH, National Institutes of Health

RDCRN, Rare Diseases Clinical Research Network

SAE, serious adverse event

SED, single enzyme defect

SLOS, Smith-Lemli-Opitz syndrome

STAIR, Sterol and Isoprenoid Research Consortium

ZSD, Zellweger spectrum disorder

1. Protocol Synopsis

Interventional Synopsis

Protocol Number:	STAIR 7012
Protocol Title:	Smith-Lemli-Opitz Syndrome: A Pilot Study of Cholic Acid Supplementation
Study Chair:	Ellen Roy Elias, M.D.
Statistician:	University of Nebraska Medical Center, TBD
Consortium:	Sterol and Isoprenoid Research Consortium (STAIR)
Participating Sites:	Children's Hospital Colorado University of Nebraska Medical Center
Activation Date:	TBD
Current Status:	NIH and DSMB Approved
Sample Size:	Up to 15 enrollees with expectation to have 12 evaluable subjects
Target Enrollment Period:	September 2020- May 2021
Study Design:	Open-label, multi-center, pilot study
Primary Objectives:	1. Determine the effect of cholic acid supplementation on serum cholesterol and related sterol biomarkers (7- and 8-dehydrocholesterol) in subjects with Smith-Lemli-Opitz syndrome (SLOS). 2. Examine the safety of cholic acid administration to subjects with SLOS by measuring adverse events.
Secondary Objectives:	1. Determine the effect of cholic acid supplementation on serum oxysterol levels, serum bile acids, and vitamin D in subjects with SLOS.
Exploratory Outcome Measures:	1. Serum bile acid levels. 2. Changes in safety monitoring tests including liver function as determined by serum transaminases, bilirubin, albumin, alkaline phosphatase, GGT and CBC.
Study Population and Main Eligibility/ Exclusion Criteria:	Subjects will be recruited through referrals from physicians at participating STAIR sites and subject referrals from the SLOS/RSH Foundation. Main Eligibility Criteria include: 1. Parent/Guardian provide written consent. 2. Subject has biochemically and genetically confirmed diagnosis of SLOS. 3. Subject is ages 2-28 years at enrollment. 4. Subject's serum cholesterol ≤125 mg/dL prior to enrollment. 5. Subject is currently on stable oral cholesterol supplementation.

	6. Subject is able to travel to one of the STAIR sites. Main Exclusion Criteria include: 1. Subject is clinically unstable. 2. Subject is unlikely to complete the study, in the opinion of the investigator. 3. Subject is taking drugs, nutraceutical, probiotics or other compounds that are known or suspected to lower serum cholesterol. 4. Subject has transaminase elevations > 3-fold above the reference range at enrollment. 5. Subject is pregnant or likely to become pregnant.
Treatment (if applicable)	
Agent:	Cholic acid (trade name Cholbam®; Retrophin, Inc.)
Dosage, Schedule, Route of Administration:	10 mg/kg/day, divided BID, administered either orally or via J- or G-Tube with a treatment duration of 2 months
Safety Issues:	Although previously administered to SLOS subjects, cholic acid has an undefined safety profile in SLOS subjects. In subjects with other disorders of cholesterol metabolism (Zellweger spectrum disorder and inherited single enzyme diseases of bile acid synthesis), worsening liver transaminases have been documented in a minority of treated subjects. These diseases are marked by inherent liver abnormalities prior to treatment. Due to the potential for worsening liver toxicity while on treatment with cholic acid, participants will be closely monitored for hepatotoxicity while on cholic acid and for one month following completion of the treatment trial.
Primary Outcome Measures:	Serum cholesterol, serum 7- and 8-dehydrocholesterol, sterol ratio, and incidence of adverse events.
Secondary Outcome Measures:	Serum oxysterol levels.
Exploratory Outcome Measures:	Serum vitamin D, serum bile acids, chemistries (AST, ALT, bilirubin, albumin).
Statistical Considerations (sample size and analysis plan):	The study will enroll up to 15 subjects with the aim for 13 evaluable subjects to complete the study.
Sponsors (federal, state, foundation	1. National Institute of Child Health and Human Development;

and industry support):	2. National Center for Advancing Translational Sciences; 3. Retrophin, Inc. will provide the study drug (cholic acid, Cholbam®).

1.1 Overview

Brief Summary

The purpose of this study is to determine whether dietary cholic acid therapy benefits subjects with Smith-Lemli-Opitz syndrome (SLOS) by leading to an increase in serum cholesterol and reduction in cholesterol precursors.

Detailed Description

People with SLOS have a deficiency of the 7-dehydrocholesterol reductase enzyme that makes cholesterol. Consequently, they exhibit deficient serum cholesterol levels and increased cholesterol precursor lipids, which are thought to be toxic. Since cholesterol is necessary for production of bile acids in the liver, which help digest dietary cholesterol from the intestine, it is likely that bile acids are not made properly and dietary cholesterol is not absorbed properly. This contributes to the cholesterol deficiency seen in SLOS. Raising serum cholesterol in SLOS subjects is expected to decrease the potentially toxic cholesterol precursor lipids, and both changes would be theoretically beneficial for affected subjects.

Objective: The objective of this study is to determine whether treatment with cholic acid (a major bile acid drug used to improve fat absorption) will increase dietary absorption of cholesterol, reverse serum cholesterol deficiency, and reduce harmful cholesterol precursor lipids. These changes would be favorable for SLOS patients. To accomplish this objective, SLOS subjects will be given cholic acid for 2 months and serum cholesterol and its precursor lipids will be measured before and while taking the drug.

Target Population: SLOS patients who are ages 2 to 28 years old at time of enrollment and are taking supplemental dietary cholesterol will be enrolled. Subjects must be clinically stable, have a serum cholesterol ≤125 mg/dl, and be able to travel to a study site. No change in supplemental dietary cholesterol intake will be allowed during the study, and dietary records will be obtained throughout the study.

Study Protocol: Subjects will be treated with cholic acid, either orally or via gastrostomy tube, daily for 8 weeks. Blood will be collected at enrollment (week-0), and cholic acid will be started once the cholesterol level is shown to be ≤125 mg/dL. Blood for research tests will be obtained at week-0 and week-8 of cholic acid treatment, and 4 weeks off cholic acid (week-12). An additional blood collection for safety labs only will take place every 4 weeks (week-0, week-4, week-8 and week-12), either at a STAIR site or locally as an option for subjects who live distantly from a STAIR site.

