1) Protocol Title

A Controlled Trial of Sertraline in Young Children with ASD

2) Author of Protocol

Dr. Randi Hagerman, MD

3) IRB Review History

This is a modification to an approved protocol.

4) Objectives

Aim 1: To evaluate the benefit of sertraline for treatment of social deficits and language delays in young children with ASD (2 through 5 years old, inclusive) in a double-blind, placebo-controlled trial.

Hypothesis 1.1: We hypothesize that sertraline will stimulate both language and socialization and improve behavior in young children with ASD compared to those who are not treated with sertraline.

Aim 2: Explore biomarkers associated with response to early treatment with sertraline in children with ASD.

Hypothesis 2.1: Levels of BDNF at baseline and change in BDNF levels will correlate to the clinical response to sertraline in young children with ASD.

Hypothesis 2.2: Polymorphisms in the brain-derived neurotrophic factor **(**BDNF), within the serotonin transporter gene (SLC6A4) and serotonin-synthesizing tryptophan hydroxylase 2 (TPH2) gene will correlate with the clinical response to sertraline.

Hypothesis 2.3: Sertraline treatment may have an immunomodulatory effect through a decrease in the pro-inflammatory (such as cytokine IL-1) and an increase in the anti-inflammatory cytokines (i.e. IL-4 and TGF1).

5) Background

Autism Spectrum Disorder (ASD) is a behaviorally defined disorder with many causes including currently estimated over 1,000 gene mutations that can lead to deficits in synaptic plasticity, neuronal migration, transcription and translation changes in addition to many other processes that are important for synapse development, CNS connectivity and developmental change. Neuroimmune mechanisms may also be active in ASD and influenced by exposure to environmental toxins. Currently the CDC has reported that 1 in 68 children at age 8 years in the general population has ASD (CDC 2014 MMWR). Known specific genetic causes of ASD that are

over 2 to 3% include fragile X syndrome (FXS) and tuberous sclerosis (TS), although many more single gene mutations, duplications or deletions can cause 1% or less of ASD.

We have chosen sertraline treatment in young children with ASD because our previous open label and our current controlled trial of sertraline in FXS+ASD have demonstrated significant benefit. There is evidence that young children with ASD demonstrate early deficits of serotonin synthesis compared to typically-developing children. There is also emerging evidence regarding the stimulation of brain-derived neurotrophic factor (BDNF) by an SSRI and the benefit of neurogenesis in animal models of intellectual disability (ID). Preliminary open-label data in ASD have shown benefit of an SSRI in preschool children with ASD. Although a multicenter controlled trial of citalopram in children with ASD ages 5-17yo did not show benefit, there has never been a controlled trial of sertraline in children <5yo with ASD. In our experience in preschool children with ASD this is the optimal time to stimulate connectivity in the brain to improve social interaction and language, and this is the time that serotonin is deficient in the brain. This double-blind, placebo-controlled trial will assess behavioral and language benefits in ASD as well as whether variations in genes that affect the level of serotonin at the synapse can predict treatment response. This work will provide much-needed rigorous evidence of a scientifically validated treatment in young children with ASD and will provide biomarker evidence of who may respond.

There is a great need to develop treatment programs early on in ASD because this is the time to rectify the synaptic abnormalities that are taking place with a developmental window which will impact the rest of their life. The benefit of early treatment with behavioral interventions has been dramatically demonstrated in young children with ASD most recently with the Early Start Denver Model (ESDM) with positive effects on behavior, cognition, and even EEG patterns. We believe the use of a low dose SSRI in early childhood in addition to the community behavioral and therapy programs will have a significant effect on behavior, language, and development in young children with ASD. Such early intervention is imperative to study at this time when the prevalence is ever higher at 1 in 68 children who are 8-years-old in the general population (CDC 2014 MMWR).

Autism is a behaviorally-defined disorder (DSM-V) that arises through one or more of a large number of genetic and/or environmental factors. Mutations in numerous recognized genes are known to be highly associated with autism, operating through mechanisms that alter/disrupt critical functions, including synaptic plasticity, the balance of inhibitory and excitatory pathways, regulation of mTOR pathways, mitochondrial function, immune function, and neuronal migration. Similar mechanisms of dysfunction are known to occur in genetic subgroups of autism such as fragile X syndrome (FXS) where the lack of the Fragile X Mental Retardation 1 Protein (FMRP) leads to dramatic up-regulation of protein production in the CNS, up-regulation of the metabotropic glutamate receptor 5 (mGluR5) pathway, and down-regulation of the GABAA pathways. This imbalance between the glutamate and GABA systems can also be seen in idiopathic autism and in the context of other mutations associated with autism. This coupling between FXS and autism suggests that treatments effective in FXS provide a rational basis for

potential efficacy in other forms of ASD. There is also preliminary evidence that low levels of FMRP occur in the brain of adults with ASD. We have seen a dramatic response of young children with FXS+ASD (2 to 6 years) to sertraline and in the interim analysis of the first 30 children of our currently funded double-blind, placebo-controlled trial of sertraline in young children with FXS. The purpose of this study is to expand our studies to children with idiopathic ASD with further investigation of biomarkers that predict treatment response; thus, providing a foundation for individualized treatment based on the biomarker profile.

Anxiety is a pervasive problem in ASD and FXS that may contribute to or exacerbate a number of the other symptoms, including deficits in reciprocal social interaction (e.g., gaze avoidance), stereotypies or repetitive behaviors (e.g., hand-flapping) and communication abnormalities (e.g. repetitive and perseverative speech) that characterize ASD. Higher ratings of anxiety were associated with more severe autism symptoms as independently rated by trained clinicians on the Autism Diagnostic Observation System (ADOS) in our study of children with FXS. Our extensive clinical experience and these findings indicate that effective anxiety treatment may reduce autism symptoms and improve social reciprocity in patients with ASD with or without FXS.

