1) Protocol Title: *Combining Lovastatin and a Parent-Implemented Language Intervention in a Multi-Modal Treatment of Fragile X Syndrome*

2) Author of Protocol

X UC Davis Researcher
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- ☐ **Private Sponsor**
- ☐ **Cooperative Group**
- ☐ **Other:** _____

3) Objectives

Aim 1. Test the efficacy of a 20 week multi-modal treatment comprised of lovastatin and the Parent-implemented Language Intervention (PILI) for children with FXS. Children will be randomized to drug or placebo in a double-blind design with all participating in the PILI. The primary endpoint will be a spoken language composite and the secondary endpoint will be challenging behavior. We will test the following hypotheses:

Hypothesis 1a. Improvements in spoken language and behavior will be greater among lovastatin-treated than placebo treated participants.

Hypothesis 1b. Improvements in parental use of the PILI-targeted strategies will be associated with greater improvements in child language and behavior, especially in the lovastatin-treated group, thereby demonstrating benefit of the combined intervention.

Aim 2. (a) Identify biomarkers in peripheral immune cells associated with FXS and the efficacy of treatment and (b) elucidate the cellular/molecular mechanisms by which lovastatin influences MEK/ERK and Rho GTPase signaling pathways and immunological abnormalities associated with FXS.

Hypothesis 2a. Lovastatin corrects core biochemical and physiological impairments by normalizing Ca2+dependent and/or Ca2+-independent regulation of the MEK/ERK signaling pathway.

Hypothesis 2b. Lovastatin normalization of MEK/ERK signaling and/or Rho GTPase signaling is mediated by its effects on isoprenoid synthesis.

Hypothesis 2c. Individuals with elevated inflammatory cytokines will be more responsive to lovastatin.

4) Background

This is the first multi-modal treatment to combine a targeted treatment for FXS, lovastatin, with an innovative parent-implemented intervention (PILI) targeting language and challenging behavior delivered through telehealth technology. Our hypothesis is that targeted treatments will be more effective when applied in combination with PILI. We also will examine whether changes in the activity of key

pathways/proteins influenced by FMRP (the mitogen-activated protein kinase (ERK) and MMP-9) are biomarkers of treatment responsiveness. Because lovastatin is also an anti-inflammatory, we will characterize MEK/ERK signaling in peripheral immune cells both and pre- and post- treatment to determine whether levels of these signaling molecules are predictive biomarkers of treatment response. We hypothesize that those individuals with elevated inflammatory cytokine profiles will be most responsive to lovastatin treatment. Once modeled in FXS, results from these studies can then be applied to other neurodevelopmental disorders including Rasopathies.

Lovastatin as a treatment for FXS: Lovastatin is an inhibitor of the rate-limiting enzyme in cholesterol biosynthesis and an FDA-approved treatment for hyperlipidemia. Lovastatin has also been proposed as a treatment for IDD, particularly for Rasopathies and Neurofibromatosis Type 1 (NF1). Lovastatin reduces activation of the small guanosine triphophatase (GTPase) Ras, which in turn reduces activity of the MEK/ERK signaling pathway. Lovastatin interferes with recruitment of Ras to the membrane, a process required to transition from inactive GDP to active GTP. The interaction of Ras with the membrane requires the prost-translational addition of a farnesyl group to the C terminus of Ras, and lovastatin inhibits Ras farnesylation by targeting the upstream mevalonate pathway.

Recent extensions of animal studies to FXS have been promising. In a study of Fmr1 knock out (KO) mouse, intraperitoneal injections of lovastatin protected the mice from audiogenic seizures that are part of the FXS phenotype. In the KO mouse, the lack of FMRP represents a loss of the translational repressor for many proteins important for synaptic plasticity. Lovastatin corrected the excessive protein production in the KO mouse, inhibited RAS-ERK1/2 signaling in hippocampal neurons, and improved the phenotype. Although lovastatin has been used to treat children as young as one year of age, FDA approval extends only to 10- to 17-years-olds and studies of efficacy for cognitive impairments in NF1 have shown benefit and safety for this age range so this study will focus on the same age group.

Parent-implemented Intervention (PILI) for FXS: McDuffie, Abbeduto, and colleagues have developed an intervention to teach parents how to interact with their children with IDD, including those with FXS, in ways that optimize language learning. The intervention integrates, in novel ways, several intervention principles with a well- documented evidence base (see grant for efficacy articles).

The PILI consists of didactic parent education sessions paired with real-time clinician coaching of parent-child interactions. Both types of sessions are aimed at enhancing parental verbal responsiveness (PVR). PVR is an interactional style characterized by talking about the child's focus of attention, exposing the child to diverse vocabulary and elaborated sentence structure, creating frequent opportunities for the child to communicate, and providing affectively positive and contingent verbal responses to child utterances. The broad goal of PVR is to expose the child to increased amounts of verbal language input slightly in advance of his/her current level of competence while providing numerous opportunities to use newly acquired language.

Numerous longitudinal studies have shown that increased PVR is associated with more positive language outcomes for typically and atypically developing children (e.g., Siller & Sigman, 2002), including those with FXS (Warren, et al., 2010). The PILI also facilitates parental use of PVR strategies in a broad range of naturally occurring interactions with the child (e.g., dinner time) outside of the intervention context. In essence, PILI turns the parent into "the agent of change" for the child – a role traditionally assumed by the speech-language clinician working directly with the child. Making the parent the agent of change can expose the child to a higher rate of language learning opportunities compared to a traditional clinician-implemented intervention, thereby making PILI highly cost effective (Ingersoll & Gergans, 2007).

Many parents find it difficult to engage in verbally responsive interactions with FXS because of the challenging behaviors displayed by children with FXS (Abbeduto et al., 2004). Put simply, being verbally responsive is not an option if a child is having a tantrum, refusing to engage in interaction, or has a narrow range of interests and preferred activities. In the case of FXS, the phenotype includes numerous challenging behaviors and comorbid conditions, including social anxiety (Cordiero et al., 2012), inattention (Cornish et al., 2004), hyperarousal (Belser & Sudhalter, 2001), symptoms of autism (McDuffie et al., 2010), repetitive behaviors (Hall et al., 2010), self-injury (Symons et al., 2003), and aggression (Leigh et al., 2012). Although the physiology of FXS contributes to these behaviors, environmental contingencies help maintain these behaviors and so, parents can be taught to prevent or reduce challenging child behaviors by modifying social-environmental antecedents and consequences or teaching more adaptive "replacement" behaviors (Hall et al.,2006; Koegel et al., 1996; Moskowitz, 2011). In the PILI, therefore, individualized strategies for addressing challenging behaviors are provided during parent education sessions and parent use of these strategies is encouraged during coaching sessions.

The PILI of McDuffie and colleagues has been delivered largely through video teleconferencing (VTC) technology, an approach that has both practical and theoretical advantages. Extensive clinic-based instruction would create an unrealistic travel burden for families. Additionally, enhancing PVR in the home has the advantage of facilitating generalization of child gains in spoken language that often do not transfer from the clinic to real world settings (Dunst & Kassow, 2008). Delivery of health care via VTC is increasingly common (Ekeland, Bowes, & Flottorp, 2010), and a growing body of research supports the use of VTC to deliver interventions to children with IDD as well as coaching to teachers, therapists, and parents (Machalicek et al., 2010, 2009a, 2009b). McDuffie, Abbeduto, and colleagues recently completed two studies examining the use of VTC to deliver parent-implemented language intervention in the home to mothers of young children with either ASD (McDuffie et al., 2013) or FXS (Oakes et al., 2013). In both studies, PVR increased equally in coaching sessions conducted in the clinic and in the family home using VTC, and parents expressed a strong preference for more VTC relative to in-clinic sessions.