2. Specific Aims (Hypothesis and Objectives)

Smith-Lemli-Opitz syndrome (SLOS) is an autosomal recessive disorder associated with multiple congenital anomalies, intellectual disability, failure to thrive, and behavioral changes. It has an estimated prevalence of 1/20,000 to 1/40,000. Patients with SLOS have deficiency of 7-dehydrocholesterol reductase (DHCR7), an enzyme that catalyzes the last step in the cholesterol synthesis pathway. Consequently, cholesterol levels are low and precursor sterols accumulate. Since cholesterol is the initial substrate for bile acid synthesis, it is likely that bile acid production is low and dietary cholesterol is not efficiently absorbed, adding to the overall cholesterol deficiency seen in this disease. The symptoms of SLOS are thought to be a result of systemic cholesterol deficiency and accumulation of sterol precursors. Although dietary cholesterol supplementation is considered the most rational approach to treatment of SLOS, dietary cholesterol has a limited effect on serum cholesterol levels of many patients, suggesting that it is not efficiently absorbed from the diet.

As the already impaired absorption of dietary cholesterol is compounded in part by deficient bile acid production, we **hypothesize** that administering cholic acid (Cholbam®, Retrophin, Inc) orally or by a gastrostomy tube to SLOS subjects with low serum cholesterol will result in an increase in serum cholesterol and a decrease in the precursor metabolites 7- dehydrocholesterol (7-DHC) and 8- dehydrocholesterol (8-DHC).

Our **objective** is to examine whether cholic acid therapy has efficacy for improving the sterol abnormalities in SLOS. This will be determined by achieving the following Specific Aims:

Specific Aim 1: We will examine whether cholic acid treatment has the ability to raise serum cholesterol and lower its precursors (7-DHC and 8-DHC) in SLOS subjects who are on dietary cholesterol supplementation.

Specific Aim 2: We will examine the effect of cholic acid supplementation on levels of serum bile acid and oxysterols, (the toxic products of oxidation of 7-DHC and 8-DHC).

Specific Aim 3: We will explore the safety of short-term cholic acid therapy in the SLOS population.

Outcome: Results of this pilot study are expected to establish whether cholic acid has *biochemical* efficacy for improving the sterol abnormalities in SLOS. If so, these results will provide a rationale for pursuing future investigations on the long term *clinical* efficacy of cholic acid in this disease.

3. Background

Patients with Smith-Lemli-Opitz Syndrome (SLOS), an autosomal recessive disorder associated with intellectual disability, congenital malformations, failure-to-thrive and behavioral challenges, have a defect in the final step of cholesterol biosynthesis, resulting from mutations in the gene for 7-dehydrocholesterol reductase (DHCR7). Patients with SLOS demonstrate both cholesterol deficiency and elevation of the precursors 7-

dehydrocholesterol (7-DHC) and 8-dehydrocholesterol (8-DHC) (See Figure 1)[1-3].The many medical issues seen in patients with SLOS are thought to arise from the cholesterol deficiency, the down-stream depletion of cholesterol metabolic products, and the accumulation and oxidation of the metabolic precursors into neurotoxic substances called oxysterols[4,5]. Thus, therapeutic approaches to SLOS include reversing the cholesterol deficiency to allow its normal utilization for downstream biosynthetic reactions, and reducing toxic 7-DHC and 8-DHC precursors and oxysterols[6,7].

Cholesterol supplementation whether via dietary means (egg yolk), cholesterol powder, or with soy-based or aqueous suspensions leads to an increase in serum cholesterol levels and a reduction in harmful precursors. Raising cholesterol levels allows cholesterol to be available for physiologically important roles including bile acid production, cortisol synthesis, RBC membrane stability, and brain growth. Although SLOS is listed as a treatable metabolic disorder causing intellectual disability[8], the complex clinical benefits of cholesterol supplementation in SLOS remain to be demonstrated in rigorously controlled intervention trials.

Treatment with antioxidants has been proposed to reduce the formation of toxic oxysterols, which cause neuronal apoptosis and retinal degeneration in an animal model of DHCR7 deficiency[9]. Moreover, antioxidant therapy has been reported to improve retinal function on serial electroretinograms in some SLOS patients[10]. Another therapeutic option which has been proposed in SLOS is the use of simvastatin to inhibit the cholesterol biosynthetic pathway and lower cholesterol precursors. Two studies reported that simvastatin was effective in reducing the plasma levels of 7-DHC, but neither one reported significant clinical improvement[11,12]. There is no reported evidence that Simvastin or other drugs which lower plasma levels of cholesterol precursors, affect brain levels of cholesterol or precursors. Thus, to date, there is no definitive treatment for SLOS and new therapies are sorely needed. Patients are routinely put on cholesterol supplementation as standard of care and often take multivitamin-mineral supplements with antioxidants. Therefore, any new therapeutic option will have to be tried in patients already supplemented with cholesterol and antioxidants.

In this study, we will investigate the efficacy of cholic acid, a bile acid produced from cholesterol, to improve intestinal cholesterol absorption, increase cholesterol levels and reduce production of toxic cholesterol precursors in patients with SLOS who are on cholesterol supplementation.

Cholesterol is a major lipid component of cellular membranes and the precursor to steroid hormones, neurosteroids and bile acids. Through its covalent modification of Sonic Hedgehog, cholesterol is also critical for certain cell signaling pathways that function in fetal and postnatal development. Cholesterol synthesis is highly regulated by feedback inhibition of the early rate-limiting step in cholesterol biosynthesis catalyzed by HMG-CoA reductase (See Figure 1). Under physiologic circumstances, changes in cholesterol levels result in compensatory variations in HMG-CoA reductase activity and bile acid biosynthesis, with bile acids playing a major positive role in intestinal cholesterol

absorption and HMG-CoA reductase controlling the synthesis of intracellular cholesterol precursors (mevalonate).

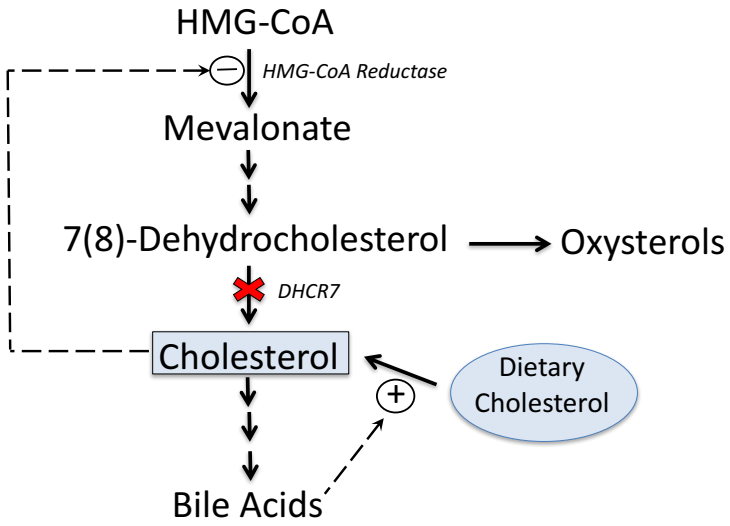

Figure 1. Abbreviated biochemical pathway for cholesterol metabolism. Dashed arrows indicate regulatory relationships for feedback inhibition of cholesterol synthesis or stimulation of dietary cholesterol absorption.

