ROBERT HENDREN, DO

Clinical Research Protocol

An examination of changes in urinary metabolites with use of folinic acid in children with autism spectrum disorder (ASD)

Protocol Number:	pending
Version Date:	11-21-17
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Development Phase:	n/a
Sponsor:	Dr. Robert Hendren, PI (UCSF)
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Principal Investigator:	Robert L. Hendren, DO Professor, Department of Psychiatry Child and Adolescent Psychiatry University of California, San Francisco 401 Parnassus Ave, Box 0984 San Francisco, CA 94143-0984 (415) 476-7198
Medical Monitor:	none
Coordinating Center:	n/a

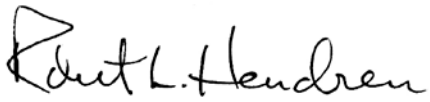
PROTOCOL AGREEMENT

I have read the protocol specified below. In my formal capacity as Investigator, my duties include ensuring the safety of the study subjects enrolled under my supervision as outlined in the protocol. It is understood that all information pertaining to the study will be held strictly confidential and that this confidentiality requirement applies to all study staff at this site. Furthermore, on behalf of the study staff and myself, I agree to maintain the procedures required to carry out the study in accordance with accepted GCP principles and to abide by the terms of this protocol.

Protocol Number: pending

Protocol Title: **An examination of changes in urinary metabolites with use of folinic acid in children with autism spectrum disorder (ASD)**

Protocol Date: 11-21-17



12/07/2017

Investigator Signature *Date*

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LIST OF ABBREVIATIONS

ABC	Aberrant Behavior Checklist
AE	adverse event
ASD	autism spectrum disorder
CBS	cystathionine beta synthase
CFR	Code of Federal Regulations
CHR	Committee on Human Research
CRF	case report form
CSF	cerebrospinal fluid
CTCAE	Common Terminology Criteria for Adverse Events
DSM-IV	Diagnostic and Statistical Manual of Mental Disorders, 4th edition
eBit	Evidence-Based Intervention and Treatment
FDA	Food and Drug Administration
FR alpha	folate receptor alpha
FRAA	folate receptor auto-antibodies
GCP	Good Clinical Practice
GSH	glutathione
GSSG	glutathione disulfide
HIPAA	Health Insurance Portability and Accountability Act of 1996
ICH	International Conference on Harmonisation
IND	Investigational New Drug
IRB	Institutional Review Board
MedDRA	Medical Dictionary for Regulatory Activities
MS	methionine synthase
MTase	methyltransferase
OHS	Oak Hill School
PedsQL	Pediatric Quality of Life Inventory
PHI	protected health information
PI	Principal Investigator
SAE	serious adverse event
SAH	S-adenosylhomocysteine
SAM	S-adenosylmethionine
SCQ	Social Communication Questionnaire
SRS	Social Responsiveness Scale
THF	tetrahydrofolate

PROTOCOL SYNOPSIS

TITLE	**An examination of changes in urinary metabolites with use of folinic acid in children with autism spectrum disorder (ASD)**
SPONSOR	Dr. Robert Hendren
FUNDING ORGANIZATION	Gift Funded from the JS Foundation
NUMBER OF SITES	1
RATIONALE	Children with autism spectrum disorder (ASD) have been found to have impaired transport of folate across the blood-brain barrier due to folate receptor auto-antibodies (FRAA) that either block or bind to the folate receptor alpha (FR alpha). This creates the condition known as "cerebral folate deficiency" where serum folate concentrations are normal but CSF folate concentrations are low. Recent studies have found that supplementing children with ASD with a reduced form of folate – folinic acid – allows for bypass of the impaired folate transport mechanism into the CSF, leading to improved behavior and language development. We propose to further examine this exciting new therapeutic possibility by conducting an open-label study of folinic acid in children with ASD to examine both changes in behavior and correlations with urinary metabolites. We previously created an outcomes database within a school for children with ASD (Oak Hill School, San Anselmo, CA) to carefully track teacher and parent-rated assessments of children enrolled in the school. In a prior study, we established the ability to provide a treatment (the phytochemical, sulforaphane) to children in the school while monitoring changers in urinary metabolites and clinical outcomes. This study found that changes in specific urinary metabolites were associated with clinical improvements, which suggested specific metabolic pathways that are involved in the treatment response. In the current study, we will use the same successful model to determine if changes in urinary metabolites are correlated with clinical changes. This study may provide insight into the mechanism of action of folinic acid and may allow for the development of a biomarker prediction model that will determine which children are most likely to benefit from this treatment.
STUDY DESIGN	Open label treatment study elucidating mechanism of action.
PRIMARY OBJECTIVE	To examine changes in urinary metabolites in children with ASD being treated with folinic acid and to determine if changes in urinary metabolites are correlated with clinical improvements.
SECONDARY OBJECTIVES	Develop a prediction model to determine which children are most likely to benefit from folinic acid.

