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Authors

Ligsay, Andrew
Hagerman, Randi J

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Review

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Review of targeted treatments in fragile X syndrome

Andrew Ligsay[1,2], Randi J Hagerman[1,3,*]

[1] *Medical Investigation of Neurodevelopmental Disorders (MIND) Institute, University of California, Davis Medical Center, Sacramento, CA, USA;*
[2] *University of California, Davis School of Medicine, Sacramento, CA, USA;*
[3] *Department of Pediatrics, University of California, Davis Medical Center, Sacramento, CA, USA.*

Summary **Fragile X syndrome (FXS) is the most common inherited form of intellectual disability, and is the leading single-gene cause of autism spectrum disorders. It is due to a loss of the fragile X mental retardation protein, which leads to molecular, behavioral, and cognitive deficits in these patients. Improvements in our understanding of its pathophysiology have led to the development of numerous targeted treatments in FXS as highlighted by metabotropic glutamate receptor antagonists and gamma-Aminobutyric acid receptor modulators. This review will summarize relevant pre-clinical data and results from clinical trials in human subjects with FXS. It will also highlight upcoming studies and future directions for clinical trials as well.**

Keywords: Fragile X syndrome, targeted treatments, clinical trials

1. Introduction

Fragile X syndrome (FXS) is the most common cause of inherited intellectual disability with prevalence rates estimated at 1:5,000 males and 1:8,000 females (*1*). Its etiology is due to a cytosine-guanine-guanine (CGG) repeat expansion mutation in the *FMR1* gene located on the long arm of the X chromosome. The normal range for individuals is up to 44 CGG repeats, whereas patients with FXS have >200. Above this threshold, the gene becomes methylated and silenced, resulting in significantly reduced or absent levels of the *FMR1* gene product (FMRP). FMRP is a RNA-binding protein that is heavily expressed in neurons (*2,3*), and functions in the stability, localization, and translation of select mRNAs (*4*).

FXS has a unique neuropsychiatric phenotype consisting of activating symptoms such as hyperactivity, anxiety, attention deficits, mood lability, sleep disturbances and increased susceptibility to seizures (*5*). FXS is also the leading single-gene cause of autism spectrum disorder (ASD), which is prevalent in approximately 60% of patients (*6,7*). Many of the genes associated with idiopathic ASD may be regulated by FMRP as well (*8*). Recent studies have shown a positive correlation between FMRP expression and cognition in individuals with FXS (*9-11*). As a whole, female patients are typically less affected (lower incidences of ASD, higher intelligence quotient (IQ) scores, less severe IQ declines in longitudinal studies) compared to males due to compensation of FMRP from their second X chromosome (*12*).

At the cellular level, FMRP plays an important role in the development and maintenance of dendritic spines, and its absence causes the spines to appear long, thin and tortuous with filopodia-like projections (*13-15*). Lack of FMRP has been associated with defects in synaptic pruning, and imbalances between long-term depression (LTD) and long-term potentiation (LTP) (*16*). A study by Pan *et al*. (2010) also showed an increase in turnover of these spines (*17*). These processes lead to abnormalities such as immature synaptic connections, alterations in synaptic plasticity, and impaired memory formation.

Increased understanding of the neurobiology and pathophysiology, coupled with advances in animal models, has paved a way for the development of numerous targeted treatments for FXS. These have

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**Address correspondence to:*
Dr. Randi J Hagerman, Medical Investigation of Neurodevelopmental Disorders (MIND) Institute, University of California, Davis Medical Center, 2825 50th Street, Sacramento, CA 95817, USA.
E-mail: rjhagerman@ucdavis.edu

already rescued cellular and behavioral defects in both the *dfmr* mutant fly and *Fmr1* knock out (KO) mouse. Research also suggests treatment at younger ages is most optimal to improve developmental trajectories for patients (*18*). Once clinical trials show safety and efficacy in adult populations, there is hope that studying outcomes in children will reveal even more positive results. The purpose of this review is to discuss the findings of major randomized clinical trials, as well as highlight upcoming studies in the field of targeted treatments in FXS.

2. Metabotropic glutamate receptor 5 (mGluR) antagonists

Perhaps the most prominent theory regarding the pathophysiology of FXS involves upregulation of mGluR-mediated processes (*19*). Normally, stimulation of mGluRs in dendrites triggers increased local protein synthesis, ultimately resulting in AMPA receptor internalization and slowing of net synaptic maturation through mGluR-mediated LTD. This is important in memory formation and brain development. Concurrently, mGluRs increase FMRP synthesis, which serves as a breaking mechanism that negatively regulates mGluR activity. The mGluR theory of FXS states that lack of FMRP allows mGluR-mediated LTD to become hyperactive, and thus lead to features associated with FXS. This theory has been validated through observed defects at the cellular level and correlating these with behavioral abnormalities across multiple animal models (*4*). Moreover, studies in *Fmr1* KO mice with partial reduction of mGluR expression showed correction of many of these abnormalities further demonstrating mGluRs play a significant role in the pathophysiology of FXS (*20*).