Patients with SLOS have deficiency of DHCR7, an enzyme that catalyzes the last step in the cholesterol synthesis pathway (See Figure 1). As noted above, low cholesterol levels should trigger a compensatory increase in HMG-CoA reductase, mevalonate synthesis and endogenous cholesterol synthesis to restore cholesterol homeostasis. In SLOS, however, the expected compensatory increase in HMG-CoA reductase is not observed, probably because cholesterol precursors such as 7-DHC and 8-DHC, and perhaps other precursors accumulating as a result from DHCR7 deficiency, substitute for cholesterol in down-regulating HMG-CoA reductase, thus maintaining a normal HMG-CoA reductase activity despite low cholesterol levels.

In individuals with intact functional cholesterol homeostasis (biosynthesis and dietary intake), approximately 50% of dietary cholesterol is absorbed. In SLOS, absorption of dietary cholesterol may be further compromised due to impaired bile acid synthesis, thus contributing to overall cholesterol deficiency. Individuals with SLOS can exhibit reduced production of bile acids[13,14]. But in spite of near total deficiency of normal bile acids in some patients, clinically evident fat malabsorption and fat-soluble vitamin deficiencies may be rare[15]. Further, mildly affected individuals have normal bile acid synthesis[16]. It is thus likely that clinically significant bile acid deficiency that would result in impaired cholesterol absorption may be limited to severely affected SLOS patients with very low cholesterol levels. Nevertheless, bile acid therapy to directly improve cholesterol intestinal absorption, whether an underlying bile acid synthesis deficiency exists or not, appears to be a viable therapeutic option for improving whole body cholesterol pool size.

Bile acid supplementation in SLOS was used in the mid 1990's along with cholesterol to promote improved cholesterol absorption. Because these early trials with cholic acid were performed soon after the metabolic error in cholesterol biosynthesis causing SLOS was first identified, there is no published evidence regarding effects of cholic acid on clinical course. However, cholic acid was well tolerated, and its use in addition to cholesterol supplementation led to an increase in cholesterol levels. Due to the decision to cease manufacturing cholic acid, it became unavailable since the late 1990's. Subsequently, ursodeoxycholic acid (Actigall) became the only bile acid that was approved for pediatric use and administered to patients with very severe disease (cholesterol levels <30 mg/dL). However, long-term use of Actigall led to eventual lowering of cholesterol levels, and thus Actigall has not been used chronically to treat patients with SLOS. Recently, the bile acid cholic acid (Cholbam®) has come back on the market. Cholic acid has been shown to improve absorption of cholesterol and other important fat-soluble nutrients. There is no data available to suggest that long term use of cholic acid might lead to a lowering of cholesterol.

In summary, cholic acid treatment may improve intestinal cholesterol absorption in severe SLOS patients with impaired bile acid synthesis, and may also be beneficial in less severely affected patients with normal or subnormal bile acid synthesis, simply by increasing the bioavailability of supplemental cholesterol. Increasing cholesterol absorption with cholic acid would secondarily increase the systemic cholesterol level and significantly decrease the levels of toxic cholesterol precursors through feedback inhibition of the cholesterol biosynthesis pathway.

4. Study Design and Methods

This will be an open label, multi-center, pilot study to assess the effects of cholic acid administration on serum sterol levels. Up to 15 SLOS subjects will be enrolled with the expectation that 13 subjects will complete the study and provide evaluable data. Subjects must have a documented serum cholesterol ≤125 mg/dL measured within 6 months prior to enrollment to qualify for the study. Informed consent will be obtained prior to completing any study procedures. Subjects will be studied in 2 phases (see Figure 2). The first phase is a "Treatment Phase". Those subjects who have a serum cholesterol ≤125 mg/dL will be qualified to be administered cholic acid (Cholbam®, Retrophin, Inc) at 10 mg/kg/day dosages given orally or via J- or G-tube divided BID for two months (weeks 0 to 8). This dose is based on weight, even in older patients. After completion of the 8-week Treatment Phase of the study, cholic acid will be discontinued and subjects will enter the "Post-treatment Phase" for 4 weeks (weeks 9-12).

We have chosen to perform this trial in patients who fall in the moderate to severely affected range, with the hope that this will allow us to see a greater affect of cholic acid treatment. Levels of cholesterol and precursors are associated with severity of disease, with those more severe patients showing lower cholesterol levels and higher levels of precursors. Although there is some variation in these values based on dietary intake and well being of the patients (intercurrent illness can worsen cholesterol deficiency and raise precursor levels, for example), these variations are not expected to impact the ability to assess affects of treatment. A severely affected patient does not suddenly appear to be

mild, and vice versa. We also will be following the Sterol Ratio of precursors divided by cholesterol levels, to help assess efficacy of treatment. The ratio does not change to a great extent over time.

Due to COVID-19 precautions and potential travel restrictions, we will incorporate optional and flexible locations for some site visits. We will adhere to all COVID-19 precautions established at each STAIR site at the time of each visit.

Figure 2 displays the design of this study, which minimizes COVID-19 risk for subjects and healthcare personnel. All subjects will be seen in person for at least two mandatory site visits: 1) at the baseline visit (week-0) and 2) at week-8 of the cholic acid Treatment Phase. During these mandatory STAIR site visits, subjects will be assessed with physical examination, vital signs, growth parameters (weight and height), and laboratory research blood specimens and safety blood tests will be obtained. Those subjects who qualify for drug treatment, based on a documented serum cholesterol ≤125 mg/dL, will be sent cholic acid after their week-0 baseline visit and started on treatment at home. At week-4 and week-12, blood will be collected for safety blood tests either at the STAIR site or, optionally for those subjects who live distantly from the STAIR site, obtained locally and shipped to the STAIR site. In addition, laboratory research blood tests will be obtained at week 12 to confirm reversal of sterol levels after discontinuation of cholic acid. For those subjects living some distance away from a STAIR site, a telehealth visit will be done at the end of week-12, whereas this final visit will be obtained either at the STAIR site or by telehealth. In this fashion, safety blood specimens will be collected at weeks 0, 4, 8 and 12. Research blood specimens will be obtained at weeks 0, 8 and 12. Subjects' STAIR clinic visits and scheduled blood draws must be met within a 5-day time interval.

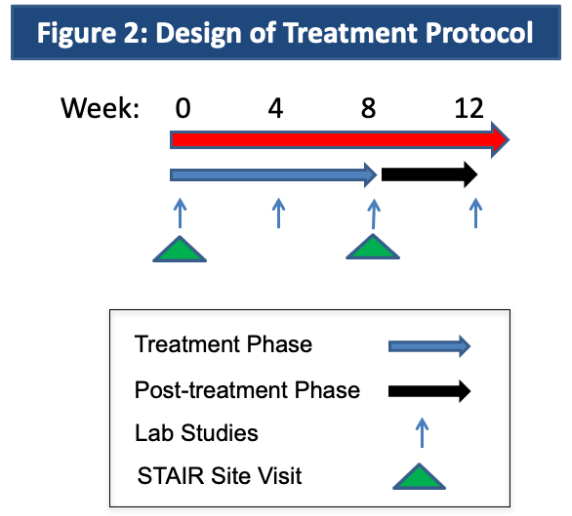

Figure 2. Protocol time line. Subjects with mean cholesterol ≤125 mg/dl at their first STAIR site visit will be placed into the Treatment Phase and administered cholic acid. Safety lab tests will be monitored while on treatment. After 8-weeks, cholic acid will be discontinued and repeat lab tests will be drawn at 12-weeks.