The initial trials of the PILI focused on 2- to 5-year-olds. There are virtually no evidence- based language/behavior interventions for older children and adolescents with FXS. Nevertheless, there is evidence that PVR continues to be important for

children with FXS well into adolescence (Brady et al., in preparation), although the linguistic content to be acquired is different for older individuals. In the present project, we adapt the PILI for 10- to 17-year-olds with FXS and their parents/caregivers by embedding the intervention in narrative activities.

Narrative as a Context for Intervention: In working with parents of 2- to 5-year-olds in PILI, McDuffie and colleagues taught them within the context of object-based play and caretaking routines (e.g., getting the child dressed) because these activities occupy a large proportion of their naturally occurring interactions. These interactive contexts are less central in the lives of older children and adolescents with FXS and their parents/caregivers. In the present project, we propose to focus on the context of joint story-telling, or narrative, activities. Children use narrative to organize and make sense of the world (Bruner, 1985). Joint book-reading is one of the earliest forms of narrative to which children are exposed and it remains an important context for acquiring and practicing language skills into adolescence. Moreover, there is substantial empirical support for the efficacy of embedding language intervention targeted into narrative interactions for older children and adolescents. Narrative based language interventions are structured around the context of a shared story-telling activity based on a book with illustrations. This type of intervention is well-suited to involving the parent/caregiver as interventionist because time spent in shared story-telling not only predicts later language outcomes but represents a routine activity, parents/caregivers can be verbally responsive by modeling more advanced vocabulary and grammar, by asking open-ended questions that prompt the child to use more advanced language, and by responding contingently to child utterances with indirect corrective feedback. The story content provides the topic of the conversational interaction while the illustrations in the book encourage child engagement, support comprehension of the shared topic, and provide visual support for child responses.

In summary, the behavioral component of the proposed multi-modal treatment will be a PILI that targets improvements in spoken language and challenging behavior for 10- to 17-year-olds with FXS by increasing PVR within picture-book based story-telling episodes. Finally, parents/caregivers will be encouraged to use the targeted strategies in other everyday interactions with their child. The intervention will be delivered to parents/caregivers in their homes by way of VTC. Considerable preliminary work will need to be conducted, however, before combining it with lovastatin. Thus, we propose to spend the first 18 months of the project conducting two small-scale studies of PILI alone. The first phase (Phase 1) of the PILI study was designed to collect preliminary data, enrolled three mothers and their sons and will be completed by end of December 2014. NIH has requested that we continue to test the preliminary efficacy of the behavioral intervention using a small N group design study prior to implementing the phase of the intervention which includes the drug Thus, the second phase of the study (Phase 2) will be a group design (N=30) with randomized assignment to a Treatment Group or a Wait List Comparison Group. Participants in the Treatment Group receive the behavioral intervention and the Wait List Comparison Group receives treatment as usual. The final phase of the study (Phase3) will be a clinical trial (N=50) in which participants are randomly assigned to receive the behavioral intervention alone or in combination with Lovastatin.

5) Inclusion and Exclusion Criteria

Subjects will be screened initially through a pre-screening form either over the phone or in person at the MIND Institute. If the subject is eligible based on the responses to the pre-screening form they will be given the option of scheduling a screening visit to complete screening assessments and confirm if language level and overall functioning are adequate to participate in the trial.

Inclusion Criteria:

1. Documentation of a full mutation with absence or deficient FMRP levels.
2. Males and females ages 10 through 17 years (Consent Forms A and B).

   Note:

   a. Pilot participants who are recruited for a one-time session using Consent Form C, will be between 6 and 23years of age with a diagnosis of Fragile X Syndrome or Down Syndrome. This will allow the researchers to obtain pilot participants from an available pool of participants who are visiting Dr. Abbeduto's lab to take part in other studies of language development in FXS.

   b. An additional cohort of three participants will be recruited using Consent Form D. These participants will be between the ages of 5 and 8 years old which will constitute a downward extension of the current project, allowing us to test the feasibility of PILI in a younger population. Participants using Consent Form D will not participate in the clinical trial combining the behavioral intervention plus lovastatin.
3. Willingness of potential study participant as well as a parent or caretaker to participate in the protocol.
4. Speech is the primary means of communication with three-word or longer utterances used on a daily basis.
5. IQ ≤70 as measured by the Leiter- R.
6. Sexually active women of childbearing potential (WCBP) must be using a medically acceptable method of birth control and have a negative qualitative serum β-human chorionic growth hormone (β-HCG) or urine pregnancy test collected at the initial screening visit. A woman of childbearing potential is defined as a female who is biologically capable of becoming pregnant. A medically acceptable method of birth control includes intrauterine devices in place for at least 3 months, surgical sterilization, or adequate barrier methods (e.g., diaphragm and foam). An oral contraceptive alone is not considered adequate for the purpose of this study. Use of oral contraceptives in combination with another method (e.g., a spermicidal cream) is acceptable. In subjects who are not sexually active, abstinence is an acceptable form of birth control and serum βHCG or urine will be tested.

Exclusion Criteria:

1. Persons who do not speak English.

The following exclusion criteria apply only to the clinical trial phase of the study (Phase 3 corresponding to Consent Form B).

2. Changes in any medications (including investigational medications) within the last month (4 weeks). All concomitant medications must have been on a stable course for at least 4 weeks prior to enrollment into the study and maintain stability throughout the course of the study.
3. Changes in behavioral therapy or educational programming during the study. This does not include scheduled school holidays.
4. Have any disease or condition (medical or surgical) at screening that might compromise the hematologic, cardiovascular, pulmonary, renal, gastrointestinal, or hepatic systems; or other conditions that might interfere with the absorption, distribution, metabolism, or excretion of the investigational product, or would place the subject at increased risk.
5. Patients who, in the opinion of the investigator, are unsuitable in any other way to participate in this study, including being unable to comply with the requirements of the study or displaying clinically significant abnormalities in safety assessments at screening.
6. Patients on prohibited medications (see package insert for more information):
    a. Potent inhibitors of CYP3A4 (itraconazole, ketoconazole, posaconazole, voriconazole, HIV protease inhibitors, boceprevir, telaprevir, erythromycin, clarithromycin, telithromycin, and nefazodone)
    b. Gemfibrozil
    c. Other lipid-lowering drugs
    d. Cyclosporine
    e. Danazol, diltiazem, dronedarone, or verapamil
    f. Amiodarone
    g. Colchicine
    h. Ranolazine
    i. Coumarin anticoagulants
    j. mGluR5 antagonists
7. History of recurrent status epilepticus.
8. Inability to withhold grapefruit and grapefruit juice from diet during the entire clinical trial.

9. Subjects unwilling to abstain from alcoholic beverages during the trial

10. Subjects who are actively suicidal.

6) Number of Subjects

We hope to enroll up to 113 participants through the entire study under the 4 consent form versions. Consent form A will be utilized to enroll up to 30 subjects and their mothers. Consent form B will be utilized to enroll up to 50 subjects and their mothers/caregivers into the clinical trial portion of the study. Up to 30 additional pilot participants may be recruited from ongoing studies here at the MIND using Consent form C. Mothers of the pilot participants enrolled under consent form C will not be involved in the clinician-child interactions. We will also recruit 3 boys with FXS between the ages of 5- and 8-years old to participate in a downward extension of the study utilizing Consent form D. This brings the total number of potential participants to 113.

7) Recruitment Methods

Initially, subjects will be recruited from the Sacramento, California metropolitan area and once local FXS populations have been exhausted, recruitment will extend nationwide to families who are capable of traveling to and from the MIND Institute to complete all necessary assessments. Patients from investigators' practices and those referred will also be eligible to participate, and may be recruited. If a physician believes his or her patient may be a good candidate for this study and the family agrees to be contacted or has approved being contacted for future research opportunities (a component of most consent forms in FXS studies), the physician will notify the study coordinator who will offer the family a flyer and may contact the family to do a prescreening form. If the patient appears to be a good candidate based on the prescreening form, they will be scheduled for a screening visit.