We and others have been treating anxiety, repetitive, and compulsive behaviors in children with ASD with SSRIs, typically fluoxetine or sertraline, for many years. The utilization of SSRIs to treat ASD increased from 6.1% in 1993 to 21.4% in 2002, and in a review by Langworthy-Lam et al., fluoxetine, paroxetine, and sertraline accounted for 61% of SSRI prescriptions in children with autism.

There have been no controlled trials of sertraline in children with ASD except for our study in FXS and ASD, which was also funded by HRSA. Previous studies of sertraline use in ASD without FXS include an open label in 42 adults with ASD that demonstrated improved aggression and repetitive behavior, and an open-label study in 9 adults with ID and ASD by Hellings et al., which demonstrated improvement. Previous studies of SSRIs in children include a controlled trial of fluoxetine in 39 children 5 to 16 years (mean age 8.2 years) with 8 weeks in each arm and a 4 week washout in between. They saw improvement in obsessive compulsive behavior but not in language; however, it would be unlikely to see improvement in language in such a short treatment period. Overall, there is a 60%-70% response rate to fluoxetine in an open-label trial for ASD and ID and a 53% response rate to fluvoxamine in a placebo-controlled trial for ASD. A recent multicenter study of citalopram in children with ASD who were 5 to 17years old demonstrated no significant benefit for citalopram use (n=73) compared to placebo (n=76). This study has dampened the enthusiasm for SSRI treatments in children with ASD. However, this study did not include children under 5 years, and this is the age that we hypothesize will have the greatest response to an SSRI. Children under 5 years not only have a developmental window for connectivity in the CNS, but those with ASD have low serotonin during this time, which sertraline can improve. Sertraline can also stimulate neurogenesis, which may further enhance development.

Our preliminary data in a subgroup of ASD, specifically young children with FXS + ASD, has demonstrated a significant benefit of low-dose sertraline in a retrospective study. In our controlled study of sertraline in FXS, the interim analysis of the data after the first 30 patients completed the trial has demonstrated a positive response with the CGI-I, demonstrating a significant benefit compared to placebo. Because of these beneficial effects, we have proposed the current study of a double-blind, placebo-controlled trial of sertraline in young children with idiopathic ASD. In 2016, the complete results of this study were published (Hess et al.), which showed that despite the fact that there was no statistically significant difference from placebo on the study's primary outcomes (MSEL expressive language age equivalent and CGI-I), analyses of secondary measures showed significant improvements, particularly in motor and visual perceptual abilities and social participation. Furthermore, post hoc analysis found significant improvement in early expressive language development as measured by the MSEL among children with FXS + ASD on sertraline. Overall, sertraline was well tolerated, with no difference in side effects between sertraline and placebo groups, and no serious adverse events occurred.

Sertraline is approved by the FDA for children 6 years and older for treatment of anxiety and obsessive compulsive disorder, but it is not approved for autism. We received an IND from the FDA to carry out our sertraline study in young children ages 2 to 6 with FXS with and without ASD, and this IND number is 113747, given to us in 2011. We have given the FDA yearly updates of our study. The PI of this study had an IND for this drug under fragile X syndrome, and this IND now includes ASD as a secondary indicated population (IND Number 113747-0001).

Sertraline has been shown to help increase the levels of serotonin in hippocampal neurons and this correlated with increased levels of the brain derived neurotrophic factor (BDNF). BDNF is the most abundant neurotrophin in the brain playing an important role during brain development and in synaptic plasticity. It has been implicated in cognitive function as well as in the pathogenesis of various psychiatric disorders. A synergistic relationship has been proposed for serotonin (5-HT) and BDNF since BDNF promotes the survival and differentiation of 5-HT expressing neurons while the presence of SSRIs stimulates the expression of BDNF. This synergistic effect has a role in mood disorders, such as depression, and makes the BDNF gene and genes related to serotonin levels attractive candidates for the study of diseased brain function and behaviors.

Besides neurogenesis, BDNF also regulates long term potentiation (LTP), and it is involved in synaptic plasticity. It primarily interacts with the protein tropomyosin-related kinase B (TrkB). Alterations in the expression of BDNF and TrkB are also associated with defects in learning and memory. Corroborating this role of BDNF, it has been shown that BDNF improves neurogenesis in animal models of Down syndrome. In the knock-out (KO) mouse model of FXS, dysregulation in BDNF/TrkB signaling affects differentiation of neural progenitor cells (NPC) and brain development.

Despite the name, BDNF is found in tissues other than the brain, including peripheral blood as this neurotrophic factor functions in the central and peripheral nervous systems; thus, BDNF expression can be easily measured in plasma samples. In fact, a study showed that blood BDNF

concentrations reflect brain-tissue BDNF levels. Therefore, for Aim 2, we will measure BDNF expression in blood to see if this correlates with treatment response to sertraline.

We will also investigate the response to sertraline in association with the different polymorphic variants, particularly in the serotonin transporter (SLC6A4), serotonin-synthesizing tryptophan hydroxylase 2 gene (TPH2), and BDNF genes, in our study patients. These genes have different polymorphic allelic variants that have been implicated in neurological conditions. Two BDNF variants have been associated with diagnosis and response to treatment in patients with neuropsychiatric disorders. Specifically, the BDNF Val66Met polymorphism has been associated with attenuated stress response in individuals suffering from depression as reviewed in a meta-analysis that examined 22 studies. The consensus scientific finding is that individuals with the met-allele have more difficulty coping with stress and major depression than individuals with a homozygous val/val genotype. This same polymorphism has also been implicated in efficacy of response to fluoxetine in an Asian population group. Thus, the variable response rate to SSRI treatment in ASD could be associated with secondary gene effects that ultimately can regulate serotonin levels in the brain.