Dietary and Drug Compliance

Dietary histories and drug (cholic acid) compliance records will be obtained throughout the study. Subjects will be required to maintain their cholesterol supplementation during the study. No changes in cholesterol medication will be permitted. Similarly, subjects who are taking antioxidants (AquADEKs™ or DEKAs) or other drugs will be required to make no changes in dose during the study. To ensure compliance, dietary histories and recordings of cholic acid and antioxidant administration will be obtained daily throughout the study using a handwritten journal. Using this journal, the subject can record the time and amount of the cholesterol supplement, the antioxidant, and the study drug taken daily. The caretaker will be asked to scan, photograph and text or fax the dietary information from the previous week to the study coordinator at Children's Hospital Colorado every Monday. The study coordinator will contact subjects by telephone on a weekly basis to review drug compliance, dietary history, clarify any adverse events or changes in medical history. The Research Dietician at Children's Hospital Colorado will review 3 days of subjects' dietary histories every week for cholesterol intake. We will ask the study subjects to return any remaining cholic acid at the end of the study, as another way to monitor compliance.

Using the patient journal, subjects will also be able to report adverse events. The site coordinator will review the drug compliance records and contact subjects weekly by telephone as needed to clarify adverse events or changes in medical history.

Laboratory Tests

The following specialized laboratory tests will be performed on fasting (>8 hour) blood specimens at weeks 0, 8 and 12:

- Serum cholesterol, 7-DHC, and 8-DHC levels will be measured by Kennedy Krieger Institute, in Baltimore, Maryland. The sterol ratio, defined below, will be calculated.

$$sterol\ ratio = \frac{[(7-DHC)\ + (8-DHC)]}{cholesterol}$$

- Serum bile acids (fractionated and total) will be measured by the Metabolic Laboratory at Mayo Clinic Laboratories in Rochester, Minnesota. These measurements will include total bile acids, cholic acid, and chenodeoxycholic acid.
- Serum oxysterols will be measured by LC-mass spectrometry at the University of Washington in Seattle, Washington.
- 25-OH vitamin D will be measured at the STAIR study sites.

At the baseline visit, serum cholesterol will be sent to confirm that the subject has a cholesterol ≤125 mg/dL prior to beginning cholic acid therapy. All other research blood specimens (cholesterol, 7-DHC, 8-DHC, oxysterols, bile acids) will be stored at the STAIR clinical sites at approximately -80 ºC and shipped to the reference labs for batch testing.

Safety Tests

The following tests will be obtained to monitor for safety of cholic acid therapy:

- CBC with differential and platelet count.

- Comprehensive metabolic panel, which includes measurements of renal function tests (creatinine, BUN), aspartate aminotransferase (AST), alanine aminotransferase (ALT), albumin, alkaline phosphatase, and bilirubin
- Gamma-glutamyltransferase (GGT).

Safety tests will be obtained at week-0, week-4, week-8 and week-12. Safety lab tests will be done immediately and not batched. The subjects' local lab results will be sent to the study PI. Results of these tests will be reviewed by the PI within approximately 3 calendar days for evidence of abnormalities. If insufficient blood is obtained from the patient for all of the above tests, we will prioritize the safety tests and serum cholesterol and cholesterol precursors over serum bile acids, oxysterols and 25-OH vitamin D.

For females of childbearing age (who have begun menstruating), a urine or serum pregnancy test will be documented at the start of the study (week 0/ baseline) before cholic acid is started and at the end of cholic acid administration (week-8).

Dietary Cholesterol Supplementation and Antioxidants

We will enroll subjects who are already on a dietary cholesterol supplementation. The form of cholesterol supplementation may include all available forms of cholesterol supplements such as dietary cholesterol in the form of egg yolk, SLOesterol, (an encapsulated cholesterol powder developed by Solace Nutrition), SONIC cholesterol (a product offered by New Beginnings), an aqueous solution provided by Johns Hopkins (150mg/ml), and a concentrated suspension of cholesterol in soy oil (200 mg/ml), compounded by a licensed pharmacy collaborating with Dr Elias. The amount of cholesterol supplementation will vary among subjects, but generally, subjects are expected to be taking cholesterol supplements at 50-150 mg/kg/day. To prevent dietary dependent changes in cholesterol status, no changes in the amount or frequency of dietary cholesterol taken during this study is allowed. A change in cholesterol intake greater than 20% based on dietary history will trigger review and contact with the subject.

It is expected that some subjects will be taking antioxidants, either in the form of AquADEKs (Actavis, Inc), available as a liquid suspension, chewable tablet or capsule, or a new comparable form called DEKAs Plus (Callion Pharma). The amount administered is based on age and weight of the subject. If taking antioxidants upon enrollment, no changes will be allowed in the antioxidant dose during this study.

Study Outcome Measures

Primary Outcome Measures: One primary outcome measure for this biochemical study will be total serum cholesterol, 7-DHC and 8-DHC, and the sterol ratio. It is hypothesized that serum cholesterol will rise and 7-DHC and 8-DHC levels will decrease. Either of these changes will result in a decrease in the sterol ratio. A second primary outcome measure is the incidence of adverse events.

Secondary Outcome Measures: A secondary outcome measure will be oxysterol level. The expectation is that oxysterols, which are produced from 7-DHC and 8-DHC, will decrease on cholic acid therapy.

Exploratory Outcome Measures: These will include serum vitamin D, bile acid levels, and changes in safety monitoring including liver function as determined by serum transaminases, bilirubin, albumin, alkaline phosphatase, GGT and CBC.

Phlebotomy Estimate: The following blood tests (and requisite maximum blood volumes) will be drawn:

- Comprehensive chemistry panel 2 ml (max)
- Complete blood count (CBC) 3 ml (max)
- Cholesterol, 7-DHC and 8-DHC 2 ml
- Bile acids 1 ml
- 25-OH Vitamin D 1 ml
- Serum gamma glutamyltransferase (GGT) 1 ml
- Oxysterols 2 ml
    - *Total volume* *12 ml*

The maximum amount of blood to be drawn for this study is 12 ml/visit x 4 visits/subject, or total 48 ml blood over a period of 12 weeks. The age range for subjects is 2-25 years. The body weight of a 2-year old child with SLOS ranges between 6 and 10 kg with 8 kg being the 50th percentile[17]. Assuming a body weight of 8 kg and a 12-ml collection of blood at 0, 4, 8 and 12 weeks, the total volume of blood collected over 12 weeks (48 ml) will be less than that the NIH Clinical Center research limit of 7 ml/kg/6 weeks. Efforts are generally made in Children's Hospitals to use small size specimens so that the amount of specimen required for a blood count and chemistries can be significantly reduced using "microtainers" in the lab. For example, the combination of a CBC, CMP and GGT would be only 1.6 ml, and it is possible to do the Chol/7-DHC and 8-DHC in only 1 ml. Older subjects in this protocol will have proportionally less blood volume taken. Therefore, the amount of blood taken for this protocol is well within the established safety limits and should cause no anemia or other complications.