Participants will also be recruited using a short recruitment statement which will be posted on the list serve of the National Fragile X Foundation and other list serves or social media associated with FXS or neurodevelopmental disorders. Recruitment flyers, brochures, and posters may be placed around UCDHS facilities or disseminated at conferences or recruitment events associated with neurodevelopmental disorders.

We are requesting a HIPAA waiver 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 is confirmed, subjects will be approached to obtain their authorization to access and use their health information for the research. Please see Section 14 for additional information regarding Data Management and Confidentiality.

8) Compensation to the Subjects

Subjects will not be compensated for their participation. Possible expenses to the subject's family include time spent at the clinic as well as expenses for travelling. Therefore subjects who would otherwise not be able to participate may be eligible for travel reimbursement for travel costs incurred as specified in question 25.

9) Study Timelines

We anticipate seeing 30 pilot participants in the first 18 months under consent form C. During this same time period up to an additional thirty participants and their mothers will be seen under consent form A, phases 1 and 2 of the study. During Phase 3, 50 participants and their parent/caregiver will be recruited into the clinical trial portion utilizing consent form B. In addition, 3 subjects will be recruited into phase 4, under consent form D. Phases 2,3, and 4 of the study, using consent forms A, B and D, will occur over the last 3.5 years of the project).

10) Study Endpoints

The primary outcome measure is a spoken language composite measured at baseline and at the end of treatment. Comparison of the two treatment groups on the outcome measure will be made through analysis of covariance, adjusted for baseline values on that measure using an intent-to-treat approach. Two-sided tests will be used with alpha at .05. With this sample size, power to detect a moderate effect size of 0.6 will be 80%, assuming equal standard deviations in the groups. The assumptions we are making to estimate power are conservative as evidenced by recent published tests of a parent mediated intervention for autism (Rogers et al., 2012) and a randomized controlled trial of a drug in FXS (Berry-Kravis et al., 2012), with samples sizes of 98 and 60, respectively, and behavioral outcome measures.

Analysis of our secondary outcome measure, the social avoidance subscale of the FXS- normed ABC, as well as the exploratory VAS and CGI will follow the same approach as the primary analysis. Additional analyses will examine potential child moderators of response to lovastatin (age, Leiter, ADOS symptom severity) using linear regression.

Aim 1. Hypothesis 1b. We will use linear regression to test the relationships between parent behaviors and child outcome at 20 weeks (i.e., spoken language composite). The regression will include main effect terms for both treatment and parent behavior as well as the interaction term, while controlling for baseline values of the parent and child measures. Various parent confounders will be

evaluated as well (e.g., SCL-90-R severity score). The main effect of the parent behavior will test the contribution of PILI, whereas the interaction term will test the value of the combined treatment. These analyses will be done separately with two parent behavior measures (parent verbal responsiveness composite and quality of caregiver involvement score).

Analyses will be repeated for secondary outcome measures.

Aim 2. In the addressing hypotheses 2a, b, and c, we will identify biomarkers associated with FXS using a regression model for marker expression with grouping of phenotype (i.e., disease state or respond to treatment) as a predictor with important clinical cofounders as covariates. The marker performance will be evaluated using receiver operating characteristic (ROC) curves to test which biomarkers are statistically better diagnostic and prognostic tests for FXS. We will further develop predictive signatures of elements involved in MEK/ERK and Rho GTPase signaling pathways in response to lovastatin using PAM, or other pattern recognized methods. The potential biomarkers are phosphorylation of S6K1, AKt, EIF4E, and ERK/MEK as well as levels of IL-1, IL-6, IL- 10, MCP-1, IL-1alpha, IL-1beta, MMP-9, and MIF.

Based on the findings of McDuffie et al. (2016, American Journal on Intellectual and Developmental Disabilities) statistical power for this study is expected to be sufficient with a subject enrollment of 50. In the McDuffie study, a treatment effect was demonstrated with the same outcome measure and same age range in a sample size of 10 per group. In this multimodal study, it is expected that the effect of the drug, if it is to be clinically meaningful, will be at last as large as the effect of the language intervention by itself in the 2016 study. In fact, the expectation is to see expect synergistic effects with the language intervention amplifying the effect of the drug.

11) Procedures Involved

Utilizing consent form C we will first recruit up to 30 participants with neurodevelopmental disorders (including Down syndrome, Autism Spectrum Disorder, and fragile X syndrome) who are between 6- and 23-years of age and who are here at the MIND Institute participating in other projects. These participants will serve as pilot subjects to enable the project clinicians who will eventually implement the PILI to practice and optimize the interaction strategies that will ultimately be taught to the mothers'/caregivers of subjects enrolled under consent forms A and B. The pilot participants will be seen for a single visit here at the MIND Institute.

Project clinicians will interact with each of these pilot participants for approximately 20 minutes using one of the wordless picture books that will potentially be used in the following phase of the study. Although the ultimate goal of the project will be to teach the parent to interact directly with the child while the clinician provides coaching to guide the parent-child interaction, these sessions will involve an interaction between the clinician and the child. The goal of these direct interactions between the project clinician and the 30 pilot participants is to practice the strategies and procedures for the narrative intervention that will be taught to parents in the next

phase of the project. That is, the project clinicians will use these interactions to: (a) determine which wordless picture books are acceptable for use in the project, (b) to refine the story scripts that parents will use to introduce the book content, and (c) to determine how many teaching episodes can be incorporated into the shared book reading activity.

We propose to conduct a study of the efficacy of the adapted PILI program during the first 18 months of the IDDRC funding period. We expect to enroll up to 30 participants and employ a series of single-case designs and PILI vs. treatment-as-usual group comparison design, with the latter group receiving no intervention other than what they are already receiving in the community and will participate only in pre- and post-treatment assessments. The treatment-as-usual participants will have the opportunity to go into the multimodal study, or clinical trial portion of the project, which will serve to maintain the participant's commitment and participation in the absence of treatment. Note that there are many reasons to believe that the adapted PILI program will be efficacious. These include evidence from other language-disordered populations of the utility of narrative as a good context for intervention for this age group and also evidence of the importance of parental verbal responsiveness (as embodied in PILI) to language development throughout childhood and into adolescence for individuals with FXS from longitudinal observational studies. Following the PILI only component, we will open enrollment into the clinical trial utilizing consent form B, and begin randomizing participants to lovastatin or placebo while receiving PILI.

In addition to the above studies, a separate study (ICF D) examining the feasibility of implementing PILI in a younger population of boys with FXS (ages 5- to 8-years of age) and their biological mothers will be conducted. We expect to recruit approximately 3 children whose participation will last approximately 3-6 months. The study implemented for this younger age group will follow similar procedures as described for the older children in consent form A, with the exception that the study materials will be adapted for children who are verbal but who are at the beginning stages of language development. Participation in this study will not require any visits to the MIND Institute; all information will be gathered through questionnaires and interactions via SKYPE.

To summarize, there will be four consent forms: one for the pilot participants who will interact with the project clinicians for the purposes of staff training (consent form C), one in Phases 1 and 2 for the purposes of collecting data on the preliminary efficacy of PILI (consent form A), one for the clinical trial during Phase 3 (consent form B) and one for the 5- 8-year old cohort (consent form D).Patients who do not receive the PILI intervention in Phase 2 will be offered the opportunity to participate in Phase 3. All participants being consented under form D will be consented over the phone. Additionally, because of the young age and developmental level of these participants (consent form D), we will not be conducting assent procedures.