Thus, the question we will investigate in Aim 2 is whether the allelic variation at BDNF, TPH2, or in the 5-HTTLPR have a correlation with treatment response to sertraline. As the pattern of response to SSRIs is variable it would be very advantageous if one could predict response and individually tailor and personalize a specific treatment. Studies of the gene for the serotonin transporter (*SLC6A4*), and in particular the 44 base pair insertion/deletion polymorphism within a repetitive unit in the promoter region (5-HTTLPR), have indicated that the long (L, insertion) form of 5-HTTLPR is functionally more active than the short (S, deletion) form. While the long allele (L) of the 5-HTT gene-linked polymorphic region (5-HTTLPR) has been correlated with shyness in grade school children, the short allele (S) has been shown to moderate the influence of stressful life events on depression. In addition, multiple studies document that individuals with social anxiety disorder or depressive disorders, who are homozygous for the high-transcribing long (L/L) genotype (which is most effective in clearing the synapse of serotonin), have consistently shown the best response to treatment with an SSRI. Several studies, although not always consistent, have examined the influences of the 5-HTTLPR polymorphism and demonstrated that individuals with the short allele (S) compared to those that are homozygous for the long allele (L/L) show increased vulnerability to develop anxiety-related traits and depression. Also there is evidence that environmental insults such as significant stress exposure in those with the S allele are at more risk to develop depression.

Thus, with Aim 2 we sought to demonstrate that patients with ASD homozygous for the L allele in the 5-HTTLPR will show a greater improvement in language and social reciprocity than those with the other genotype. This hypothesis is supported by studies that documented individuals with social anxiety disorder or depressive disorders that are homozygous for the high-transcribing long (L/L) genotype (which is most effective in clearing the synapse of serotonin), consistently have the best treatment response to SSRIs. Establishing a link between

treatment response and this polymorphism would identify a useful genetic trait that would be very important when selecting effective treatment options.

BDNF, as described above, has been shown to play a role in intellectual disabilities, ADHD. Differences in BDNF expression due to the different activity of the allelic variants could lead to altered brain development in ASD at very early stages of development that contribute to the synaptic plasticity defects in ASD. Thus, we expect that the BDNF (Val/Met) may act as a modifier of disease severity and that those homozygous (Met/Met) and heterozygous (Val/Met) may have less response than those with the (Val/Val) genotype.

We will also assess the contribution of the serotonin-synthesizing tryptophan hydroxylase 2 (TPH2) gene, which is involved in serotonin biosynthesis in the brain, and has been associated with a number of psychiatric disorders, including ADHD, autism, and major depression. The rs1178997 SNP in the promoter of TPH2 results in disruption of a transcription factor binding site and has been reported to decrease promoter activity by 22%, so we will also assess this allelic variant.

Mucosal serotonin (5-HT) has been shown to modulate the immune response potentially influencing intestinal inflammation. In addition, serotonergic receptors have been characterized in lymphocytes, monocytes, macrophages, and dendritic cells suggesting a role for 5-HT in immune cell function. Several lines of evidence have identified the existence of immune dysfunction ASD that can affect core features of ASD that ultimately lead to altered neurodevelopmental processes. Cytokines are regulators of host responses to infection, immune responses, inflammation, and trauma. Some cytokines act to make disease worse (pro-inflammatory); whereas, others serve to reduce inflammation and promote healing (anti-inflammatory). Interleukin (IL)-1 and tumor necrosis factor (TNF) are pro-inflammatory cytokines. Pro-inflammatory cytokines and serotonergic homeostasis have both been implicated in the pathophysiology of major psychiatric disorders. It has been demonstrated that inflammatory cytokines can activate p38 mitogen-activated protein kinase (MAPK), which induces a catalytic activation of the serotonin transporter.

The role of inflammation in ASD prompts us to investigate the cytokine response to sertraline treatment. We will document whether the abnormal cytokine profiles in ASD change with sertraline treatment and whether these changes correlate with treatment response.

In the treatment of autism, Chugani has emphasized the importance of developmental windows for pharmacological intervention aimed at setting brain developmental programs back on course. In her PET studies using a ^{11}C methyl-L-tryptophan as a tracer to estimate serotonin synthesis, she has demonstrated that humans undergo a period of high brain serotonin synthesis capacity during early childhood, in the first five years and that this developmental process is disrupted in children with autism. Children with autism show a gradual increase in serotonin synthesis capacity that is significantly lower on average than typically developing children in the first 6 years and then it becomes gradually higher by 7 years of age. Since serotonin is known to be an important factor involved in postnatal synaptogenesis, one approach to the treatment of autism would be the use of an SSRI in children less than the age of

5 years when serotonin synthesis capacity is lower in autistic children compared to non-autistic children. The goal of such treatment would be to provide a more normal modulation of synaptic plasticity for a finite period time when the serotonin input is critical for brain connectivity. Currently Chugani and colleagues have a multicenter study assessing the benefit of buspirone in children 2 through 5 years of age with autism.

Our experience in using sertraline in young children with ASD comes from the clinical experience of the PI, Randi Hagerman MD, in the clinical follow-up of children with ASD who were started with an SSRI between 2 to 5 years. In addition, at the MIND Institute we have carried out a controlled trial of buspirone in young children with ASD as part of the multicenter trial of Dr Chugani, but her evaluation of the data is not yet complete.

6) Inclusion and Exclusion Criteria

Inclusion Criteria

1. Documentation of ASD with DSM-V criteria as well as a standardized autism assessment such as the Autism Diagnostic Observation Schedule (ADOS) or the Autism Diagnostic Interview–Revised (ADI-R).
2. Subject between the ages of 24-72 months of age.
3. A reliable parent or caregiver who can report the side effects and communicate effectively with the research team.
4. Stable medications during the two months prior to enrollment.
5. Currently receiving interventions in the community or school for ASD.

Exclusion Criteria

1. Current or past SSRI treatment.
2. Other serious co-morbid medical disorders affecting brain function and behavior, including uncontrolled seizures.