Cholic Acid

Cholic acid (brand name Cholbam®, available from Retrophin, Inc.) is a natural bile acid synthesized in the liver and secreted in bile. It is a white or off-white powder that is supplied in 50 mg and 250 mg two-piece gelatin capsules. It should be stored at room temperature (69º – 77º F).

Cholbam® is approved by the FDA for the treatment of bile acid synthesis disorders due to single enzyme defects (SEDs) and for adjunctive treatment of peroxisomal disorders including Zellweger spectrum disorders (ZSD) in patients who exhibit manifestations of liver disease, steatorrhea or complications from decreased fat soluble vitamin absorption. Owing to their inherited defect in cholesterol and bile acid synthesis, SLOS subjects are considered to be SEDs. The recommended dosage of Cholbam® is 10 to 15 mg/kg administered orally once daily, or in two divided doses, in pediatric patients and in adults.

Cholic acid is a primary bile acid synthesized from cholesterol in the liver. Bile acids

facilitate fat digestion and absorption by forming mixed micelles, and facilitate absorption of fat-soluble vitamins in the intestine. Endogenous bile acids including cholic acid enhance bile flow and provide the physiologic feedback inhibition of bile acid synthesis. Cholic acid enhances absorption of lipids from the intestine and has additional metabolic effects. The loss of cholic acid is normally compensated by increased *de-novo* synthesis of cholic acid from cholesterol to maintain the bile acid pool in healthy subjects. In patients with SLOS, this compensation mechanism is expected to be disrupted due to the impaired synthesis of cholesterol.

Cholbam® should be taken with food. For subjects who are unable to swallow the capsules, the capsules can be opened and the contents mixed with infant formula or soft food such as mashed potatoes or apple puree (for older children and adults) in order to mask any unpleasant taste. Many of the more severely affected patients to be enrolled in this study are fed via G-tube which will obviate the taste issue. One of the reasons for picking patients >2 years of age is to simplify the dosing calculation as the patients will not be that tiny. Cholbam comes in 2 sizes of capsule, so that we can use the smaller 50 mg capsule in the smaller patients.

On entering the treatment phase of the trial, the parents/ caregivers will be trained at the STAIR clinical site in preparing and administering the medication using the aliquot method for those subjects who are unable to swallow capsules and must be administered cholic acid as a liquid. A study staff member will directly observe each caregiver to certify that the caregiver can accurately prepare and administer the correct dose of the study drug without assistance. Using video conferencing (such as FaceTime, Skype or Zoom), a study staff member will observe the caregiver prepare and administer the study drug correctly at least 1-3 times or until the study team is assured that the family members are able to administer accurate dosing.

4.1 Inclusion Criteria

SLOS subjects with the following characteristics will be enrolled:

1. Ages 2-28 years at enrollment.
2. Subjects (or their parents/legally-authorized representative) must provide signed informed consent.
3. Assent must be obtained from those subjects ages 7-17 years (or 7-18 years, for some STAIR sites) who are intellectually capable of understanding this study.
4. Diagnosis of SLOS based on clinical features and biochemical/genetic confirmation.
5. Subjects are capable of traveling to the STAIR study site.
6. Fasting serum cholesterol ≤125 mg/dL within the prior 6 months must be established before enrolling in the study. This level of cholesterol will be confirmed at the first STAIR visit prior to starting cholic acid therapy.
7. Clinically stable at the time of enrollment (see below for definition of clinical conditions which would preclude enrollment)
8. Subjects must be on a constant dietary cholesterol intake for at least 3-months prior to treatment with cholic acid.

9. Subjects must agree to make no changes in cholesterol supplementation during the STAIR study.
10. SLOS subjects who are taking antioxidants or drugs for neurologic/psychiatric symptoms may be included if they agree to make no changes in the medication dose during this study.
11. Subjects must have internet access and computer (or tablet, smart phone) to allow telehealth visits and/or observation of drug preparation.

4.2 Exclusion Criteria

Subjects with the following characteristics are excluded from this study:

1. Subject has an unstable clinical condition that, in the judgment of the principal investigator, would prevent completion of the study. Medically unstable patients would include those patients with severe liver disease, those patients with complex birth defects such as severe heart disease or renal dysplasia, those patients with severe respiratory compromise requiring tracheostomy, or patients not likely to survive longer than 1 year.
2. Subject has transaminase elevations (>3-fold above the reference range) at baseline.
3. Subject is taking drugs (statins, bile acid sequestrants, ezetimibe) or nutraceuticals (red yeast rice, phytosterols, polycosanol, high dose omega-3 fatty acids) that are known or suspected to lower serum cholesterol levels.
4. Subject is pregnant.

4.3 Recruitment of Participants

SLOS subjects will be recruited from each of the STAIR sites listed in Participating Institutions/Investigators. Most subjects will be already known to the site PIs and followed in their centers. We will also recruit subjects with the assistance of the Smith-Lemli-Opitz/RSH Foundation and their website, and families will be notified of this study at the annual SLOS Foundation Family Conference. The study will be posted on www.clinicaltrials.gov. Finally, we may advertise the study to medical geneticists at the national meetings of the American Society of Human Genetics and American College of Medical Genetics.

4.4 Retention Strategies

Retention of subjects in the study will be encouraged by conducting weekly telephone calls and promptly addressing any issues, complications or adverse events that may arise. Also, to encourage retention, efforts will be made to reimburse costs for travel expenses and hotel accommodations for subjects for their STAIR site visits.

4.5 Data Elements

Every individual who provides consent will be assigned a unique numerical identifier that will ensure anonymity throughout the study. Study personnel at each STAIR site who interact with the subject will know them by name and have access to their HIPAA identifiable data, however this information will not be shared with the entire study personnel group. Each STAIR site that enrolls subjects will upload anonymous subject data into a REDCap database that is maintained by the DMCC.

4.6 Schedule of Events

The schedule of event for this study is shown in the Table 1.