Translating these to human trials has not shown the dramatic benefits seen in the animal models. In an open label, single-dose trial of fenobam in 12 adults with FXS (6 males and 6 females), half of the subjects showed at least 20% improvement in prepulse inhibition (PPI) – a measure of sensory gating – within 1 hour of dosing, and most subjects showed clinical improvement in areas of hyperactivity and anxiety (*21*). It was also well tolerated in these participants and no serious adverse events were reported. However, the company failed financially because of an unrelated trial in ASD and therefore further trials in FXS could not be carried out.

In a double-blind, placebo-controlled crossover trial of AFQ056 (mavoglurant) in 30 adult males with FXS, no significant improvements were observed in the primary or secondary endpoints in the overall population while taking study drug compared to placebo (*22*). However, a small subgroup of participants with fully methylated *FMR1* promoter regions showed significant improvements in stereotypic behavior, hyperactivity, and inappropriate speech as measured by the Aberrant Behavior Checklist-Community Edition (ABC-C) as well as in the overall ABC-C total score. These results encouraged two larger, multinational double-blind, placebo-controlled and parallel group trials of mavoglurant: one in adolescents (Phase III trial) and one in adults (Phase II trial) (*23*). In each study, participants were stratified into partial or full *FMR1* methylation groups and then randomized to placebo or one of three doses of mavoglurant: 25 mg BID, 50 mg BID, or 100 mg BID. Over 170 adults and 130 adolescents were randomized in these studies, but neither showed significant improvement on any test measures regardless of dose or methylation status. However, many families saw improvement in behavior and cognition particularly in follow- up open label continuation of mavoglurant that for some lasted longer than a year. The benefits of the open label study however were not controlled and could not be accurately captured by outcome measures. A multicentered controlled trial of mavoglurant will be studied in children with FXS ages 3 to 6 yo and is planned in combination with parent implemented language intervention (PILI) carried out through skype. It is likely that a younger age combined with an intensive learning program and outcome measures that assess cognition through language will demonstrate improvement with this mGluR5 antagonist.

The mGluR5 antagonist, basimglurant, was studied in two multinational, double-blind placebo-controlled trials. One trial was designed for adolescents and adults ages 14 to 50 years (*24*), and the other was designed for children ages 5 to 13 years (*25*). Subjects in the older study were randomized to placebo, basimglurant 0.5 mg daily, or basimglurant 1.5 mg daily; whereas age-equivalent dosages to match the adult steady state exposure levels was used for the child study (0.5 mg equivalent = 0.2 mg daily in 5-8 yo and 0.3 mg daily in 9-13 yo; 1.5 mg equivalent = 0.6 mg daily in 5-8 yo and 0.9 mg daily in 9-13 yo). In total, 122 adults, 63 adolescents, and 47 children were randomized. Neither study showed significant improvement on primary or secondary test measures in favor of basimglurant. However, post-hoc analysis showed males with low *FMR1* methylation and subjects who were not taking concomitant antipsychotic medication had slightly improved performances on select test measures while taking basimglurant. Again, many families found this medication also beneficial to their children with FXS but the benefit could not be precisely captured on the outcome measures.

Fenobam, mavoglurant, and basimglurant were all generally well tolerated by participants and showed good safety profiles in their respective trials. However, they have failed to show efficacy on designated test measures, with positive results being largely limited to those found in post-hoc analyses. The achievements of

mGluR5 NAMs from preclinical work have not fully translated to successes in human patients yet, and it is likely that the pathophysiology of FXS in humans is more complex than projected by animal models.

3. γ-Aminobutyric acid (GABA) modulators

The GABA system is one of the main inhibitory components of the central nervous system (CNS), and recent evidence shows GABAergic dysfunction in FXS animal models (*26-30*). This is believed to contribute to activating behavioral symptoms described previously, and has been implicated in fragile X-associated cellular abnormalities as well (*31-33*).

The GABA system functions through two major receptor subtypes: a $GABA_A$ ion channel and a metabotropic G-protein coupled $GABA_B$ receptor. $GABA_A$ receptors are reduced in the neocortex, cerebellum, and hippocampus of the *Fmr1* KO mouse with deficits more pronounced at younger ages (*27,34,35*). These receptors consist of multiple subunits, and those containing an extrasynaptic δ-subunit are diminished by 50% in certain areas of the KO mouse brain (*29*). FMRP has been shown to directly bind to mRNA encoding for $GABA_A$ subunits serving as a possible stabilization factor, and reintroduction of FMRP can correct δ-subunit mRNA to normal levels (*34*). Further, PET imaging in human subjects with FXS indicate significant reductions in $GABA_A$ receptor availability throughout the CNS (*36*), which suggests the $GABA_A$ deficit is not simply an artifact of the KO mouse. Trials of $GABA_A$ agonists in animal models have shown positive results by restoring neuronal excitability in the amygdala to normal levels, mitigating anxiety and hyperactive behaviors, and rescuing the audiogenic seizure phenotype (*30,33,34*). Neuroactive steroids, in particular, could be well suited to treat FXS because they potentiate the effects of $GABA_A$ receptors containing the δ-subunit.