7) Number of Subjects

We will recruit a total of 72 patients with ASD over three years, in hopes of having 20 subjects complete the trial each year (for a total of 60 subjects completing the trial). Due to a delayed activation and several periods of low recruitment due to study staff turnover in the past few years, we will enter a no-cost extension year to reach enrollment targets by July 2018.

8) Recruitment Methods

Subjects will be recruited through the MIND Institute Subject Tracking System (STS) and referral from UCDHS clinicians. If needed, approved study flyers will be posted in the regional center, UCDHS clinics, social networking sites, community providers' offices, and given to an autism support groups. Additionally, the investigator plans to do educational presentations on use of sertraline in neurodevelopmental disorders, which may lead to recruitment into this study.

Potential subjects who are interested in participating in the study will be pre-screened either via telephone or on site by the research team utilizing the approved Oral Informed Consent Script for Research. This will also allow potential subjects to ask study-related questions and discuss the study in depth with the research team.

We are requesting a waiver of HIPAA Authorization for recruitment purposes only. The research team will identify potential subjects and access medical records to verify eligibility prior to the treating physician and research coordinator approaching the potential subject. Consent to participate and HIPAA Authorization to access additional information in the medical records will be obtained prior to enrollment into the study. The protected health information will not be reused or disclosed to outside persons or entities.

The review of subjects' medical records is for limited information and only to determine eligibility. The data are derived from clinically indicated procedures and there is minimal risk to the subjects' status, employment, or insurability. Only research personnel will access medical records via EMR, and all personnel are required to use the “Quick Disclosure” function in EMR to document review. Without an initial review of the medical record for screening purposes, it would not be possible to identify potential subjects and confirm their applicability for study participation. Once eligibility in confirmed, subjects will be approached to obtain their authorization to access and use their health information for the research.

9) Compensation to the Subjects

There will not be any compensation available to subjects for completing any or all of the study procedures.

10) Study Timelines

Screening and enrollment began upon IRB approval of this protocol and associated documents. Enrollment has continued for approximately two and a half years and will be extended for an additional year in order to reach the full target and to have the final participant finish before the end of 2018.

Each participant will be involved in this trial for a period of six months. This will include three visits to the UC Davis MIND Institute – a combined screening and baseline visit, a 3 month interim visit (±2 week), and a 6 month end-of-treatment visit (±2 week). Additionally, there will be weekly phone calls for the first month of treatment, and subsequent monthly phone calls if no visit is scheduled during that month.

Participants will be active in this trial until about December of 2018, at which point we expect that the final participant will have completed the trial period.

Final data analysis of data collected during the trial period of each participant will be analyzed after the final participant has completed the trial, which is expected to be about December 2018.

11) Study Endpoints

Our primary study endpoints will be changes, from baseline to 6 months, in both the expressive language raw scores and the combined age equivalent scores as measured by the MSEL. All other measures listed below in Section 12 are secondary study endpoints.\

12) Procedures Involved

The schedule of assessments is listed below:

Assessments	Screening/ Baseline Visit	Three month visit (+2 week)	Six Month Final Visit (+2 week)
Informed Consent	X		
Inclusion/Exclusion Criteria	X		
Medical History	X		
Physical Exam	X	X	X
Adverse Events	X	X	X
Concomitant Medications	X	X	X
Vital Signs	X	X	X
Blood Draw	X	X[a]	X
Mullen Scales of Early Learning (MSEL)	X		X
Preschool Language Scale, 5th ed. (PLS-5)	X		X
Autism Diagnostic Observation Schedule (ADOS) or Autism Observation Scale for Infants (AOSI)	X		
Vineland Adaptive Behavior Scale, 2nd ed. (VABS-II)	X		X
Clinical Global Impression Scale (CGI)	X	X	X
Visual Analogue Scale (VAS)	X	X	X
Sensory Processing Measure, Preschool ed. (SPM-P) or Sensory Processing Measure (SPM)	X		X
Preschool Anxiety Scale, Revised ed. (PAS-R)	X		X
DSM V Checklist	X		
Social Responsiveness Scale (SRS)	X		X
Eye Tracking[b]	X		X
Aberrant Behavior Checklist (ABC)[c]	X	X	X
Suicidality Checklist[c]	X	X	X
Dispense Study Drug	X	X	

Demographics:

At Screening, basic demographic information will be collected on all study subjects, including but not limited to date of birth, race, ethnicity, socioeconomic status, education of parents, parental occupation, and parental salary range.

Medical Examination:

This will be carried out by Dr. Hagerman or her associate and will include growth percentiles, vitals, a detailed medical history, physical, neurological examination, and review of medical records, as applicable. In addition a detailed family history may be obtained, if medically

necessary. A medical history and exam will be conducted at the Screening/baseline visit. A medical exam and a review of recent history will take place at each subsequent visit.

Mullen Scales of Early Learning (MSEL; Mullen, 1995):

The MSEL was standardized in children from birth to 68 months of age. It includes five subscales: gross motor, fine motor, visual reception, expressive language, and receptive language. The subscales yield alpha coefficients between .75 and .83. Test-retest values range from .76 to .96 across ages and scales, and inter-rater reliability from .91 to .96. NVMA and NVIQ will be computed (with latter being twice the T score). The MSEL will be a baseline and 6-month outcome measure.

Preschool Language Scale-Fifth Edition:

The PLS-5 assesses auditory comprehension and expressive communication for children birth to 7 years 11 months. The measure examines the child's attention, play, gestures, social communication, semantics, language structure, integrative language skills and emergent literacy skills. The PLS-5 has expanded coverage of early play behaviors, concepts, Theory of Mind, as well as emergent literacy skills. The PLS-5 also has a means by which to track progress using Growth Scores to monitor a child's skills. PLS-5 was standardized with a sample of 1400 children, including children with disabilities, selected to be representative of the U. S. population in terms of ethnicity, gender, geographic region, and parent education level based on U.S. Census figures (March 2008 update). Of the sample, 45% consisted of minorities, the largest percentage to date. The PLS-5 will be a baseline and 6-month outcome measure.