NUMBER OF SUBJECTS	40
SUBJECT SELECTION CRITERIA	Inclusion Criteria: Age 5-22, enrolled at Oak Hill School, diagnosis of autism established by DSM-IV criteria and clinician examination. Exclusion Criteria: Seizure disorder, serious medical illness within the last 6 months as judged by the study PI. Known allergy to folate.
TEST PRODUCT, DOSE, AND ROUTE OF ADMINISTRATION	Folinic acid 2mg/kg per day (maximum 50mg/day) in two equally divided doses with half the target dose given during the first 2 weeks.
CONTROL PRODUCT, DOSE AND ROUTE OF ADMINISTRATION	There is no control product in this study.
DURATION OF SUBJECT PARTICIPATION AND DURATION OF STUDY	Subjects will be on study for 4.5 months **Screening:** up to 2 weeks. **Treatment:** 3 months **Follow-up:** 1 month (1 additional month after treatment) The total duration of the study is expected to be 12 months. 3-6 months for subject recruitment; 4 months for final subject follow-up; 2 months for data analysis and manuscript preparation.
CONCOMMITANT MEDICATIONS	Allowed: all Prohibited: none
EFFICACY EVALUATIONS	Parent (Aberrant Behavior Checklist, Social Responsiveness Scale, Pediatric Quality of Life) Teacher (Student Progress Form, Aberrant Behavior Checklist, Social Responsiveness Scale) Urinary Metabolomics (Baseline and 3 months)
PRIMARY ENDPOINT	• Correlation between mean change in Aberrant Behavior Checklist and mean change in urinary metabolites.
SECONDARY ENDPOINTS	• Correlations between changes in urinary metabolites and all secondary outcome measures.
OTHER EVALUATIONS	None

SAFETY EVALUATIONS	All study subjects are enrolled at the Oak Hill School and will be observed all school days by teachers, who are aware of student participation in the study. Parents, teachers, and enrolled students will be advised to report any new medical problems to study investigators, who will evaluate all potential adverse events and report to the CHR according to established guidelines.
PLANNED INTERIM ANALYSES	No interim analyses planned given the short-term duration of the study, the established safety of the treatment, and the lack of randomization (so no ability to compare rates of AE's between active and placebo treatment).
STATISTICS **Primary Analysis Plan**	Correlations between changes in mean levels of urinary metabolites and changes in mean behavioral measures (0 to 3 months) will be done using a spearman rank correlations test using STATA. We prospectively define correlations with an absolute value of ≥ 0.6 to be potentially involved in the mechanism of action. All metabolites with this correlation will be mapped to known metabolite pathways to examine pathway changes.
Rationale for Number of Subjects	We are recruiting all children currently enrolled at Oak Hill School (current enrollment = 36). We anticipate that we will enroll 20 students in the current study, but we will allow up to 40 (if school enrollment increases).

1 BACKGROUND

1.1 Overview of Clinical Studies

Cerebral folate deficiency in ASD: Prior studies have linked abnormalities in the metabolism of folate, an essential B vitamin, to ASD.[1] These abnormalities may relate to the ability of a child with ASD to transport folate across the blood-brain barrier. Children with ASD have been found to have impaired transport of folate across the blood-brain barrier due to auto-antibodies that either block or bind to the folate receptor alpha (FR alpha). (The antibodies are known as folate receptor auto-antibodies - (FRAA)). This creates the condition known as "cerebral folate deficiency" which describes an individual with normal serum folate concentrations but low concentrations of folate in the cerebrospinal fluid (CSF) due to impaired ability to transport folate across the blood-brain barrier.[2]

High prevalence of FRAA in ASD: Children with ASD have been found to have a high prevalence of FRAA. In a study of 93 children with ASD, 60% had blocking FRAA, and 44% had binding FRAA.[3] Blocking antibodies interfere with binding of folate to the FR alpha and binding antibodies bind to the FR alpha and cause an antibody-mediated immune reaction. The rates of FRAA in ASD are higher than the 4-15% prevalence reported in healthy adults and the 3% prevalence reported in developmentally delayed non-autistic children.[2] Up to 23% of children with ASD who underwent lumbar puncture were reported to have abnormally low CSF folate concentrations.[4] Together, this evidence suggests that low CSF folate levels may contribute to the abnormal physiology and clinical symptoms of ASD.

Reduced forms of folate can bypass impaired folate transport across the blood-brain barrier: Folinic acid, a reduced form of folate, is able to bypass the normal blood-brain barrier transport mechanism and increase CSF folate levels through a secondary transport mechanism that involves a reduced-folate transporter. Case reports have found that high-dose folinic acid supplementation markedly improves CSF folate levels in children with ASD and low CSF folate.[2]

Folinic acid supplementation improves language and aberrant behavior in ASD: In a recent (2016) randomized controlled trial of folinic acid supplementation vs. placebo in 48 children with ASD and language delay, children randomized to folinic acid had statistically significant improvements in language and aberrant behavior.[2] Furthermore, improvements were greater in the subgroup of children who were positive for FRAA, suggesting that children with more severe cerebral folate deficiency were more likely to benefit (FRAA was used a proxy for CSF folate levels, since it was not possible to obtain CSF folate on study participants due to the invasive nature of obtaining CSF).