Table 1: Schedule of Events

Phase:	Treatment			Post-Treatment
Visit Site:	STAIR	Local (or STAIR)	STAIR	Local (or STAIR)
Timepoint:	Week 0 Baseline	Week 4**	Week 8	Week 12
Assessment		± 5 days	± 5 days	± 5 days
Eligibility and Written Consent	X			
Demographics	X			
Physical Exam	X		X	
Medical History	X			
Concomitant Medications	X	X	X	X
STAIR Site Labs/Local Labs*	X	X	X	X
Dietary History	X	X	X	X
Teaching and observation of drug preparation	X			
Pregnancy Test	X		X	
Weekly phone calls	Inquire about AEs, dietary history recordings, change in medications, etc.			
Adverse Events Reporting	Will be included in weekly phone calls.			
Patient-Reported Dietary History	This data will be collected daily and analyzed weekly			

* These labs will be drawn at the STAIR site or locally for subjects.
** Note that weeks 4, 8 and 12 refer to time after the start of cholic acid therapy.

5.0 Data and Safety Monitoring Plan

The study protocol will be reviewed and approved by the NIH before submission to the STAIR IRB of Record for approval. Participant enrollment may only begin with IRB approved consent forms.

Adverse events (AEs) will be recorded from each subject on a weekly basis by the local clinic coordinators at each study site. This will be done at routinely scheduled weekly telephone calls and include all contact initiated by the subjects themselves. AEs will include medical illnesses, change in medical condition, suspected side effects of cholic acid, complications of phlebotomy, etc. AEs will be reported to the Study Chair who will review them weekly. The Medical Review Officer and Study Chair, together with input

from the study site PIs, will decide whether AEs rise to the level of seriousness or unusual character or frequency as to suspend the study.

In addition, this study will be reviewed every 6 months by the UNMC Data Safety Monitoring Board (DSMB) for safety concerns, complicating issues, and study progress.

5.1 Study Oversight

The Study Chair (Dr. Ellen Roy Elias) has primary oversight responsibility of this clinical trial. The UNMC appointed Data Safety Monitoring Board (DSMB) has oversight responsibility of the Data Safety Monitoring Plan (DSMP) for this clinical trial. The DSMB will review accrual, patterns and frequencies of all adverse events, and protocol compliance every 6 months. The DSMB makes recommendations to the NIH regarding the continuation status of the protocol.

Each site's Principal Investigator and their research team (co-Investigators, research nurses, clinical trial coordinators) are responsible for identifying adverse events. Aggregate report detailed by severity, attribution (expected or unexpected), and relationship to the study drug/study procedures – will be available from the DMCC for site review. Adverse events will be reviewed monthly by the research team. A separate report detailing protocol compliance will also be available from the DMCC for site review on a monthly basis. The research team will then evaluate whether the protocol or informed consent document requires revision based on the reports.

5.2 Definitions and Standards

An adverse event is defined as: "…an unfavorable and unintended sign, symptom or disease associated with a participant's participation in a study."

Serious adverse events include those events that: "result in death; are life-threatening; require inpatient hospitalization or prolongation of existing hospitalization; create persistent or significant disability/incapacity, or a congenital anomaly/birth defects."

An unexpected adverse event is defined as any adverse experience…the specificity or severity of which is not consistent with the risks of information described in the protocol.

Expected adverse events are those that are identified in the research protocol as having been previously associated with or having the potential to arise as a consequence of participation in the study

All reported adverse events will be classified using the current version of the Common Terminology Criteria for Adverse Events (CTCAE) developed and maintained by CTEP at National Cancer Institute.

5.3 Expected/Known Risks/Discomforts/Adverse Events Associated with Study Intervention and Procedures: Definition of Expected Adverse Events

The risks of cholic acid therapy in SLOS are expected to be low, in part due to the endogenous nature of cholic acid which is normally made in the liver and excreted into the intestine. Based on the 3 studies in patients with single enzyme defects (SED) of bile acid synthesis and Zellweger syndrome spectrum disorders (ZSD), diarrhea was identified as the most frequent AE, occurring in 2% of this population. In addition, the following AEs were noted at frequencies of 1% or less: reflux esophagitis, malaise, jaundice, nausea, abdominal pain, intestinal polyp, skin lesions, urinary tract infections and peripheral neuropathy. In this population, cholestasis and liver dysfunction was seen in 58/79 (86% of patients). Five patients exhibited worsening liver function on cholic acid. Five additional patients without liver dysfunction at the time of cholic acid initiation developed liver dysfunction on the drug. In contrast to SED and ZSD, SLOS is not usually associated with liver disease, although some SLOS subjects do have mildly elevated serum transaminases. Consequently, it is not expected that cholic acid therapy will lead to liver related AEs. Nevertheless, safety monitoring for worsening of the liver will be done at least monthly and more frequently if clinically indicated.

Risks of Phlebotomy:

- ***Procedure and risks***: In general, phlebotomy is considered to carry a very low risk of complications. Venous blood is typically removed from an accessible site such as the arm using a small needle. Blood drawing will cause some mild temporary pain and carries a small risk of bleeding, bruising and/or infection at the puncture site.
- ***Protections from risk****:* To decrease discomfort of the needle poke in children, we will use topical anesthetic EMLA cream applied to the skin 30 minutes in advance of the procedure. To minimize associated risks of bleeding and bruising, only experienced phlebotomists will draw blood for our studies. Bleeding and bruising at the puncture site is usually easily controlled with the application of direct pressure and a bandage. Bruising at the puncture site is occasionally seen, but is not harmful. Using standard phlebotomy procedures with sterilization of the skin and gloved personnel, infection from routine venous phlebotomy is exceedingly rare. It can be treated effectively with antibiotics, if necessary. The minimal amount of blood necessary to carry out our STAIR studies will be collected. We will not exceed 7 ml of blood/kg body weight per 6 weeks, which is considered safe in children and will not cause anemia or related symptoms.

5.4 Reporting Timeline

- Within **24 hours** of learning of any Serious Adverse Event (SAE), investigators must report it to the study PI and DSMB if it:
  - Is considered life-threatening/disabling or results in death of subject

  -OR-
  - Is Unexpected/Unanticipated
- Investigators must report all other reportable SAEs within **5 working days** (of learning of the event).

- All other (suspected) reportable AEs must be reported to the DSMB within **20 working days** of the notification of the event or of the site becoming aware of the event.

5.5 RDCRN Adverse Event Data Management System

Upon entry of a serious adverse event, the DMCC Data Management system will immediately notify the Study Chair, site PIs, and any additional agencies (if applicable-industry sponsor, CTEP, etc) of any reported adverse events via email.

Serious adverse events: The Medical Review Officer (MRO) determines causality (definitely not related, probably not related, possibly related, probably related, definitely related) of the adverse event. The MRO [and Retrophin] may request further information if necessary and possibly request changes to the protocol or consent form as a consequence of the adverse event. A back-up notification system is in place so that any delays in review by the MRO beyond a specified period of time are forwarded to a secondary reviewer. The DMCC REDCap system maintains audit trails and stores data (and data updated) and communication related to any adverse event in the study.