Clinical Global Impression Scale (CGI):

This scale is a standard assessment for medication studies because it allows the clinician to utilize the history from the parents or caretaker and incorporate it into a clinical rating for the clinical follow-up of the patient through the treatment trial. (Guy, 1976; Psychopharmacology, 1985).

The CGI-S is a 7-point scale that requires the clinician to rate the severity of the patient's illness at the time of assessment, relative to the clinician's past experience with subjects who have the same diagnosis. The CGI-S will be captured at Screening. The 7-point scale is as follows:

Normal	1
Borderline Ill	2
Mildly Ill	3
Moderately Ill	4
Markedly Ill	5
Severely Ill	6
Extremely Ill	7

The CGI-I is a 7-point scale that requires the clinician to assess how much the subject has improved or worsened relative to a baseline status. The CGI-I will be captured at the 3-month visit and the 6-month visit. The response options include the following:

Very Much Improved	1
Much Improved	2
Minimally Improved	3
No Change	4

Minimally Worse	5
Much Worse	6
Very Much Worse	7

Visual Analogue Scale (VAS):

Parents will mark on a visual line measuring 10 cm with one side marked "worst behavior" and the other side marked "best behavior". They will assess 3 key behaviors that are targeted for this trial (e.g., aggression, hyperarousal with stimuli, anxiety, hyperactivity). For each behavior they will mark their impression of the behavior at each visit, demonstrating how they have changed from the baseline. The horizontal marks are measured in cm distance so that we can see improvements or worsening of behavior over this time period. The VAS will be a baseline, 3-month, and 6-month outcome measure.

Autism Diagnostic Observation Scale (ADOS) or Autism Observation Scale for Infants (AOSI):

The ADOS (Lord, et al., 2000) and AOSI are semi-structured standardized interviews administered directly for the purposes of diagnosing autism. The ADOS uses developmentally appropriate social and object-based interactions in a 30-45 minute interview to elicit symptoms of autism in four areas: social interaction, communication, play, and repetitive, restrictive behaviors. The ADOS consists of different modules, each directed at a particular level of language ability and is thus appropriate to use across subjects of varying ages and functioning levels. A previously completed ADOS or AOSI performed 6 months prior to the Screening/Baseline visit can be used to determine study eligibility. In this instance, the ADOS or AOSI will not need to be done at the Screening/Baseline visit. This assessment will not be repeated at the 3-month or 6-month visits because its purpose is solely for eligibility determination and characterization of the study population at baseline.

Vineland Adaptive Behavior Scale-Second Edition (VABS-II):

The VABS-II (Sparrow, Cicchetti, & Balla, 2005) is an update of the VABS developed by Carter et al., 1998 that was designed to assess handicapped and non-handicapped persons in their personal and social functioning and is appropriate for individuals of all ages. The VABS-II is a survey that is administered to a parent or caregiver using a semi-structured interview format and is organized around four Behavior Domains: Communication, Daily Living Skills, Socialization, and Motor Skills. Each subtest is scored with a standard score X=100 ± 15. The VABS was successfully utilized to establish level of adaptive functioning for individuals with FXS (Berry-Kravis et al., 2006; Zingerevich et al., 2009). Internal consistency for each domain was measured utilizing the split-half method and coefficients ranging from .83 to .90 were established. Test retest reliability was calculated to be .81 to .86 and inter-rater reliability was .62 to .78. The VABS will be a baseline and 6-month outcome measure.

Sensory Processing Measure (SPM):

The SPM is designed to measure sensory processing difficulties in multiple environments (at home, at school, and in the community) for children aged 5 to 12 years old. The SPM provides norm-referenced standard scores for two higher level integrative functions (praxis and social participation) and five sensor sensory systems (visual, auditory, tactile, proprioceptive, and vestibular functioning. Within each system, the SPM offers descriptive clinical information on processing vulnerabilities, including under- and over- responsiveness, sensory-seeking behavior, and perceptual problems. The SPM will be a baseline and 6-month outcome measure.

Sensory Processing Measure-Preschool (SPM-P):

The SPM-P is designed to integrate seamlessly with the SPM (as described above) but is targeted for a younger population (ages 2 to 5 years old). For most patients we will use the SPM-P; for those over 5 years old the SPM or the SPM-P will be used as appropriate based on the participant's cognitive age and ability. As with the SPM, the SPM-P will be a baseline and 6-month outcome measure.

Preschool Anxiety Scale – Revised (PAS-R):

The PAS-R is a revision of an earlier measure (Preschool Anxiety Rating Scale, Spence et al., 2001). It is designed to assess symptoms of anxiety and fears in young children (aged 6 and below) as reported by their parents. The measure provides 4 subscales tapping generalized anxiety, social anxiety, separation anxiety, and specific fears. The total scale and 4 subscales showed strong internal consistency (alphas = .72-.92), 12-month stability (rs = .60-.75) and maternal/paternal agreement (rs = .60-.75). Scores on the scale also showed expected correlations with a measure of emotional distress, diagnosed anxiety disorders, and behavioral indicators of anxiety. The PAS-R will be a baseline and 6-month outcome measure.

Diagnostic and Statistical Manual of Mental Disorders-V:

Per the DSM-V (APA 2013), Autism Spectrum Disorder (ASD) is characterized by severe and pervasive impairment in several areas of development: reciprocal social interaction skills, communication skills, and the presence of stereotyped behavior, interests, and activities. The qualitative impairments that define these conditions are distinctly deviant relative to the individual's developmental level or mental age. The DSM-V will not be repeated at the 3-month or 6-month visits because its purpose is solely for eligibility determination and characterization of the study population at baseline.