11 (a). Efficacy/Behavioral Assessments

Parent-Implemented Language Intervention (Consent Form Versions A, B, and D): The parent-implemented language intervention will be delivered entirely in the participants' homes through VTC over 20 weeks. Each session will be delivered by an ASHA-certified Speech Language Pathologist (SLP) in collaboration with a Board- Certified Behavior Analyst (BCBA).

At the end of the baseline assessment procedures (described subsequently), the SLP and BCBA will provide an overview of the intervention program. The parent/caregiver will be given a MacBook Pro laptop computer and tablet with preinstalled VTC software (e.g., Skype). Project staff will demonstrate use of VTC software and arrange a time for a practice session once the family returns home. The first VTC intervention session will be scheduled within 5 weeks of baseline. VTC support will be provided by the Administrative Core.

Participants will receive parent education sessions covering strategies to support language development and to reduce challenging behaviors. Each parent education session will focus on (a) introducing or reviewing the rationale, procedures, and examples of parent use of PVR and behavioral strategies and (b) providing the parent/caregiver with reflective feedback and opportunities for joint problem solving during review of videos from previous sessions. Parent education sessions also will include power-point presentations and written handouts. All written, graphic, and video- recorded materials for parent education sessions as well as the recorded parent and parent/child sessions will be accessible to the parent/caregiver and clinician using a cloud-based system (e.g., Dropbox). Parent education sessions will not involve the child.

Following delivery of the parent education sessions, families will participate in one 45-min parent/child coaching session per week. The procedures for the parent education and coaching sessions are adapted from McDuffie et al. (2013). All sessions will be scheduled at the family's convenience and delivered in the home by VTC (e.g., Skype). The parent/child coaching sessions will be embedded within the context of an interactive, shared story-telling activity. Wordless picture books appropriate for 10- to 17-year- olds with IDD will be used during the intervention. During coaching sessions, the SLP will provide real-time guidance to the parent/caregiver to increase use of verbally responsive language input including: (a) models of diverse vocabulary; (b) models of targeted grammatical constructions; (c) intonation prompts and open- ended questions to prompt child communication; and (d) recasts of child utterances to provide contingent feedback in the form of more advanced language that expresses the child's intended meaning. The BCBA will be available during the coaching sessions to assist with the management of challenging behaviors.

Parents/caregivers will also complete an independent homework session each week with their child to provide additional practice in effectively implementing the shared-story book reading activity. Parents/caregivers will record and upload this session to the clinical team using a cloud based system (e.g., Dropbox). The clinical team will watch the session and provide written and oral feedback to the parent/caregiver based

on their observations of the parent/child interaction. Finally, the parent/caregiver and child will participate in one additional book sharing activity during each week which will serve as the context for data collection. Intervention targets will be individualized. Language goals for the child will be selected based on the results of the baseline spoken language sampling procedures. PVR targets for the parent/caregiver will be selected based on results of the baseline shared storytelling and structured interaction procedures. Child challenging behavior goals will be selected based on the results of the baseline Functional Behavior Assessment. The SLP and BCBA will coordinate their planning to ensure that targets and procedures form a consistent and coherent plan. As is customary in PILI, the parent/caregiver will be involved in prioritizing targets.

Child Assessment Measures

The following measures will be used to verify that participants meet screening criteria, verify that random assignment has yielded equivalent groups, and provide variables that can be examined as potential moderators of treatment response. To ensure accurate scoring and for study training purposes some assessments may be audio and/or video recorded at the pre and post visits. Any assessments or study procedures deemed not appropriate for a subject based on their cognitive abilities and behaviors will not be completed with the exception of those required for inclusion/ exclusion criteria and safety assessments.

i. Clinical Evaluation of Language Fundamentals – 4 (CELF-4): ICF A and B

A standardized norm- referenced assessment that provides subtests assessing sentence comprehension, following directions, formulated sentences, and written language (15 min).

ii. Leiter International Performance Scales: ICF A and B

A published, standardized, norm-referenced test designed to measure developmental functioning and IQ for individuals ranging in age from 2 years to adulthood. Minimal verbal directions are used in the administration to allow assessment of cognitive ability independent of language ability. The tasks require matching shapes, completing visual sequences through card placements, and detecting sequences and component parts. The figure ground, form completion, sequential order, and repeated patterns subtests will be administered to determine Brief IQ (45 min).

iii. Comprehensive Assessment of Spoken Language (CASL): ICF A and B

A norm referenced, orally administered standardized test used to measure language comprehension, language expression, and language retrieval in the following linguistic domains: lexical/semantic, syntax, and pragmatics. The test has age-based norms which can be used to detect language impairment (45 min,).

iv. Test for Reception of Grammar – 2 (TROG-2): ICF A and B

A norm referenced, standardized test of grammar comprehension. For each item, the participant points to the one drawing from a choice of four that matches the meaning of a word, phrase, or sentence spoken by the examiner

(10 min).

v. ***Peabody Picture Vocabulary Test – 4 (PPVT-4): ICF A and B***
A norm referenced, standardized test of vocabulary understanding. For each item, the participant points to the one picture out of four that corresponds to a single word spoken by the examiner (10 min).

vi. ***Expressive Vocabulary Test – 2 (EVT-2): ICF A and B***
A norm referenced, standardized test of spoken vocabulary. For each item, the participant names the pictured object (10 min).

vii. ***The Autism Diagnostic Observation Schedule-Second Edition (ADOS-2; Lord et al., 2012): ICF A and B***
The assessment will provide a continuous metric of autism symptom severity for use in characterizing the participant sample and as a potential moderator of treatment response. The ADOS is a gold-standard measure of current symptoms of autism. Total scores will be used to assign a severity score. Scores range from 1 to 10, with higher scores reflecting more severe impairment (45 min)

viii. ***Functional Assessment of Challenging Behavior (FACB): ICF A and B***
A functional behavior assessment (FBA) of child challenging behavior will be used to inform the behavior plan developed by the BCBA. The FBA will have three components: (1) The Functional Assessment Interview Form (FAI; O'Neill et al., 1997), which is a structured interview with the parent/caregiver to learn about the antecedents, consequences and topographies of child challenging behavior; (2) Questions About Behavioral Function (QABF; Matson & Vollmer, 1995): A caregiver-report survey to assess the potential functional value of the challenging behaviors for the individuals; and (3) Brief Experimental Functional Analysis (Wacker et al., 2004). An examiner-implemented procedure in which potential antecedents and consequences are experimentally manipulated to identify the environmental variables controlling the challenging behaviors (45 min).

ix. ***Child Spoken Language: ICF A and B***
The conversation and narration procedures described in Preliminary Studies will be administered at the baseline and post-treatment visits. Alternate versions of conversation and narration materials will be administered at the two visits and counterbalanced.