Folate may support the 3 interdependent pathways involved in folate (THF)-dependent methionine transmethylation/transsulfuration metabolism. These pathways are involved in DNA synthesis, cellular methylation, and redox homeostasis, and an adequate cerebral supply of folate may allow for improved functioning of these pathways and clinical improvements.

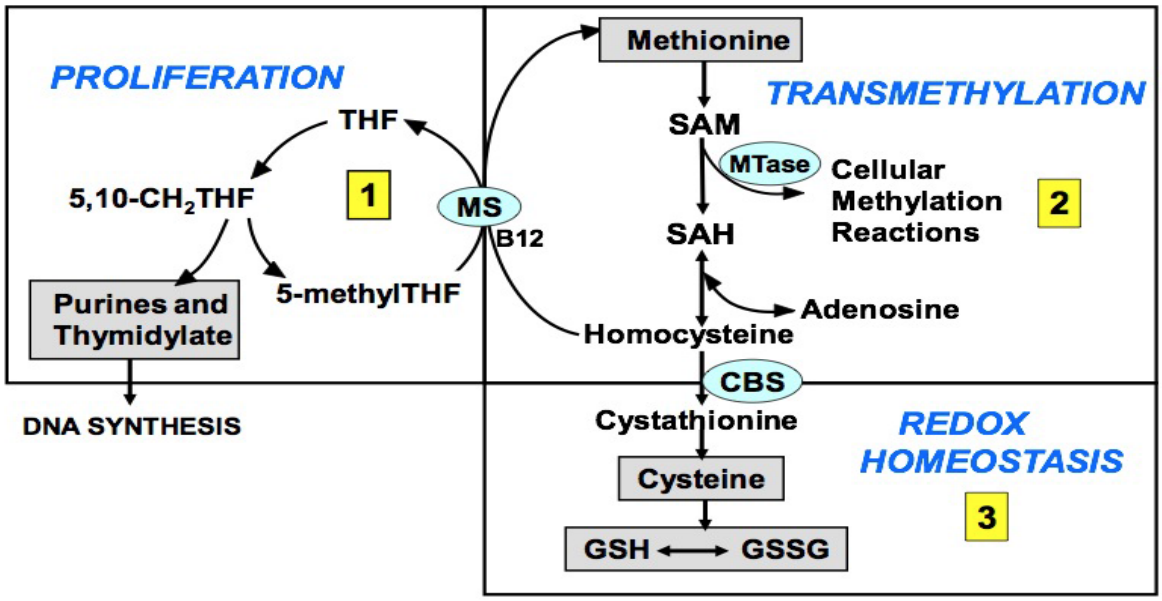

Figure 1. *This is an overview of the 3 interdependent pathways involved in folate (THF)-dependent methionine transmethylation/transsulfuration metabolism. The vital importance of these three interconnected pathways is underscored by their essentiality for error-free DNA synthesis (Folate Cycle Pathway 1); for cellular methylation (epigenetic) capacity (Transmethylation Pathway 2); and for the maintenance of glutathione (GSH) redox homeostasis (Transsulfuration Pathway 3). Because these 3 pathways regulate the distribution of precursors for DNA synthesis (proliferation), DNA/histone methylation (epigenetics) and glutathione synthesis (redox/antioxidant status), the homeostatic balance between these pathways is essential to support normal cell programming and ontogeny during prenatal and post-natal development.*

In summary, children with ASD are known to have a high prevalence of cerebral folate deficiency, likely caused by folate receptor auto-antibodies (FRAA), which block the normal transfer of folate from the bloodstream to the CSF. Prior studies have documented that supplementation with reduced forms of folate can bypass the impaired transport mechanism and restore normal cerebral folate levels. A recent randomized controlled trial found that folinic acid supplementation improved language and behavior in children with ASD. We therefore propose to conduct an open-label study of folinic acid in children with ASD to determine if clinical improvements are correlated with changes in metabolites known to be related to the folate pathways. This study will gain information needed to determine a possible metabolomic mechanism of action of folinic acid and allow for the development of a prediction model to identify children with ASD who are mostly likely to respond to treatment.

2 STUDY RATIONALE

2.1 Risk / Benefit Assessment

Risk of treatment with folinic acid: In a prior randomized controlled trial of the same dose and duration of treatment with folinic acid in 48 children with ASD, there were no serious side effects and no side effects that were more common with folinic acid than placebo treatment.