Social Responsiveness Scale (SRS):

The SRS distinguishes autism spectrum conditions from other child psychiatric conditions by identifying the presence and extent of autistic social impairment. This 65-item rating scale measures the severity of autism spectrum symptoms as they occur in natural social settings. Completed by a parent or teacher in 15 to 20 minutes, the SRS provides a clear picture of a child's social impairments, assessing social awareness, social information processing, capacity for reciprocal social communication, social anxiety/avoidance, and autistic preoccupations and traits. The SRS will be a baseline and 6-month outcome measure.

Eye Tracking:

In a series of eye tracking tasks participants will be shown stimuli, either objects or emotional faces and their visual reaction times as measured by mean saccade latencies and overall fixations to predefined areas of interest will be recorded. The tasks aim to index visuospatial attention via passive viewing methods that will not overly tax the participants, and may be sensitive to subtle changes over time that will not be evident in more gross behavioral measures. The eye tracking protocol also includes collection of demographic information and completion of the Carey Temperament Scales (CTS). The CTS is a collection of parent-report questionnaires, each covering a specific age range between 1 month and 12 years, that examines nine categories of behavioral and temperamental style as defined in the New York Longitudinal Study (NYLS). Eye tracking will be a baseline and 6-month outcome measure.

Aberrant Behavior Checklist-Community (ABC-C):

The ABC is a parental survey consisting of 58 questions designed to measure the severity of autism-associated behaviors, including irritability, lethargy, stereotypy, hyperactivity and inappropriate speech. The irritability subscale includes self-injurious and aggressive behavior. The total score range within the ABC is 0–174, with higher scores indicating more severely affected behavior. The ABC has been previously approved by the IRB as it is part of the MIND Institute Protocol Repository (MIPR, IRB # 200513093). The ABC will be captured at baseline, 3 months, and 6 months.

Clinical Assessment of Suicidal Ideation and Behavior:

This assessment is a series of "yes" or "no" questions. The clinician will ask if the subject understands the concept of suicidal ideation. If the answer is "No", the remaining questions about the subject's behavior will be asked to the caregiver. If the answer is "Yes", the remaining questions will be directed to the subject. This assessment will be used in combination with the ABC irritability subscale to assist the clinician in assessing any possible suicidal ideation or behavior. Although we have never seen this side effect in children under 6 years old, this has been added to follow the FDA draft Guidance for Suicidal Ideation and Behavior (attached for reference). This assessment will be administered at screening, 1 month, 3 months, and 6 months.

Additional Information Regarding Above Assessments:

Measures administered may be audio or video recorded for future reference or coding. All recording will be stored in compliance with all applicable guidelines.

The MSEL, CGI, ADOS, VABS-II, SPM, SPM-P, and VAS assessments listed above are part of the MIND Institute's Protocol Repository (MIPR) and have been approved under the human subjects protocol #232909. The PLS-5 has been included as part of this protocol's initial submission for approval. The remaining assessments are computer-based and are described in full above.

Laboratory Blood Tests

Laboratory assessments will be collected at the Screening/Baseline visit and the 6-month visit. If blood is unable to be completed at the Screening/Baseline visit, then sample collection will be attempted at the 3-month visit. Both safety blood samples and biomarker blood samples will be collected. Safety laboratory assessments include complete metabolic panel and complete blood count. Biomarker assessments will evaluate DNA, RNA, FMR1 mutation status, BDNF, SLC6A44, TPH2, 5-HTTLPR, and cytokine response. Approximately 30 mL (6 tsp) of blood will be collected per blood draw.

13) Data and Specimen Banking

Each sample will be coded by a unique numerical identifier. There will be a total of two blood draws throughout the study. Each blood draw will have a total volume of up to 30ml. Safety labs will be processed through the UC Davis Pathology Laboratory. Biomarker samples will be delivered to Dr. Tassone's lab at the UC Davis MIND Institute's Wet Lab for processing and storage. Samples will be kept until the end of the study and subsequent analysis, at which point they will be destroyed. Alternatively, on the study consent form, participants may indicate permission to allow for samples to be kept for a period of 15 years after the end of the study.

14) Data Management and Confidentiality

The clinical study data will be entered into and stored in REDCap, which is managed by the Biomedical Informatics Program of the UC Davis Clinical and Translational Science Center. Vanderbilt University, with collaboration from a consortium of institutional partners, has developed a software toolset and workflow methodology for electronic collection and management of research and clinical trial data. REDCap (Research Electronic Data Capture) data collection projects rely on a thorough study-specific data dictionary defined in an iterative self-documenting process by all members of the research team with planning assistance from the Biomedical Informatics Program. Iterative development and testing processes result in a well-planned data collection strategy for individual studies. The REDCap system provides secure, web-based applications that are flexible enough to be used for a variety of types of research, provide an intuitive interface for users to enter data and have real time validation rules (with automated data type and range checks) at the time of entry. These systems offer easy data manipulation with audit trails for reporting, monitoring, and querying patient records as well as and automated export mechanism to common statistical packages (SPSS, SAS, Stata, R/S-Plus). REDCap servers are housed in a local data center at UC Davis Health System and all web-based information transmission is encrypted. REDCap was developed specifically around HIPAA-Security guidelines. For example, upon login, an approved username and password is required. REDCap has been disseminated for use locally at other institutions and currently supports 240+ academic/non-profit consortium partners on six continents and over 26,000 research end-users (www.project-redcap.org).

All research personnel on this IRB protocol will have some type of access to this database. The data management and biostatistics team (Patrick Adams and Dr. Danh Nguyen) will have full access since they will create the data dictionary and address data requests. Research coordinators, technicians, and assistants will enter the data into the database. All research personnel will have current GCP training for investigators and staff involved in research involving human subjects.