Neurobehavioral Analysis Core staff blind to hypotheses and participant characteristics will transcribe/code. A spoken language ability composite will be obtained for each assessment time by taking a weighted mean of variables reflecting talkativeness, fluency, intelligibility, vocabulary diversity, and syntactic complexity in combined narrative and conversational samples. This composite will provide a broad-based and robust measure of spoken language ability and serve as the primary outcome measure. Exploratory analyses will evaluate the separate contributions of the variables included in the composite (30 min).

x. The Social Communication Questionnaire (SCQ): ICF D

A widely used informant report checklist that evaluates communication and social skill deficits common to those with symptoms of autism. This brief questionnaire will be completed by the participant's parent/caregiver (10 min).

xi. The Vineland Adaptive Behavior Scales (VABS): ICF D

The VABS is a widely used informant report checklist that will be used to assess four domains: communication; self-care; social skills; and maladaptive behavior. It will be completed by the participant's parent/caregiver (30 min).

xii. Childhood Autism Rating Scale, Second Edition (CARS-2): ICF D

An examiner completed behavioral rating scale used to evaluate symptoms of autism following a direct observation of a child. The parent-child structured interaction will be used as the context which is evaluated for this measure.

xiii. The Aberrant Behavior Checklist (ABC): ICF A, B, and D

A behavior rating scale completed by the parent/caregiver that is frequently used to examine treatment effects on challenging behaviors for individuals with FXS. The scale is comprised of 58-items in the following domains: (1) irritability, agitation, crying; (2) lethargy, social withdrawal; (3) stereotypic behavior (4) hyperactivity, noncompliance; and (5) inappropriate speech (15 min). The Sansone et al. (2011) FXS-normed ABC scoring measures will be used. The social avoidance subscale score will be a secondary clinical endpoint.

xiv. The Repetitive Behavior Scale-Revised (RBS-R; Lam & Aman, 2007): ICF A and B

A conceptually derived and empirically validated informant report scale that assesses the presence of a wide variety of repetitive behaviors frequently associated with autism in the domains of: Ritualistic Sameness, Self Injury, Stereotypic Behaviors, Restricted Interests, and Compulsive Behaviors (10 min).

xv. The Anxiety Depression and Mood Screen (ADAMS): ICF A and B

An informant report questionnaire that serves as a screening instrument to determine anxiety, mood, and depression among individuals with intellectual disability. The respondent is asked to rate the extent to which their child displays target symptoms. (10 min).

Parental Assessment Measures:

i. Kaufman Brief Intelligence Test (K-BIT; Kaufman & Kaufman, 2004): ICF A and B

Assessment will be administered to determine IQ (25 min).

ii. Symptom Checklist-90-R (SCL-90-R: Derogatis, 1993): ICF A, B, and D

This assessment is used to determine the presence of mental health symptoms via the global severity index standard score (10 min).

iii. The Five-Minute Speech Sample (FMSS): ICF D

A procedure designed to measure expressed emotion in the parent's attitude toward their child. The sample is elicited by instructing the parent/caregiver simply to talk about the child for five minutes without interruption from the examiner. The FMSS is audio recorded, transcribed, and coded for dimensions of emotional climate, such as maternal criticism, emotional over-involvement, and warmth. These dimensions have been found to relate to maternal behavior and to predict symptom change in individuals with autism, schizophrenia, and many other conditions (5 min).

iv. Parenting Stress Index (PSI): ICF A, B, and D

A 36-item measure of the degree of stress in the parent- child relationship (10 min).

v. Parenting Sense of Competency Scale (PSOC): ICF A, B, and D

A 16-item measure of parenting satisfaction and perceived self-efficacy (10 min.).

vi. Family Background Questionnaire: ICF A, B, and D

Parent report of family background information, for example parent's educational attainment, parents' occupation, school setting, son's diagnoses, additional family members, child medications, household income. Information from this questionnaire will allow us to describe our sample in detail.

vii. Post Treatment Survey: ICF B

A 32 item parent questionnaire regarding participation in the study intervention.

Parent-Child Assessment Measures:

Parent use of targeted intervention strategies and child spoken language will be assessed during joint book reading within two contexts at both the pre- and post-treatment assessment. The contexts will be 1) at the clinic visit, and 2) in the family home via distance teleconferencing. The parent/caregiver and child will look at a wordless picture book together and the parent/caregiver will be instructed to look at each page of the book and tell the story with his/her child as he/she usually would. A set of two wordless picture books will be used in each sampling context and the books will be randomly assigned to participants and counterbalanced at the pre- and post-assessment time points. The following parent and child variables will be obtained from each context and average to create composite variables. Parent variables: story-related talking, open-ended questions, use of expansions, use of intonation prompts. Child variables include: Mean length of utterances, number of different words, story-related talking, intelligibility, mazes, off-topic utterances, and repetitive utterances. Each sample will last approximately 15 minutes.

i. Parent/Child Narrative Task 1(Clinic): ICF A and B

The parent/caregiver will tell a story with the child using the wordless picture books *Frog Where are You* (version A) or *One Frog Too Many* (version B). The parent/caregiver will be encouraged to look through/familiarize themselves with the book in advance of the language sampling session (15

min, Pre/Post Consent Form Versions A and B).

ii. Parent/Child Narrative Task 2 (Home): ICF A, B, and D

Parent/caregiver will tell a story from a wordless picture book from a selection of books that will not be used during the intervention. The parent/caregiver will be encouraged to look through/familiarize themselves with the book in advance of the language sampling session.).

iii. Parent/child structured interaction – Child: ICF D

The parent will interact with the child during four activities: joint book reading, preparation of a snack, completion of a goal-directed construction activity, and free play. The mother will be instructed to participate and involve the child as much as possible. Sessions will be coded to determine maternal use of responsive verbal interaction strategies, child spontaneous and prompted communication acts, and child challenging behaviors (20 min).

The following are exploratory measures:

i. The Clinical Global Impressions Scale–Severity (CGI-S) and Improvement (CGI-I) (Busner & Targum, 2007): ICF B

These standardized assessment instruments are widely used in drug trials to enable the clinician to utilize history from the parents or caregiver and incorporate it into a clinical rating, first for severity, and then for clinical follow-up. The CGI-S will be used at the pre-treatment assessment to judge symptom severity and the CGI- I will be used at the 3 month and 6 month visits.

ii. The Visual Analogue Scale (VAS): ICF B

The measure will be used to assess parental impressions of progress in two key symptoms: spoken language impairment and social impairment. The distance of the mark from one end is used as the outcome variable for analysis. The VAS will be administered at each visit. The VAS has been effectively used as an outcome measure in other FXS drug trials (Erickson et al., 2011; Paribello et al., 2010; Leigh et al 2013).

11 (b). Medical/Safety Assessments (Consent Form B):

The schedule of procedures is also listed in a table format below:

Assessment	Screening/Baseline Visit 1 (Week 1, Day 0)	Visit 2 (Week 10 ± 3 Days)	Visit 3 (Week 20 ± 3 Days)
Informed Consent	X		
Inclusion/Exclusion Criteria	X		
Medical History	X		
Vital Signs	X	X	X
Physical Exam	X	X	X
Neurological Exam	X	X	X

Concomitant Medications	X	X	X
Adverse Events	X	X	X
Clinical Labs	X	X	X
Biomarkers	X	X	X
Leiter-R (45 min.)	X		
ADOS-2 (45 min.)	X		
CGI-S	X		
CGI-I		X	X
VAS	X	X	X
Language Assessments			
CELF-4 (15 min.)	X		X
CASL (45 min.)	X		X
TROG-2 (10 min.)	X		X
PPVT-4 (10 min.)	X		X
EVT-2 (10 min.)	X		X
FACB (45 min.)	X		
Child Spoken Language (30 min.)	X		X
Parent Narrative Task 1 (15 min.)	X		X
Parent Narrative Task 2 (15 min.)	X		X
Parental Assessments			
K-BIT-2 (maternal) (25 min.)	X		
Parent Questionnaires			
ABC (15 min.)	X	X	X
ADAMS (10 min.)	X	X	X
RBS-R (10 min.)	X	X	X
PSI (10 min.)	X		X
PSOC (10 min.)	X		X
Family Background Questionnaire (10 min.)	X		
SCL-90 (maternal) (10 min.)	X		X
Post Treatment Survey			X
Dispense Medication	X	X	

Randomization to lovastatin or placebo will be conducted by the UCDMC pharmacy using a computer algorithm. IDS will over-encapsulate the active study medications and prepare the placebo capsules prior to dispensing. Equivalence of the groups on these stratification variables, as well as other variables (e.g., child autism symptom severity and maternal IQ), will be tested, with any significant differences accounted for by inclusion of covariates in the primary analyses.