Folinic acid is closely related molecule to the B vitamin, folate. It is approved to help with the adverse of effects of certain medications such as methotrexate, which depletes folate in the body. Folinic acid is also approved to be used in combination with chemotherapeutic agents in certain cancers.The identification of side effects of folinic acid is somewhat confounded by the combined use of potent chemotherapeutic drugs. Adverse effects of folinic acid may include: skin rashes, itching, facial flushing, nausea or vomiting. Acute allergic reactions have been reported – but considered rare.

Potential benefits: There is a possibility that children enrolled in this study will experience some beneficial effects related to treatment with folinic acid. As noted above, in a prior study of folinic acid in ASD, children were noted to have improvements in aberrant behavior and language development. Also, adult studies have found improvement in mood in patients treated with supplements containing folinic acid. Finally, there is biological plausibility that children with impaired cerebral folate transfer may have some beneficial physiological, and therefore clinical, effects when cerebral folate levels are raised through folinic acid treatment.

3 STUDY OBJECTIVES

3.1 Primary Objective

The primary objective is to determine if there is a correlation between changes in behavior and changes in urinary metabolites in children with ASD who receive folinic acid treatment for 12 weeks. This will provide insight into the potential mechanism of action.

3.2 Secondary Objectives:

Secondary objectives include an examination of the correlation between changes in urinary metabolites and other outcome measures, including the Social Responsiveness Scale (SRS) (parents and teachers) and the Pediatric Quality of Life Inventory (PedsQL) (parents). It is possible that specific urinary metabolites are related to different clinical domains, and we will therefore examine correlations in each of these domains.

STUDY DESIGN

3.3 Study Overview

This is an open-label, 12-week study examining the effects of folinic acid on behavior in children with ASD and the correlation between behavior change and urinary metabolites.

Total duration of subject participation will be four months – 3 months while taking the study supplement and one month of wash-out. Total duration of the study is expected to be 12 months.

4 CRITERIA FOR EVALUATION

4.1 Primary Endpoint

- Correlation between changes in the Aberrant Behavior Checklist and urinary metabolites.

4.2 Secondary Endpoints

- Correlation between changes in all outcome measures and changes in urinary metabolites.

4.3 Safety Evaluations

- Patient reported adverse events, which will be collected through spontaneous reporting as well as specific queries at 1 and 3 months of treatment.

5 SUBJECT SELECTION

5.1 Study Population

Children and young adults between the ages of 5 and 22 who are enrolled at the Oak Hill School (OHS) in San Anselmo, CA (a school for children with autism or related neurodevelopmental disorders). OHS is the site for our ongoing outcomes study using a secure web-based outcome platform where enrolled children (n=40) have regular outcomes completed by teachers (weekly) and parents (quarterly) (UCSF CHR approval number 13-11086). A prior intervention study of the supplement, sulforaphane (UCSF CHR approval number 15-17002) demonstrated the feasibility of conducting an open-label study to examine changes in urinary metabolites and clinical measures with this population and the web-based outcome platform.

5.2 Inclusion Criteria

1. Male or female, enrolled at OHS, age 5-22 and with a diagnosis of ASD.
2. ASD diagnosis is established DSM-IV criteria, child observation by an expert clinician, and score on the Social Communication Questionnaire, completed by parents).

3. Written informed consent obtained from the subject's legal representative and ability for subject to comply with the requirements of the study.

5.3 Exclusion Criteria

1. Presence of a condition or abnormality that in the opinion of the Investigator would compromise the safety of the patient or the quality of the data.
2. Known allergy to any component of the folinic acid product.

6 CONCURRENT MEDICATIONS

All subjects should be maintained on the same medications throughout the entire study period, as medically feasible, with no introduction of new chronic therapies.

6.1 Prohibited Medications and Treatments

Study subjects must not currently be taking folinic acid or have used this supplement in the last 6 months.

7 STUDY TREATMENTS

Since this is an open-label study, all subjects will receive folinic acid. Subjects, parents, teachers, and clinicians are not blinded to treatment status. There is no control product being used.

7.1 Formulation of Product –

Folinic acid is prepared as a calcium salt. The capsules are dye-free, milk-product free, and vegetarian and are provided in 10 mg available for weight-based dosing (2mg/kg with maximum dose of 50mg/day divided in two doses). The capsules are produced by Lee Silsby Pharmacy (Cleveland Heights, OH). Certificate of analysis has been obtained by an independent analytical service and is part of the IND packet, approved by the FDA.

7.1.1 Dosage/Dosage Regimen -

Study participants will receive weight-based dosing (2mg/kg per day) divided in 2 equal doses with maximum daily dose of 50mg. If unable to swallow, the capsules may be administered by piercing the capsule and mixing the contents into soft food including yogurt, oatmeal, drinks.

7.1.2 Dispensing

Study staff will dispense the study supplement, labelled with each participant's unique study ID number, after the participant has completed all necessary enrollment procedures.

7.1.3 Administration Instructions

Each participant will be instructed to take the weight-based dose, by mouth twice a day with meals.
Doses may be mixed with liquid or soft foods.