Analysis will be based on the intention-to-treat principle. The analysis of treatment efficacy will be based on the analysis of covariance (ANCOVA) with outcomes (receptive and expression language at 6-month follow-up with corresponding baseline measure as a covariate). The chosen endpoint at 6-month, based on preliminary data, provides a reasonable treatment time period to assess changes in the response. The proposed method adjusts for potential baseline differences between treatment arms despite randomization, if any. The sample size/power for the ANCOVA is based on sample size needed for a t-test by a variance inflation factor 1-2 where there is the correlation between baseline and follow-up measures. From our preliminary data, the receptive and expressive scores have ranges from approximately 0.2 to 0.5. Thus, we provide the power for the t-test and power for the ANCOVA will be higher depending on this factor. We estimate clinically relevant effects sizes from our preliminary data at follow-up visit for (sertraline /placebo) as follows: receptive language score mean 18.1/23.8, SD=7.0; expressive language score mean 13.6/20.7, SD 8.4. For these effect sizes of the 2 primary measures, the ANCOVA will have power of at least 80% (or have between 0.2-0.5) at level alpha=0.016 with the proposed total sample size n = 60.

For CGI, we will use the standard t-test for 6-month follow-up assessment. For n = 60 the t-test to detect a one point CGI change (from 4 = no change to 3 = minimally improved) and conservatively assuming a SD = 1, the power is 91% at alpha level of 0.016. We will use similar regression and ANCOVA/ANOVA methods to analyze other secondary variables.

15) Provisions to Monitor the Data to Ensure the Safety of Subjects

A DSMB will be established that will include two physicians who are independent of the study but who have significant expertise in carrying out psychopharmacological interventions in children with ASD. In addition, an independent statistician will be a member of the DSMB. The DSMB will examine unblinded adverse event(s) and clinical data annually to recommend whether the trial can be safely continued. The investigators and staff will remain blinded throughout the trial.

16) Withdrawal of Subjects

The study team reserves the right to withdraw subjects from the study for reasons including, but not limited to, adverse events and non-compliance. The caregivers of a subject may also withdraw their son or daughter from the study.

If a subject is withdrawn from the study early, caregivers will be asked to return to the MIND Institute for a termination visit which will include a visit with a study physician who can assess for adverse events and general safety of the subject.

17) Risks to Subjects

Risks associated with participation in this study pertain to the study medication, the possible ineffectiveness of treatment, and the blood tests. A potential risk is the chance of infection or bleeding at the site where the venous blood draw is completed. This site will be carefully cleaned and a band aid will be applied. Other potential risks are the side effects of sertraline, which include hyperarousal, hyperactivity or activation especially if the dose is too high.

In adolescents, SSRIs have induced suicidal ideation in rare circumstances, but we have never seen this side effect in children under 6 years old. According to the FDA Draft Guidance for Suicidal Ideation and Behavior section III.C, this study falls under trials involving patients where it is reasonable to omit assessments of suicidal ideation. However, because the study does involve an antidepressant drug, we are implementing the irritability subscale of the ABC and the Clinical Assessment of Suicidal Ideation and Behavior Checklist to aid clinicians in this risk assessment. Clinicians will perform the assessments at each visit, and at the one-month phone call (scheduling a follow-up, face to face interaction if needed). The study physician will discontinue the medication if there is any concern about suicidal ideation or behavior.

Weekly phone calls during the first month will be made by study personnel to evaluate the presence of side effects. Subsequent phone calls will be monthly except for months 3 and 6, when a visit will take place. The study physician will perform a phone call after one month of treatment to assess for adverse events as well as perform the Clinical Assessment of Suicidality and ABC – Irritability Subscale. Dr. Hagerman's contact information will be provided to all subjects' parents for emergencies related to the study.

All the data obtained from this study will be kept confidential. Only research personnel on the IRB-approved protocol will have access to the data. Electronic data will be password protected and physical data will be stored in a locked file room. However, just like with other personal information kept by your health care providers, your banks, and others, even these safeguards cannot guarantee absolute protection of the data. If private information gets into the wrong hands, it can cause harm. Although rare, there are reported cases of breaches that have resulted in discrimination in insurance or employment.

18) Vulnerable Populations

This study intends to enroll developmentally disabled children with ASD who will be too young to consent to participate to this study. As such, reliable and competent caregiver(s), such as (a) parent(s) or other legal guardian, must give consent to participate in this study. The caregivers of the participant will be informed of all known risks and potential benefits associated with the study.

Because this study involves young children ages 2 through 5 years old (inclusive) with developmental disorders, all study staff will pay special attention to the potential for the extreme sensitivities, multiple stressors, and other possible risks associated with the vulnerable population under study. We have done research with developmentally disabled and cognitively impaired individuals of all ages for many years. Consent for individuals with cognitive disability is always signed by parent or caregiver, and no study-specific procedures will be done for any subject prior to consent.

19) Multi-Site Research

This is a single-site research study.

20) Community-Based Participatory Research

Not applicable.

21) Sharing of Results with Subjects

Participants may request a research report with scores from some of the cognitive testing completed at the baseline and final visits. Participants will not be unblinded at any point during their enrollment. This is to prevent unconscious biases that may present as the trial progresses and to ensure quality data. Subjects will be unblinded once all study procedures and all statistical analyses for all subjects have been completed.

22) Setting

<u>The UC Davis MIND Institute:</u>

Participants will be evaluated at the MIND Institute Research Clinic. The MIND Institute is a multi-purpose research facility focusing on patients with autism spectrum disorders and other neurodevelopmental disorders. The basic (wet labs) and clinical buildings, total approximately 100,000 ft^2, were completed in 2002 and are co-localized on the UC Davis Medical Center campus in Sacramento. The MIND Institute is well equipped with multiple patient exam rooms available for screening IQ measures as well as questionnaires, surveys, and physical exams. This will be the main study site, as recruitment, screening, and patient visits will occur at the MIND Institute. The MIND MICRO committee has approved space usage for this study.

23) Resources Available

For a full list of research personnel associated with this study, please refer to the associated personnel list. All study personnel will be familiar with the protocol and research procedures.

Computers: All key personnel have up-to-date personal computers with high-speed internet and printer connections.

Offices: The MIND Institute has office space available for all associated research personnel. All office space includes up-to-date computers, high-speed internet access, printers and telephones with private voice mail.