Non-serious expected adverse events: Except those listed above as immediately reportable, non-serious expected adverse events that are reported to or observed by the investigator or a member of his/her research team will be submitted to the DMCC in a timely fashion (within 20 working days). The events will be presented in tabular form and given to the MRO and UNMC DSMB on a bi-annual basis. Local site investigators are also required to fulfill all reporting requirements of their local institutions.

The DMCC will post aggregate reports of all reported adverse events for site investigators.

5.6 Study Discontinuation

The NIH and local IRBs (at their local site) have the authority to stop or suspend this trial at any time. This study may be suspended or closed if:

- Early stopping rules have been met.
- Accrual has been met.
- The study objectives have been met.
- The Study Chair / Study Investigators believe it is not safe for the study to continue.
- The DSMB recommends suspending or closing the trial
- The NIH suspends or closes the trial.
- The FDA suspends approval of Cholbam for human use.
- Retrophin withdraws use of Cholbam.

Premature discontinuation of this study will also occur under the following circumstances:

- Adverse events (AEs) are determined to occur in a significant proportion of subjects or the AEs are serious enough that the subjects are judged to be at high medical risk if participation in the study continues. An adverse event would include hospitalization, or marked worsening liver function or GI symptoms severe enough to prevent the patient from taking the cholic acid.

- Failure to retain subjects and complete the study.

The study involves treating SLOS subjects with cholic acid. For this purpose, AEs will be reviewed by the Medical Review Officer and DSMB to determine if the AE is related to the drug.

5.7 Subject Withdrawal and/or Cholic Acid Dose Interuption/Reduction

The following conditions will result in withdrawal of the subject from the study:

- Withdrawal of consent
- Withdrawal by the participant
- Withdrawal by the investigator
- Intercurrent illness or event that precludes further visits to the study site or ability to evaluate disease (e.g.-mental status change, serious illness, pregnancy).

Withdrawal from the study will be permitted at any time with the agreement of the Principal Investigator and/or SLOS subject (or parent/legal authorized representative). Withdrawal may be necessitated for clear evidence of non-compliance, adverse effects of the drug, onset of concurrent illness, or new SLOS complication that prevents continuation. All data acquired prior to termination for the reasons outlined below will be included in the primary analysis unless the subject provides a written request to delete all of their data from the data base and subsequent analyses. Every effort will be made to conduct a final study visit with the participant and participants will be followed clinically until, if applicable, all adverse events resolve.

Cholic Acid Dose Reduction

1. During the Treatment Phase, administration of cholic acid may be interrupted for up to 3 successive days if the subject develops an acute illness and cannot tolerate the drug, for example due to vomiting or diarrhea. Interruption of drug for a longer period of time (>5 days) may trigger withdrawal from the study, upon review and agreement by the Principal Investigator and site investigators or the Medical Review Officer.

2. In addition to a temporary complete interruption of cholic acid administration, evidence of worsening liver function may prompt a reduction in drug dosage. Worsening of liver function is defined as:
   - AST, ALT and/or GGT >3 times above baseline measurements or >300 IU in any patient and/or
   - A total bilirubin value >3 mg/dL (if the baseline level is <10) and/or
   - onset of serious gastrointestinal symptoms not clearly associated with a limited infectious illness

Worsening of liver function will trigger a review by the Prinicipal Investigator and reduction of cholic acid dosage to 5 mg/kg/day. Monitoring labs will be obtained 1 week after lowering the dose. If the subject does not tolerate this lower dose, based on symptoms or persistently abnormal test results, the subject may be withdrawn from the study.

5.8 Data Quality and Monitoring Measures

As much as possible data quality is assessed at the data entry point using intelligent on-line data entry. Data element constraints, whether independent range and/or format limitations or 'relative' referential integrity limitations, can be enforced by all methods employed for data input. QA reports assess data quality post-data entry. As we note, data quality begins with the design of the data collection forms and procedures and incorporates reasonable checks to minimize transcription and omission errors. Of the more important quality assurance measures are the internal validity checks for reasonableness and consistency.

- Data Monitoring: The DMCC identifies missing or unclear data and generates a data query to the consortium administrator contact.
- Data Delinquency Tracking: The DMCC will monitor data delinquency on an ongoing basis.

5.9 Quality Control: Study Related Procedures

Study related procedures will be outlined in a Manual of Operations (MOO), which will be distributed to each of the study sites. The MOO will include descriptions of methods for blood collection, processing and storage. Instructions will be included for shipment of specimens to the Study Chair for batch shipment to reference laboratories for testing.

6. Statistical Considerations

Data from this study will be submitted to the DMCC located at the Cincinnati Children's Hospital & Medical Center. Physical examination, laboratory studies, vital signs, and growth parameters assessed at week 0 and week 8 will be tabulated. The difference in total serum cholesterol, 7-DHC and 8-DHC, sterol ratio, oxysterol levels, serum bile acids, and liver function from the baseline visit (week 0) to week 8 visit will be calculated for each participant. The average change and 95% confidence intervals for each of these measurements will be estimated.

Safety data will be summarized for all treated subjects using appropriate tabulations, and descriptive statistics. The incidence rate of each adverse event will be estimated and reported with exact 95% confidence intervals.

Sample size: This pilot study is intended to explore preliminary signals for efficacy as well as examine safety of cholic acid supplementation. The primary purpose of the study is to detect a difference in pre-and-post treatment mean serum cholesterol level of one group. The sample size was based upon a sufficient number of participants to begin such explorations prior to pursuit of a randomized controlled trial and historical variation in serial serum cholesterol levels in SLOS subjects. We conservatively use the population effect size of 0.80 and the significance level (alpha) of 0.05 using a two-sided one-sample t-test. With an accrual of 13 evaluable participants, an estimated standard deviation of 5 mg/dl with mean 61 mg/dl for the cholesterol measurements, a within subject correlation of .8 (between pre and post testing) and a two-sided alpha of 0.05, there is greater than 80% power to detect a change in total serum cholesterol of greater than 10 mg/dl. Under the same assumptions, for sterol ratio of 7-DHC/cholesterol, based on a standard

deviation of 0.21, there is greater than 80% power to detect a change of greater than 0.07. To adjust for 15% dropout rate, 15 SLOS patients will be recruited for the study. There will be no interim analysis of the data.

7. Data Management

The following data will be obtained:

- Demographics: age (years and months), date of birth, gender.
- Physical measurements, including vital signs, weight, height, blood pressure.
- Laboratory: serum cholesterol, 7-DHC, 8-DHC, oxysterols, serum bile acids (total bile acids, cholic acid, chenodeoxycholic acid, comprehensive chemistry profile (including AST, ALT, bilirubin), GGT and complete blood count (CBC) including platelets.

All study data will be collected via systems created in collaboration with the DMCC and will comply with all applicable guidelines regarding patient confidentiality and data integrity.

All research data will be collected on data forms and submitted online to the DMCC REDCap database. Participant identifiers will be used to protect participant confidentiality and safety.