7.2 Supply of Study Supplement at the Site

The study supplement will be stored by the study site at controlled room temperature, 20 to 25ºC (68 to 77ºF). Participants will be instructed to also store the product at room temperature, consistent with labelled instructions and stability testing.

7.2.1 Study Supplement Accountability

An accurate and current accounting of the dispensing and return of study supplement for each participant will be maintained on an ongoing basis by a member of the study site staff. The number of study supplement capsules dispensed and returned by the participant will be recorded.

8 STUDY PROCEDURES AND GUIDELINES

A Schedule of Events representing the required testing procedures to be performed for the duration of the study is diagrammed in Appendix 1.

8.1 Clinical Assessments

8.1.1 Concomitant Medications

All concomitant medication and concurrent therapies will be documented at the Screening visit. Dose, route, unit, frequency of administration, and indication for administration and dates of medication will be captured.

8.1.2 Demographics

Demographic information (date of birth, gender, race) will be recorded at the Screening visit.

8.1.3 Medical History

Relevant medical history, including history of current disease and information regarding underlying diseases will be recorded at Screening visit.

8.1.4 Adverse Events

Information regarding occurrence of adverse events will be captured throughout the study. Participants will be informed to contact a study staff member if they experience a new medical problem – this information will be recorded on an Adverse Event form (including entries for duration, severity, outcome, treatment and relation to study supplement). AE forms will be reviewed by a study investigator and reported to the UCSF CHR according to established guidelines. We will be carefully monitoring patients for the occurrence of any adverse events through active questioning and 1 and 3 months as well as instructions for patients to report any new medical problem immediately to a study team member. All adverse events will be reviewed by a study investigator within 24 hours and reported to the Committee on Human Research according to standard guidelines.

8.2 Clinical Laboratory Measurements

Urinary Metabolomic Profile – all participants will have urine collected (fasting, 8am target collection time) at Screening and Close-out (3 months). Urine will be stored in sterile containers at -80 degrees Celsius and analyzed in one batch after all participants have completed the study. Metabolomic assessment will be conducted by Metabalon (Research Triangle, NC). Metabalon is one of the largest and most established laboratories conducting state-of-the art metabolomics assessments of urine, plasma, and other clinical samples. Metabalon has 160 employees including more than 40 PhDs with diverse backgrounds in molecular biology, biochemistry, bioinformatics, and statistics. They have completed over 4,000 metabolomic projects for over 700 clients. Their analyses have been used in more than 500 peer-reviewed publications including numerous articles in *Cell, Nature,* and *Science*. (www.metabalon.com)

9 EVALUATIONS BY VISIT

9.1 Visit 1: Screening visit – this visit will involve a face-to-face meeting with the study participant and their parent/legal guardian. A study team member will explain the entire study and obtain signed informed consent. Study inclusion/exclusion criteria will be reviewed and eligible participants will go on to complete all screening activities as below.

1. Review the study with the participant and obtain written informed consent.
2. Assign the participant a unique study ID number
3. Record demographics data.
4. Record medical history.
5. Record concomitant medications.
6. Collect urine specimen.
7. Dispense study supplement
8. Schedule subject for Baseline visit
9. Record study subject weight

9.2 Visit 2 (Baseline) – NOTE – for this study, which will take place at the Oak Hill School, all enrolled children will begin the study supplement on the same date (exact day TBD based on parent/teacher schedules and CHR approval date). This will improve the logistics of the study because teachers regularly complete outcome assessments using our established on-line outcomes platform at quarterly intervals (that match their school-based quarterly progress reports). This "batch" enrollment of participants has several advantages: 1) all participants will begin and end on the same date, which we believe will support the overall study compliance and enthusiasm, as all children/families will be involved in the study at the same time; 2) outcome assessments are a natural part of the study schedule, since they normally are completed by the teachers at 3 months intervals and will therefore occur as children complete the 3-month treatment; 3) compliance and reminders to parents/guardians and teachers will be simplified, since all participants will be on the same schedule and all

reminders can be sent in batches; 4) variation from time/season effects will be minimized, since all children will be experiencing the same school environment at the same period in the study.

1. Parents complete Baseline Outcome Measures.
2. Teachers complete Baseline Outcome Measures.
3. Subject starts taking study supplements at this time.
4. Reinforce study directions, contact information, emphasize need to report new medical problems (possible AE's).

9.3 Visit 3: Follow-up 1(one-month visit): TBD

1. Parents complete Follow-up Outcome Measures.
2. Teachers complete Follow-up Outcome Measures.
3. Adverse event form completed by parents, reviewed by study investigators.
4. Study supplement instructions reviewed.

9.4 Visit 4: Follow-up visit 2 (three month visit): TBD

1. Parents complete Follow-up Outcome Measures.
2. Teachers complete Follow-up Outcome Measures.
3. Adverse event form completed by parents, reviewed by study investigators.
4. Urine collected (fasting, 8am specimen, stored at -80 degrees Celsius)
5. Study supplement stopped. Medication diary and used and unused study supplement bottle collected and counted.
6. Record study subject weight.