24) Prior Approvals

The Year 1 initial submission was submitted on 18-Sep-2014, and a response addressing analyst concerns from 7-Oct-2014 was subsequently submitted on 24-Oct-2014. Initial approval was granted on 25-Nov-2014. Year 2 renewal was submitted on 26-Aug-2015 and approved 14-Sep-2015. Year 3 renewal was submitted on 29-Jul-2016 and approved on 9-Aug-2016. Year 4 renewal submission is due by 23-Jun-2017.

25) Provisions to Protect the Privacy Interests of Subjects

Research charts will be kept on the subjects and subject information will be coded to protect confidentiality. All charts will be kept in a locked cabinet of a locked file room. The identifiers used to identify subjects will be kept in locked offices and/or locked cabinets, and the electronic database containing personal information will be kept on a secure computer network accessible only to PI's research team. If information from the study is published or presented at scientific meetings, subject names and other information that could identify subjects will not be used.

The subjects' health information, along with the identifiers, will be kept with the investigator as mentioned, until the conclusion of the study, or when immediate access is no longer required. Thereafter, the information may be transferred to a records and information management company for long-term storage, or to a UCDMC long-term storage facility. Archiving, storage, and destruction of all study materials will comply with the MIND Institute's Archiving SOP# 500-017.

All study personnel will have access to study records, data, and specimens. If required, access to study records and data will be made available to representatives of HRSA. Enrolled subjects will be made aware that study personnel, and representatives of HRSA, will have access to their records. This will be included in the consent form and will also be thoroughly reviewed during the consenting process. In addition, every attempt will be made to ensure that the personal and medical information of the subject will be kept private; however, we cannot guarantee total privacy. The subject's personal and medical information may be given out if required by law (for example, reporting sensitive information (such as child abuse) to state or local authorities if necessary).

26) Compensation for Research-Related Injury

It is important that subjects promptly tell the person in charge of the research if they believe that they have been injured because of taking part in this study. If subjects are injured as a result of being in this study, the University of California will provide necessary medical treatment. Depending on the circumstances, the costs of the treatment may be covered by University or the study sponsor or may be billed to the subject's insurance company just like other medical costs. The University and the study sponsor do not normally provide any other form of compensation for injury.

27) Economic Burden to Subjects

There is no charge for the subject to participate in this study. Neither the subject nor his or her insurance carrier will be charged for taking part in the research. All costs associated with the study will be paid by the sponsor/department.

There may be indirect costs associated with participation including travel and time taken off work to meet appointments. Possible expenses to the subject's family include time spent at the clinic as well as expenses for traveling. All eligible research subjects, who would not otherwise be able to participate due to the cost of traveling to the MIND Institute (e.g., flights, gas, lodging), will be offered up to $400 travel reimbursement per visit for actual costs incurred.

Costs of travel may include, but are not limited to, economy airfare, car mileage, lodging, bridge tolls, parking garage fees, taxis, rental cars, and/or gas reimbursement for rental cars. Reimbursement will be based off of paid receipts submitted by families per UCDMC reimbursement policy. Travel reimbursement is based off of employee reimbursement guidelines enforced by the Department of Psychiatry and Behavioral Sciences and University policy. Reimbursement checks will be mailed 6-10 weeks from submission of paid receipts following the completed study visit.

28) Consent Process

If requested, the study consent form will be provided to potential subjects prior to visiting the MIND Institute.

Consenting will take place at the UC Davis MIND Institute by research personnel delegated by the PI to consent. Procedures for obtaining consent will be followed as outlined in HRP-090. Consenting and all study measures and assessments will be done in English, as some of the measures used through this study are only available in English. All participants will be under the age of 18; thus, we will require the consent of a parent, guardian, or other legally responsible adult with documentation of authority to sign on behalf of the participant in order to be enrolled in this study. All subjects, parents, or LARs (as applicable) will sign a HIPAA authorization for research.

Consent to enroll in this study will preferentially be obtained from both parents/guardians of the participant; however, only one parent will be required to consent to participate in this study. The investigator feels that given the potential benefit and low-risk safety profile of the investigational product, it is sufficient to obtain permission from one parent.

29) Process to Document Consent in Writing.

The consent will conform to HRP-090 “Informed Consent Process for Research” and HRP-091 “Written Documentation of Consent”.

30) Drugs or Devices

We will treat children with ASD ages 24 to 72 months in a randomized, double-blind, placebo-controlled trial of sertraline (brand name Zoloft) for 6 months. Randomization to sertraline or placebo will be carried out by UCDMC Investigational Drug Services Pharmacy, and dosing will be determined based on patient age at enrollment. The study drug will be administered in liquid form (20 mg per cc).

For patients aged 2 to 3 years (inclusive) at enrollment, sertraline liquid or placebo liquid in a dose of 2.5 mg per day (0.125 ml) will be used. For those aged 4 years through 5 years (inclusive) at enrollment, the starting dose will be 5.0 mg perday (0.25 ml). The liquid can be mixed with water, ginger ale, lemon or lime soda, lemonade, or orange juice, as per the package insert. The UCDMC Investigational Drug Services Pharmacy will store and dispense the study drug.

As previously described, starting dose is assigned based on age at enrollment. After enrollment, dose modifications may be made at the discretion of the study physician; for example, if a subject aged 4-5 years (inclusive) at time of enrollment is experiencing an adverse event while at the 5 mg daily dose, the study physician may decrease the subject’s dose to 2.5 mg daily if clinically indicated. However, subjects started at 2.5 mg based on enrollment age will not undergo dose increase to 5 mg during the trial. In other words, if a subject aged 2-3 years (inclusive) at time of enrollment turns 4 years of age during her study participation, she will nevertheless continue at the original dose of 2.5 mg per day.

The PI of this study had an IND for this drug under fragile X syndrome, and this IND now includes ASD as a secondary indicated population (IND Number 113747-0001).