The dose will range from 10 to 40 mg of lovastatin or placebo depending on tolerance of the dose. The dose will start at 10 mg per day and will be increased by 10 mg increments on a weekly basis from study weeks 1 through 4 as listed below. Dosing will occur once per day in the evening. Dose tolerance will be assessed through a weekly phone call or email contact conducted by a study physician during the titration period and a dose decrease can be made if necessary. If a dose decrease is made between weeks 2 and 4 no further dose titrations will occur and subject will be considered on a stable dose throughout the remainder of the titration period and study.

If more than one dose change is needed, the subject will be discontinued from the study. Once a stable dose is reached, subjects will remain at the designated dose through visit 3 and will be contacted approximately every other week by study staff to assess for possible adverse events and verification of proper dosing. Study participants will be dispensed study medication at visits one and two and will discontinue dosing after completing visit 3.

Week 1: 10 mg (Day 1-7)
Week 2: 20 mg (Day 8-14)
Week 3: 30 mg (Day 15-21)
Week 4: 40 mg (Day 22- 28)
Week 5-20: 40 mg or OTD (Day 29- 140)

Our dosage model takes into consideration the biphasic and pleiotropic effects of statins. The biphasic effect can be explained by the higher affinity for the enzymes that catalyze non-sterol product formation than for the cholesterol biosynthesis enzymes. Low doses of statins mainly affect cholesterol synthesis, while high doses affect mevalonate-derivative intermediates, which are essential for the prenylation of proteins and Ras activation (Li, et al., 2007). The pleiotropic effects of statins suggest that the sustained use of statins (more than 2 weeks) causes PTEN up regulation, which inhibits the PIK3/AKT pathway; and that higher acute dose administration of statins will inhibit Ras, which is our targeted effect. (Ludman, et al., 2009).

Adverse events (AE) will be documented along with any changes in study medication dose or use of other medications. Each AE will be scored for severity, frequency, and relatedness to lovastatin. All serious adverse events will be reported to our IRB as required by reporting guidelines. The DSMB will also review the study data and assess for safety on a regular basis.

Safety labs, listed below, will be conducted at all three visits through the UC Davis health system pathology laboratory. These laboratory tests will assess for creatine phosphokinase (CPK) as rare irritation to the muscle have been reported with statin treatment. The liver function studies will be utilized to rule out irritation to the liver and the urinalysis will screen for rhabdomyolysis. The lipid panel will assess that cholesterol and triglycerides remain within the normal range. Due to the processing requirements for the lipid panel, blood draws will be done while subjects are fasting. If there is any sign of myopathy, muscle pain or allergic reaction the medication will be discontinued. Pregnancy testing will only be conducted on female participants of child-bearing potential.

Safety Lab Tests

Chemistry	**Hematology**	**Urinalysis**
Creatine Kinase	Hemoglobin	pH
Potassium	Hematocrit	Color
Chloride	WBC	Clarity
Carbon Dioxide	RBC	Specific Gravity
BUN	MCV	Occult blood
Creatinine	MCH	Urobilinogen
Glucose	MCHC	Ketones

Calcium	RDW	Protein
Protein	MPV	Glucose
Albumin	Platelet Count	Pregnancy spot urine
ALP	Neutrophils	
AST	Lymphocytes	
ALT	Monocytes	
Bilirubin	Eosinophil	
Cholesterol	Basophils	
HDL cholesterol		
Triglycerides		
Serum Pregnancy (βHCG)		

In addition to the 7ml of blood for safety labs, we will collect approximately 30 ml of blood at each visit for biomarker analysis of immune function, MEK/ERK, RhoGTPase, and Ca2+ signaling, and cell activation.

Cytokine and chemokine levels will be measured in plasma using a Luminex based multiplex technology to analyze IL-1, IL-6, IL-10, MCP-1, IL-1alpha, IL-1beta, MMP-9 levels, and MIF. These will be measured using plasma according to the manufacturer's instructions. A control plasma sample will be run on each plate for normalization between plates. The plates will be read on a Bio-Plex 100 (BioRad) and analyzed using Bio-Plex Manager software using a 6-point standard curve. Levels of cytokines and chemokines that fall below the limit of detection (LOD) are assigned a value of LOD/2 to allow for $\log_n$ transformation of raw data. Plasma levels of TGF- beta will be determined by ELISA as previously described (Ashwood et al., 2008).

To determine the effects of lovastatin treatment on TLR4 activation, we will use isolated PBMCs and stimulate the TLR4 receptor with LPS for 48 hr. The supernatants will be collected and analyzed for IL-1, TNF-alpha, IL-6, MMP-9, and MIF using luminex multiplex analysis. Quantification of Molecular Biomarkers: MMP-9 activity will be measured in plasma of participants, which will be obtained from whole blood samples by centrifugation and aliquots will be stored at -80°C until use. Samples will be coded and the code revealed after results have been obtained. MMP activity will be quantified by zymogram analysis. Levels of total and phosphorylated ERK ½ will also be assessed by Luminex. In addition, kinetic analysis of the early phases of ERK activation will be employed to determine the feasibility of using pERK in PMBCs as an indicator for responsiveness to lovastatin treatment. PMBCs will be isolated from CPT tubes, counted, and stimulated by PMA to activate protein kinase C-mediated phosphorylation of ERK. Cells will be fixed at different time points by the addition of methanol, permeabilized and immunostained for phospho- ERK and analyzed in a flow cytometer as described in Weng, et al. (2008).

The mechanistic effects of lovastatin on MEK/ERK signaling and Rho GTPase activation will also be studied. The goal of these studies is to determine: (1) the

effects of lovastatin on Ras GTPase and Rho GTPase (RhoA, Cdc42 and Rac1) activation in antigen presenting cells (APCs); (2) the effects of lovastatin on Ca2+ signaling in APCs; and (3) the relative contribution of cholesterol versus isoprenoid synthesis inhibition to lovastatin effects on these signaling events in APCs. The data obtained in these mechanistic studies will provide insight on therapeutic targets which may be useful in developing more targeted treatments and provide the groundwork for expanding this work into relevant animal models to examine mechanisms by which lovastatin improves neurodevelopmental outcomes. APCs will be isolated from PBMCs collected from children with FXS (where baseline values will be compared to treatment samples) by panning for cells that adhere to a polystyrene tissue culture dish. High content imaging, which enables cell based detection of up to five distinct fluorescent markers simultaneously in a multi-well format plates (up to 96 wells per plate) will be used to determine: 1) specific APCs as identified by cell surface markers (as described in Enstrom et al., 2010); and 2) activation of Ras and Rho GTPases as identified by immunocytochemical localization of these signaling molecules within the cell, with activated versus inactivated forms indicated by plasma membrane versus cytoplasmic localization, respectively (Bar-Sagi and Hall 2000).

Real-time Ca2+ oscillations measured with FLIPR will also be studied. This rapid throughput technique will be used to measure the onset and properties of synchronized Ca2+ oscillations in human monocyte cell cultures at baseline, mid-treatment, at the end of the trial. This method permits simultaneous measurements of intracellular Ca2+ transients in a 96-well format using the FLIPR® Tetra imager rapidly (10Hz) and in real-time as we described recently (Cao et al 2012). Cells are loaded with Fluo-4 and recordings from each of 96-wells are simultaneously acquired for 10 min. We will analyze Ca2+ responses evoked by ATP challenges (1-200μM) in monocytes to correlate whether differential responses are correlated with treatment response as well as cytokine/chemokine profiles.