9.5 Visit 5 Follow-up visit 3 (four month visit, one-month off study supplement): TBD

1. Parents complete Follow-up Outcome Measures.
2. Teachers complete Follow-up Outcome Measures.

10 ADVERSE EVENTS REPORTING AND DOCUMENTATION

10.1 Adverse Events

An adverse event (AE) is any untoward medical occurrence in a clinical investigation of a patient administered a pharmaceutical or supplement product and that does not necessarily have a causal relationship with the treatment. An AE is therefore any unfavorable and

unintended sign, symptom or disease temporally associated with the administration of an investigational product, whether or not related to that investigational product.

The study visits include a direct question for parents/caregivers regarding the occurrence of AEs since the last study visit. All information will be recorded in the site's source documents. Adverse events will be recorded in the patient CRF. Adverse events will be described by duration (start and stop dates and times), severity, outcome, treatment and relation to study supplement, or if unrelated, the cause.

AE Severity

The National Cancer Institute's Common Terminology Criteria for Adverse Events (CTCAE) Version 3.0 will be used to assess and grade AE severity.

Table 1. AE Severity Grading

Severity (Toxicity Grade)	**Description**
Mild (1)	Transient or mild discomfort; no limitation in activity; no medical intervention or therapy required. The subject may be aware of the sign or symptom but tolerates it reasonably well.
Moderate (2)	Mild to moderate limitation in activity, no or minimal medical intervention/therapy required.
Severe (3)	Marked limitation in activity, medical intervention/therapy required, hospitalizations possible.
Life-threatening (4)	The subject is at risk of death due to the adverse experience as it occurred. This does not refer to an experience that hypothetically might have caused death if it were more severe.

AE Relationship to Study Supplement

The relationship of an AE to the study supplement will be assessed using the guidelines in

Table 2.

Table 2. AE Relationship to Study Supplement

Relationship to Supplement	**Comment**
Definitely	Previously known toxicity of agent; or an event that follows a reasonable temporal sequence from administration of the supplement; that follows a known or expected response pattern to the suspected supplement; that is confirmed by stopping or reducing the dosage of the supplement; and that is not explained by any other reasonable hypothesis.
Probably	An event that follows a reasonable temporal sequence from administration of the supplement; that follows a known or expected response pattern to the suspected supplement; that is confirmed by stopping or reducing the dosage of the supplement; and that is unlikely to be explained by the known characteristics of the subject's clinical state or by other interventions.

Possibly	An event that follows a reasonable temporal sequence from administration of the supplement; that follows a known or expected response pattern to that suspected supplement; but that could readily have been produced by a number of other factors.
Unrelated	An event that can be determined with certainty to have no relationship to the study supplement.

10.2 Serious Adverse Events (SAE)

An SAE is defined as any AE occurring at any dose that results in any of the following outcomes:

- death
- a life-threatening adverse experience
- inpatient hospitalization or prolongation of existing hospitalization
- a persistent or significant disability/incapacity
- a congenital anomaly/birth defect

Other important medical events may also be considered an SAE when, based on appropriate medical judgment, they jeopardize the subject or require intervention to prevent one of the outcomes listed.

10.3 Serious Adverse Experience Reporting

We will document all SAEs that occur (whether or not related to study supplement) per UCSF CHR Guidelines. The collection period for all SAEs will begin after informed consent is obtained and end after procedures for the final study visit have been completed.

We will report all AE's and SAE's according to UCSF CHR guidelines.

11 EARLY DISCONTINUATION OF STUDY SUPPLEMENT

A subject may be discontinued from study treatment at any time if the subject, the investigator, or the Sponsor feels that it is not in the subject's best interest to continue. The following is a list of possible reasons for study treatment discontinuation:

- Participant legal guardian withdrawal of consent.
- Participant is not compliant with study procedures.
- Adverse event that in the opinion of the investigator would be in the best interest of the participant to discontinue study supplement.
- Protocol violation requiring discontinuation of study treatment.
- Lost to follow-up.

If a participant is withdrawn from treatment due to an adverse event, the subject will be followed and treated by the Investigator until the abnormal parameter or symptom has resolved or stabilized.

All participants who discontinue study supplement will be asked to continue the outcome assessment as scheduled.

11.1 Withdrawal of Subjects from the Study

All subjects are free to withdraw from participation at any time, for any reason, specified or unspecified, and without prejudice.

Reasonable attempts will be made by the investigator to provide a reason for subject withdrawals, and this information will be recorded in the subject's source documents.

12 PROTOCOL VIOLATIONS

A protocol violation occurs when the subject, or investigator, fails to adhere to significant protocol requirements affecting the inclusion, exclusion, subject safety and primary endpoint criteria. Protocol violations for this study include, but are not limited to, the following:

- Failure to meet inclusion/exclusion criteria.
- Improper use of schedule/dose of study supplement.