7.1 Registration

IRB approval for the protocol must be on file at the DMCC before accrual can occur from the clinical site.

The DMCC will use a system of coded identifiers to protect participant confidentiality and safety. Each participant enrolled will be assigned a local identifier by the enrollment site. This number can be a combination of the site identifier (location code) and a serial accession number. Only the local site will have access to the linkage between this number and the personal identifier of the subject.

7.2 Data Entry

Data collection for this study will be accomplished with online electronic case report forms. Using encrypted communication links, on-line forms will be developed that contain the requisite data fields.

7.3 Laboratory Data Flow

If possible and practical for this pilot study, the DMCC will provide laboratories with on-line forms and/or electronic data exchange mechanisms - depending on their capabilities and needs - to enter, update and obtain relevant data. Online forms exist to verify specimen receipt, report specimen issues and submit test results for specimens. The preferred method to exchange data electronically is through the Specimen Management System Web Service. The Web Service allows laboratories to obtain specimen shipment information, receive individual specimens or specimen shipments, report specimen issues and communicate specimen aliquots in a secure manner (test result submission is

planned). The DMCC will also support uploading of files electronically. All transactions are logged and validated for both methods.

7.4 Study Records Retention

Hard copies of data forms will be stored at each STAIR study site that enrolls subjects. Forms will be stored for at least 5 years after completion of the study.

7.5 Protocol Deviations

Protocol deviations are defined as any significant alteration of the proposed procedures. Deviations will be reported to the site PI and recorded. Protocol deviations will also need to be reported to the IRB and DMCC. Deviations will include, but are not limited to, the following:

- Failure to obtain proper written consent.
- Failure to obtain required blood specimens on time (± 5 days).
- Inadequate administration of cholic acid dose.
- Failure to return to study site visit on time (± 5 days).
- Inappropriate specimen collection, change in drug dosing or other data collection.

8. Human Subjects

8.1 Good Clinical Practice Statement

This clinical trial will be conducted in accordance with the ethical principles that have their origin in the Declaration of Helsinki, and that are consistent with Good Clinical Practice and all applicable regulatory requirements.

8.2 Benefits

Subjects will receive no clinical benefit from participating in this study. It is hoped that the information learned in this study will be able to help others with SLOS in the future.

8.3 Risks

Risks of participating in this study are summarized in Section 5.3 and consist of risks associated with cholic acid therapy, phlebotomy and potential loss of confidentiality.

8.4 Inclusion of Subjects

Since SLOS is not limited to any particular ethnic minority or socio-economic group or gender, we anticipate enrolling any and all subjects who are diagnosed and satisfy the inclusion criteria.

Inclusion of children

Because the majority of subjects with SLOS are children and relatively few adults with SLOS are known, we expect that children will be the major group studied. We will recruit subjects between 2 years to 28 years of age and of both genders.

Inclusion of women
Women and girls will be included in the study. SLOS is an autosomal recessively inherited condition that affects males and females equally, so females should be equally represented in this study. However, since this disorder is uncommon, STAIR investigators will have limited control over the gender mix of the study population.

Inclusion of Minorities
SLOS affects all ethnic and minority groups. Owing to the rarity of SLOS in the United States population, STAIR investigators will have limited control over the race and ethnicity mix of the study population. In order to increase the enrollment of minority subjects, we will recruit from a national population, so that subjects in all regions of the U.S. with ethnically and racially diverse populations may be recruited.

8.5 Written Informed Consent

Written informed consent will be obtained from each participant or their parents/ legally authorized representative before any study-specific procedures or assessments are done and after the aims, methods, anticipated benefits, and potential hazards are explained. The participant's willingness to participate in the study will be documented in writing in a consent form, which will be signed by the participant with the date of that signature indicated. The investigator will keep the original consent forms and signed copies will be given to the participants. It will also be explained to the participants that they are free to refuse entry into the study and free to withdraw from the study at any time without prejudice to future treatment. Written and/or oral information about the study in a language understandable by the participant will be given to all participants.

Assent will be obtained from children ages 7-17 years (7-18 years for subjects studied in the Nebraska STAIR site). Written assent forms will be provided using age appropriate language. It is recognized that most subjects with SLOS have intellectual disability and may not be capable of providing informed assent. We will therefore rely on the judgment of the child's parents/guardian/legal authorized representative for guidance concerning their ability to fully comprehend this study and provide assent.

8.6 Process of Consent

Informed consent will be obtained in one of the following manners. Those SLOS subjects who are routinely followed at one of the STAIR sites will be notified of the study at the time of their clinic visit or by telephone. Informed consent will be obtained from the parent and/or subject's legally-authorized representative (LAR) in the clinic setting. Ample time will be given for the parent/LAR to review the study information and provide written consent.

Alternately, since some SLOS subjects are located throughout the United States, initial contact concerning enrollment will be made with the parents and/or LAR by telephone. The study will be described in detail and any questions addressed at the time of initial contact. Parents/LARs will be mailed the consent forms to review. A follow up telephone contact will be made to clarify any additional questions about the study. Parents/LARs who provide verbal assent for enrollment of their child will be asked to travel to the STAIR

center, where written consent will be obtained in person.

8.7 Certificate of Confidentiality

This research is covered by a Certificate of Confidentiality from the National Institutes of Health. The researchers with this Certificate may not disclose or use information, documents, or biospecimens that may identify the participant in any federal, state, or local civil, criminal, administrative, legislative, or other action, suit, or proceeding, or be used as evidence, for example, if there is a court subpoena, unless the participant has consented for this use. Information, documents, or biospecimens protected by this Certificate cannot be disclosed to anyone else who is not connected with the research except, if there is a federal, state, or local law that requires disclosure (such as to report child abuse or communicable diseases but not for federal, state, or local civil, criminal, administrative, legislative, or other proceedings, see below); if the participant consents to the disclosure, including for their medical treatment; or if it is used for other scientific research, as allowed by federal regulations protecting research participants.

The Certificate cannot be used to refuse a request for information from personnel of the United States federal or state government agency sponsoring the project that is needed for auditing or program evaluation by the U.S. Department of Health and Human Services and/or the National Institutes of Health, which is funding this project or for information that must be disclosed in order to meet the requirements of the Federal Food and Drug Administration (FDA). The Certificate of Confidentiality does not prevent a participant from voluntarily releasing information about themselves or their involvement in this research. If a participant wants research information released to an insurer, medical care provider, or any other person not connected with the research, the participant must provide consent to allow the researchers to release it.

Even with the Certificate of Confidentiality, the investigators continue to have ethical obligations to report child abuse or neglect and to prevent an individual from carrying out any threats to do serious harm to themselves or others. If keeping information private would immediately put the study participant or someone else in danger, the investigators would release information to protect the participant or another person. The Certificate of Confidentiality will also not be used to prevent disclosure as required by federal, state, or local law, such as reports of child abuse and neglect, or harm to self or others.

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10. Appendices

None