To determine the relative contribution of cholesterol versus isoprenoid-dependent mechanisms to lovastatin effects on these signaling molecules, we will determine whether selective inhibitors of cholesterol synthesis (zaragozic acid) or isoprenoid synthesis (perillic aid, manumycin A, GGTI-298, FTI-277) phenocopy lovastatin and whether supplementation with mevalonate, cholesterol or isoprenoids (farnesol, geranylgeraniol, farnesyl phyrophosphate or geranylgeranyl pyrophosphate) reverse lovastatin effects at a cellular level as described in Kim et al., 2009. These assays will first be optimized using PBMCs isolated from consenting members of the laboratory before being applied to PBMCs isolated from children enrolled in the clinical trial.

12) Data and Specimen Banking

Retention of Records:

Records and documents pertaining to the conduct of this study and the distribution of IMP, including eCRFs, Informed Consent Forms, laboratory test results, and medication inventory records, must be retained by the Principal Investigator for at least 2 years after completion or termination of the study, or for the length of time

required by relevant national or local health authorities, whichever is longer. After that period of time, the documents may be destroyed, subject to local regulations. Records transferred to another party will be de-identified.

Specimen Banking:

Samples will be stored until the conclusion of the study after which only samples of subject's consenting to further storage of study samples after the study will be stored.

13) Data Management and Confidentiality

Confidentiality:

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. When the investigator or any regulatory agencies no longer require the information (but no sooner) than 5 years, the documents will be securely shredded.

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 the IRB. Enrolled subjects will be made aware that study personnel, and representatives of the IRB will have access to their records. This will be included in the consent form and will also be thoroughly reviewed during the consent 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.

The results and data are de-identified to protect patient confidentiality.

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

During the clinical trial portion of the study the subject will have a medical assessment initially by a study physician to ensure that it is safe for the subject to be enrolled in the study. Weekly telephone calls will take place in the first month to document any side effects and make dose adjustments if necessary
Subsequent calls to monitor subject safety will take place approximately every other week. Visits will include a medical examination with vitals and growth parameters and a review of behavior and side effects. Safety labs will be conducted at all three

visits to ensure the subject is safe to enroll and continue in the study.

We will carefully monitor study participants for any significant worsening of mood, agitation, and impulsivity in our phone calls and in the follow-up medical assessments.

An independent data safety and monitoring board (DSMB) will be established and consist of 2 physicians who are not affiliated with the study but have significant expertise in conducting psychopharmacological interventions in children with FXS and/or autism. In addition, an independent statistician will be a member of the DSMB. The first DSMB will examine un-blinded adverse events and clinical data after six months from first subject enrolled into the clinical trial component to determine if the trial is safe to continue. DSMB meetings will be held annually thereafter until all subjects have completed the study. The investigators and staff will remain blinded throughout the trial unless there is an adverse event requiring unblinding, when medical treatment for a subject would be different based on whether or not they were receiving active study drug.

Any serious adverse events which occur to any subject after being randomized into this study and up until 30 days after the last dose of the study drug will be followed up for outcome and reported to the IRB according to their reporting guidelines. Mild and moderate adverse events considered related to the study medication, but not listed in the consent form will be reported to the IRB according to the reporting guidelines. Serious adverse events will be reviewed by the principal investigator for indications of a change in the overall risk assessment, or need for modification of the consent form.

Written standard operating procedures (SOPs) will be followed to ensure that the clinical trial component is conducted and data are generated, documented (recorded), and reported in compliance with the protocol, GCP, and the applicable regulatory requirements. Quality control (QC) will be applied to each stage of data handling. Monitoring, as defined in ICH GCP, Section 1.8, "The act of overseeing the progress of a clinical trial, and of ensuring that it is conducted, recorded, and reported in accordance with the protocol, SOPs, GCP, and the applicable regulatory requirement(s)" will be conducted throughout the conduct of the study.

The clinical investigator and the institution will permit trial-related monitoring, audits, IRB review, and regulatory inspection (FDA or other regulatory body) by providing direct access to source data/study documents.

15) Withdrawal of Subjects

The investigator has the right to discontinue or withdraw a subject from the study at any time. In addition, subjects have the right to voluntarily discontinue study drug or withdraw from the study at any time for any reason. Reasons for discontinuation of study drug or withdrawal from the study may include, but are not limited to, the following:

- Subject withdrawal of consent at any time
- Any medical condition that the investigator determines may jeopardize the subject's safety if he continues in the study

- Investigator determines it is in the best interest of the subject
- Subject non-compliance

Discontinuation from Study Drug:
Subject must discontinue study drug if they experience any of the following:

- Hematological events
- Subject unable to continue to comply with study requirements

Subjects who discontinue study drug prematurely will be asked to return to the clinic for a study completion/early termination visit and may undergo follow up assessments. The primary reason for premature study drug discontinuation should be documented on the appropriate eCRF.

Withdrawal from Study:
Every effort should be made to obtain information on subjects who withdraw from the study. The primary reason for withdrawal from the study should be documented on the appropriate eCRF. Subjects will not be followed for any reason after consent has been withdrawn.

16) Risks to Subjects

Venipuncture:
The risks of blood collection from a vein include discomfort at the site of the needle stick, possible bruising and swelling around the site of the needle stick, rarely an infection or blot clot, and uncommonly feeling faint or dizzy from the procedure. Due to the type of safety labs being conducted for this study, fasting blood draws are required, which may cause discomfort to the subject.

Privacy:
There may also be risks to subjects' privacy. Study staff at the MIND Institute will store study records and other information in a secure location and will grant access only to those who require it. However, even these safeguards cannot guarantee absolute protection of the data.

Unidentified or unforeseen risks:
The results of the subject's lab tests may be outside the normal range (too high or too low). If this occurs, the study doctor will talk to him or her and may ask to have him or her repeat one or more tests during the study. Since the study medicine is experimental when taken alone or in combination with other medications, it may cause other adverse effects that are currently still unknown. If any new information comes out that might affect the subject's decision to continue with the study, he or she will be informed.

Sometimes people have an allergic reaction to medicines. Allergic reactions may be mild (rash, hives) to severe (an acute or sudden drop in blood pressure to shock levels with loss of consciousness and/or associated with seizures, including the possibility of death).

Lovastatin Risks:
Lovastatin has generally been well tolerated and adverse reactions have usually been

mild and transient. Patients will be advised to report promptly any symptoms that may indicate liver injury, including fatigue, anorexia, right abdominal discomfort, dark urine, or jaundice. The package insert lists all risks associated with the medication and adverse reactions possibly, probably, or definitely related to study medication are listed below:

More Common	Less Common
• Feeling weak • Abdominal pain • Constipation • Diarrhea • Upset stomach • Flatulence • Nausea • Muscle Cramps • Muscle pain • Dizziness • Headache • Rash • Blurred vision • Irritability	• Chest pain • Acid reflux • Dry mouth • Vomiting • Leg pain • Shoulder pain • Joint pain • Insomnia • Burning, prickling, or tingling • Hair loss • Itching • Eye irritation

Lovastatin may elevate creatine phosphokinase and transaminase levels, which may be considered in the differential diagnosis of chest pain. Lovastatin is also less effective in patients with the rare homozygous familial hypercholesterolemia and may raise serum transaminases. Due to possible risks associated with lovastatin, liver enzymes will be checked at all three study visits.

17) Potential Benefits to Subjects

Patients may receive no direct benefit from participation in this study. However, participation will help investigators better understand autism and contribute to the scientific knowledge of FXS as well as potentially lead to improved testing and treatment options.

Half of the participants will receive the Parent-Implemented Language Intervention (PILI) under consent form A, the remaining half of participants will have the option to participate in the clinical trial portion of the study under consent form B, at which point they will be guaranteed to receive the PILI intervention.

All participants will receive the Parent-Implemented Language Intervention (PILI) under the clinical trial portion of the study, consent form B. In addition to PILI, half of the patients will be randomized to take lovastatin and may experience a beneficial response to lovastatin including heightened response to PILI with language improvements and reduction of problematic behaviors of FXS including ADHD,

aggression, and anxiety. All participants under consent form B will also benefit by receiving physical exams, cognitive assessments, and blood tests.