When a protocol violation occurs, it will be discussed with the investigator and a Protocol Violation Form detailing the violation will be generated. This form will be signed by the Investigator and reported to the UCSF CHR according to established guidelines.

13 STATISTICAL METHODS AND CONSIDERATIONS

Prior to the analysis of the final study data, a detailed Statistical Analysis Plan (SAP) will be written describing all analyses that will be performed. This information will be posted on clinicaltrials.gov to ensure that data analysis proceeds as defined a priori. Any modifications to the plan provided below will be noted on clinicaltrials.gov before the first subject is enrolled.

13.1 Analysis of Primary Endpoint

The primary analysis in the study is the correlation between the mean change in each urinary metabolite and the mean change in participant behavior as measured by the ABC using the spearman rank correlation test in Stata. The primary outcome measure is the correlation between urinary metabolites and the total ABC score.

13.2 Analysis of Secondary Endpoints

Secondary outcome measures will use the same spearman rank correlation test in Stata, comparing each urinary metabolite with each of the other outcome measures (SRS, PedsQL). Because this study is exploratory in nature, no adjustments will be made for multiple comparisons, as recommended by Rothman.[5]

Safety and tolerability data will be summarized in a tabular format, which will be included in the final study report/manuscript.

Adverse event rates will be coded by body system and MedDRA classification term. Adverse events will be tabulated by treatment group and will include the number of patients for whom the event occurred, the rate of occurrence, and the severity and relationship to study supplement.

13.3 Sample Size

The study is designed to detect a correlation coefficient (Pearson's r) between the mean urinary metabolite change and the mean behavioral measure change of 0.6, which is considered to indicate a moderate-to-strong correlation. Using a two-sided alpha of 0.05 and a Beta of 0.1 (90% power), our required sample size is 25 participants to detect this degree of correlation. Since we may incur up to 20% dropout (5 participants), our target sample size is 30 participants, but we will allow up to 40 to provide the study opportunity to all children enrolled in the target school (Oak Hill School).

14 DATA COLLECTION, RETENTION AND MONITORING

14.1 Data Collection Instruments

This study will take place at Oak Hill School, where we have established an ongoing outcomes study that uses a web-based platform for data entry for parents and teachers. This platform is secure and has been reviewed for adequacy by the UCSF CHR (#13-11086). Data gathered for the study will be electronically coded into a password-protected database. All uploaded files will be stored in a database not in the server's file system, and the storage format will be encrypted. We will use the same secure platform for all data collection for the current study.

14.2 Data Quality Control and Reporting

All data entered in this on-line system undergoes a rigorous quality control evaluation and pre-specified logic checks. All entries are date and time stamped, and any modifications to study data are flagged – the editor and time/day of edits are also recorded. As a result, the study database is cleaned continuously and will be ready for analysis at the completion of the last study subject visit.

14.3 Archival of Data

The database is safeguarded against unauthorized access by established security procedures; appropriate backup copies of the database and related software files is maintained.

14.4 Availability and Retention of Investigational Records

All study data is available, as need and required, for review by appropriate regulatory authorities. In this case, the UCSF CHR is the responsible regulatory authority. A file for each subject will be maintained and will include the signed Informed Consent and copies of all source documentation related to that subject. The Investigator ensures the reliability and availability of source documents from which the information on the CRF was derived.

14.5 Subject Confidentiality

All study forms containing PHI are kept in a secure database (named "eBit" for Evidence-Based Intervention and Treatment) that meets FDA criteria for electronic database security.

In eBit, only UCSF study coordinators are able to create online parent and teacher accounts, which are password-protected. Data gathered for the study will be electronically coded into a password-protected database. All uploaded files onto eBit will be stored in a database not in the server's file system, and the storage format will be encrypted. eBit uses a separate platform to handle the PHI (including parent's email addresses) that will then communicate directly with Oak Hill platforms. This will keep the PHI separate from the actual health data and will meet all the IRB and HIPPA requirements that eBit and the UCSF IT group have agreed upon.

eBit is deployed on the Microsoft Azure secure cloud platform, which, according to their website, uses "industry-standard encrypted transport protocols between user devices and Microsoft datacenters, and within datacenters themselves." More information about Microsoft Azure's security can be found at the following link: https://www.microsoft.com/en-us/TrustCenter/Security/default.aspx . This is the same server that was used in our previously approved, and still active, CHR application for our Oak Hill School outcomes study. The UCSF IT group has reviewed the terms of this web platform and has ensured that it meets all data security requirements.

15 ADMINISTRATIVE, ETHICAL, REGULATORY CONSIDERATIONS

The study will be conducted according to the Declaration of Helsinki, Protection of Human Volunteers (21 CFR 50), Institutional Review Boards (21 CFR 56), and Obligations of Clinical Investigators (21 CFR 312).

15.1 Protocol Amendments

Any amendment to the protocol will be written by the Investigator and submitted to the UCSF CHR. Protocol amendments will not be implemented without prior written approval from the UCSF CHR except as necessary to eliminate immediate safety hazards to patients. A protocol amendment intended to eliminate an apparent immediate hazard to patients may be implemented immediately, provided the UCSF CHR is notified within five working days.

15.2 Institutional Review Boards and Independent Ethics Committees

The protocol and consent form will be reviewed and approved by the UCSF CHR prior to study initiation. Serious adverse experiences regardless of causality will be reported to the UCSF CHR in accordance with the standard operating procedures.

The UCSF CHR's written unconditional approval of the study protocol and the informed consent form will be in the possession of the Investigator before the study is initiated.

Protocol and/or informed consent modifications or changes will not be initiated without prior written UCSF CHR approval except when necessary to eliminate immediate hazards

to the patients or when the change(s) involves only logistical or administrative aspects of the study. Such modifications will be submitted to the UCSF CHR and written verification that the modification was submitted and subsequently approved will be obtained.

15.3 Informed Consent Form

Informed consent will be obtained in accordance with the Declaration of Helsinki, ICH GCP, US Code of Federal Regulations for Protection of Human Subjects (21 CFR 50.25[a,b], CFR 50.27, and CFR Part 56, Subpart A), the Health Insurance Portability and Accountability Act (HIPAA, if applicable), and local regulations.

The consent form generated by the Investigator (attached) must be acceptable to the UCSF CHR. The written consent document will embody the elements of informed consent as described in the International Conference on Harmonisation and will also comply with local regulations.

A properly executed, written, informed consent will be obtained from each subject prior to entering the subject into the trial. Information will be given in both oral and written form and each subject's legal representatives) will be given ample opportunity to inquire about details of the study. A copy of the signed consent form will be given to the subject's legal representative and the original will be maintained with the subject's records.

15.4 Publications

The publication or presentation of any study results will comply with all applicable privacy laws, including, but not limited to, the Health Insurance Portability and Accountability Act of 1996.

15.5 Investigator Responsibilities

By signing the Agreement of Investigator form, the Investigator agrees to:

1. Conduct the study in accordance with the protocol.
2. Personally conduct or supervise the study (or investigation).
3. Ensure that the requirements relating to obtaining informed consent and CHR review and approval meet federal guidelines, as stated in § 21 CFR, parts 50 and 56.
4. Ensure that all associates, colleagues and employees assisting in the conduct of the study are informed about their obligations in meeting the above commitments.
5. Maintain adequate and accurate records in accordance with §21 CFR 312.62.
6. Ensure that an IRB that complies with the requirements of §21 CFR part 56 will be responsible for initial and continuing review and approval of the clinical study.
7. Promptly report to the IRB all changes in the research activity and all unanticipated problems involving risks to subjects or others.
8. Seek IRB approval before any changes are made in the research study, except when necessary to eliminate hazards to the patients/subjects.

9. Comply with all other requirements regarding the obligations of clinical investigators and all other pertinent requirements listed in § 21 CFR part 312.

APPENDIX 1. SCHEDULE OF STUDY VISITS - UPDATE

	VISIT 1 Screening/Month -1	VISIT 2 Baseline/ Month 0	VISIT 3 Follow-up 1/ Month 1	VISIT 4 Follow-up 2/ Month 3	VISIT 5 Follow-up 3/ Month 4
Study Clinic Visit	X			X	
Informed Consent	X				
Assign Study ID and eBit log in	X				
Demographics	X				
Medical History	X				
Urine Sample (including sibling if consented)	X			X	
Weight	X			X	
Dispense Study Supplement	X				
Administer Study Supplement		X			
Concomitant Medication Review	X	X	X	X	X
Adverse Experiences		X	X	X	X
Parent Assessments (Online)					
SCQ		X			
ABC		X	X	X	X
SRS		X	X	X	X
PedsQL		X		X	
Teacher Assessments (Online)		**Teacher will complete Weekly Progress Report every week starting at baseline to month 4**			
ABC		X	X	X	X
SRS		X	X	X	X

References:

1. Vahabzadeh A, McDougle CJ. Maternal folic acid supplementation and risk of autism. *JAMA.* Jun 05 2013;309(21):2208.
2. Frye RE, Slattery J, Delhey L, et al. Folinic acid improves verbal communication in children with autism and language impairment: a randomized double-blind placebo-controlled trial. *Molecular psychiatry.* Oct 18 2016.
3. Frye RE, Sequeira JM, Quadros EV, James SJ, Rossignol DA. Cerebral folate receptor autoantibodies in autism spectrum disorder. *Molecular psychiatry.* Mar 2013;18(3):369-381.
4. Shoffner J, Trommer B, Thurm A, et al. CSF concentrations of 5-methyltetrahydrofolate in a cohort of young children with autism. *Neurology.* Jun 14 2016;86(24):2258-2263.
5. Rothman KJ. No adjustments are needed for multiple comparisons. *Epidemiology.* Jan 1990;1(1):43-46.