18) Vulnerable Populations

The patient population to be studied is developmentally disabled children with fragile x syndrome. We have done research with developmentally disabled and cognitively impaired individuals of all ages for many years. The consent process for individuals without the cognitive ability to consent will be completed by the parent, caregiver, or legally authorized representative (LAR), and no procedures will be done with any subjects without consent of either subject or parent/caregiver/LAR.

19) Sharing of Results with Subjects

At the conclusion of the study, the possible medical benefit from the study will be reviewed with the subject and his or her parent/guardian. Any laboratory work considered standard of care will be available for patients at their request. Psychological assessment scores may be communicated to the subjects if it does not impact future testing. If a subject has to be unblinded for emergency purposes then the subject will be notified by the principal investigator or study physician. Unblinding will be conducted in accordance with the MIND Institute's SOP# 500-014: Study Subject Unblinding Process. The results of the study may also be published or presented at scientific meetings including peer reviewed scientific journals that will be made publicly available through PubMed Central. In addition, after publication the results will be posted on the MIND Institute website under clinical trials

20) Setting

The MIND Institute Research Clinic has a large waiting room with divided areas for each family, 10 examination rooms, a consult room, a chart/supplies room, a vitals room, and 2 coordinator stations totaling approximately 15,000 square feet. There are one-way mirrors and a state of the art digital video recording systems in each of the 4 observation rooms. It is in the clinical research space that subjects will be seen. We have a research phlebotomy lab adjacent to the patient evaluation rooms, where all blood samples will be drawn for this study. The private family rooms may be utilized for recruitment purposes and the MIND Institute also has many non-patient areas where subject phone contacts and study records can be stored.

The PILI assessments will be completed through secure video teleconferencing so families may complete the PILI in the comfort of their homes.

21) Resources Available

Drs. Hagerman, Leigh, and Angkustsiri will follow patients through their treatment with the study drug, until they have completed the study. Designated qualified staff will conduct cognitive and behavioral assessments as required for the study. Coordinators and research assistants will be responsible for contacting and screening potential patients, scheduling and coordinating all patient appointments and visits, coordinating the processing of all samples, meeting with study monitors, as well as organizing all relevant supplies and data for the study. Transcriptionists will be

responsible for transcribing and coding all language samples for purposes of deriving the primary outcome measures. The number of physicians, coordinators, and raters is sufficient to successfully carry out the protocol to completion in the specified timeline. Study protocol specific training will occur and be documented prior to staff conducting assessments or procedures and all study staff will be approved and designated by the principal investigator.

The UC Davis Medical Center Investigational Drug Services pharmacy will store, over-encapsulate the active study medications, prepare the placebo capsules, and dispense the study drug.

22) Prior Approvals

All research staff will be required to complete CITI training per UCD research requirements and will be adequately trained to perform their allotted tasks as approved by the principal investigator.

23) Provisions to Protect the Privacy Interests of Subjects

Sections 12, 13, and 14 address various ways the privacy interests of subjects are protected.

In addition, subject medical information obtained by this study is confidential. Data generated by this study must be available for inspection by representatives of the USA FDA, national and local health authorities, and the IRB.

With the subject's permission, medical information may be given to his or her personal physician or other appropriate medical personnel responsible for his or her welfare.

Study Records will be maintained on site in non-patient areas accessible only to study staff. The informed consent process and study assessments conducted by study physicians or raters will be carried out in private areas to ensure patient privacy and only approved study staff will have access to subject information.

24) Compensation for Research-Related Injury

If the subject is 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 may be billed to the subject's insurance company just like other medical costs. The University does not normally provide any other form of compensation for injury.

25) 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.

Possible expenses to the subject's family include time spent at the clinic as well as expenses for travelling. Subjects will not be paid just for taking part in this study (i.e., compensated). However, all eligible research subjects, who would not otherwise be able to participate due to the cost of travelling to the MIND Institute (e.g., flights,

gas, lodging), will be reimbursed for actual costs incurred.

Since fragile X syndrome is a rare disorder, we often need to recruit nationwide to meet recruitment goals and more importantly to provide an opportunity for the non-local families to participate in research that is not available near their residence. Each family's travel expenses will vary greatly due to their geographic location.

Costs of travel may include, but are not limited to economy airfare, car mileage, lodging, bridge tolls, parking garage fees, taxis, rental cars 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 typically be mailed 4-10 weeks from submission of paid receipts following the completed study visit.

26) Consent Process

Patients will be recruited and consented at the UC Davis MIND Institute. The consent will conform to HRP-90 and HRP-091.

The permission will be obtained from both parents unless one parent is deceased, unknown, incompetent, or not reasonably available, or when only one parent has legal responsibility for the care and custody of the child. If the child has the cognitive capacity to assent, then they will be assented to participate. Study staff will assess the capacity to consent using the Capacity Assessment Checklist for Informed Consent with Cognitively Impaired Subjects. Parents or LARs whose child turns 18 throughout the course of the study will be consented with the surrogate decision maker form.

All parents or LARs will sign a HIPAA authorization for research.

27) Process to Document Consent in Writing

We will be following “SOP: Informed Consent Process for Research, HRP-090” and “SOP: Written Documentation of Consent, HRP-091.”

28) Drugs or Devices

The investigational product will be dispensed by bottle number by the Investigational Drug Services Pharmacy (IDS). All investigational product supplies must be kept in a secure locked cabinet or location, with access limited to authorized clinical investigation personnel. The contents of each bottle will be blinded using labels that contain a unique bottle number. The randomization schedule will match a subject number to the unique bottle number and an appropriate dose. Upon completion of screening evaluations for each subject, the Investigator or appropriate designee will contact IDS to randomize subject and dispense investigational product. The Pharmacist at IDS will select the correct bottle of investigational product and distribute the bottle to the Investigator or designee. Only IDS will be unblinded as to the contents of each bottle of investigational product.

Parent(s)/guardian(s) will be given detailed written dosing and storage instructions.

All financial interests have been disclosed. No conflicts of interest have been identified for this study. The investigator will act in compliance with the study protocol and FDA regulations as stipulated in 21 CFR 312.

Lovastatin is an FDA-approved medication for hyperlipidemia and has an established adverse events profile explained in the package insert.

We have utilized lovastatin clinically in five children with FXS and seen a response in enhanced language in all of them. For instance, the mother of an 11-year-old old boy with autism and FXS who was started on lovastatin (20mg a day) stated that he "came out of the fog" and he started verbalizing more by utilizing new phrases for the first time and "using more combinations of new words". His eye contact improved, as did his overall behavior. Although his mother stated that he is still "spinning things", she adds that "he is now using pretend play which is new for him". There were no side effects and his cholesterol and other laboratory studies remained normal. These data and the considerable safety and efficacy data for treating cognitive impairments in NF1 (Acosta, 2011) suggest a controlled trial in FXS is warranted.

	Applicable to:		
FDA Regulation	***IND***	***IDE***	***Abbreviated IDE studies***
21 CFR 11	*X*	*X*	
• Electronic Records and Signatures			
21 CFR 54	*X*	*X*	
• Financial Disclosure by Clinical Investigators			
21 CFR 210	*X*		
• Current Good Manufacturing Practice in Manufacturing, Processing, Packing, or Holding of Drugs			
21 CFR 211	*X*		
• Current Good Manufacturing Practice for Finished Pharmaceuticals			
21 CFR 312	*X*		
• Investigational New Drug Application			
21 CFR 812		*X*	*X*
• Investigational Device Exemptions			
21 CFR 820		*X*	
• Quality System Regulation			