Ganaxolone is a synthetic analog of the neuroactive steroid allopregnanolone, and has been previously used in the treatment of epilepsy and post-traumatic stress disorder. It is well tolerated in both pediatric and adult populations, and initial trials in the KO mouse have specifically improved seizures and showed a dose-dependent reduction in stereotypic and repetitive behaviors (*30,34*). Moreover, unlike benzodiazepines which also target GABA receptors, ganaxolone does not show tolerance allowing potential for long-term use (*37*). There is currently a double-blind, placebo-controlled crossover trial of ganaxolone in children with FXS being conducted at the UC Davis MIND Institute (NCT01725152). The study is now closed to enrollment, and safety and efficacy results will be available later this year.

On the other hand, $GABA_B$ receptors have been shown to lower presynaptic glutamate release (*38*); therefore, targeting $GABA_B$ in FXS may both increase the inhibitory effect of the GABAergic system and decrease input from excess mGluR activation. Treatment with racemic baclofen, a GABAB receptor agonist, rescued overactive protein synthesis and AMPA receptor internalization in FXS animal models, and reduced audiogenic seizures and abnormal dendritic spine density as well (*39*). These results spurred a phase II double-blind, placebo-controlled crossover trial with 4-week treatment periods separated by a washout (*40*). Sixty-three subjects with the FXS full mutation were randomized, and the drug was flexibly titrated in each treatment period and continued at optimal dose for 4 weeks total. Multiple behavioral and cognitive assessments were performed throughout each arm of the study, but failed to show significant improvement in the primary endpoint of the study, the ABC-C Irritability Subscale. However, there was significant improvement on the Visual Analogue Scale (VAS) and on the ABC-C_{FX} (*41*) Social Avoidance (SA) subscale ($p = 0.008$). Blinded treatment preference by clinicians ($p = 0.05$) and parents ($p = 0.09$) showed trends in favor of arbaclofen, as well as improvements on the CGI-S ($p = 0.09$) and CGI-I ($p = 0.15$). Furthermore, post hoc analysis revealed subjects that were more socially impaired (as designated by baseline ABC-LSW scores) showed significant improvements in favor for arbaclofen in multiple assessments including the CGI-S ($p = 0.009$), CGI-I ($p = 0.02$), the Vineland Adaptive Behavior Scale (VABS) Socialization Subscale ($p = 0.03$), ABC-C SW subscale ($p = 0.07$), and ABC-C_{FX} SA subscale ($p = 0.04$). Arbaclofen showed no safety issues as well.

Two large 8-week placebo-controlled trials of arbaclofen were subsequently conducted: a flexible dose trial in adolescents and adults (ages 12-50 years; $n = 125$, 119 completed) and a fixed dose (3 doses and placebo groups) trial in children (ages 5-11 years; $n = 172$, 159 completed). The older trial failed to show efficacy over placebo in the primary outcome measure, the ABC-C_{FX} SA) or any secondary measures (*42*). However, the trial in children showed a positive trend on the ABC-C_{FX} in the highest dose group compared to placebo, and found significant improvement in several key secondary outcomes as well. Unfortunately, the development of arbaclofen was been terminated due to financial constraints.

Finally, acamprosate is a GABA agonist that has properties at both $GABA_A$ and $GABA_B$ receptors. It is FDA approved to treat alcohol withdrawal, and showed positive results in an initial open-label trial of 3 adults with FXS on the CGI-I and in areas of communication (*43*). In a subsequent 10-week trial in 12 children with FXS (*44*), nine subjects met criteria for treatment response (CGI-I score of 1 or 2 and a ≥ 30% improvement on the ABC-SW). There was also significant improvement relative to baseline in the ABC-Hyperactivity subscale ($p = 0.04$), Social

Responsiveness Scale (SRS, $p = 0.005$), the ADHD-Rating Scale ($p < 0.0001$), and in the communication domain on the VABS ($p = 0.03$). Acamprosate was also found to be safe in these participants. The study also looked at levels of soluble amyloid precursor protein (sAPP) and sAPPalpha in blood samples (*45*) as these are known to be elevated in ASD (*46-48*). Treatment with acamprosate was able to normalize elevated levels of sAPP and sAPPalpha in these patients (*45*), and the authors suggest sAPP and sAPPalpha may be viable biomarkers to assess treatment response in future studies. Acamprosate is currently being tested to determine whether effects on hyperactivity and social functioning observed in the open-label studies can be verified in a small placebo-controlled trial in FXS (NCT01911455).

4. Minocycline

In addition to its involvement in both mGluR and GABA pathways, FMRP negatively regulates the translation of the protein matrix metalloproteinase 9 (MMP-9), with lack of FMRP leading to elevated MMP-9 activity in FXS (*49*). This dysregulation has also been associated with immature dendritic spine morphology (*50,51*). However, novel research in *Fmr1*/*Mmp*-9 double KO mice revealed dendritic spines were similar to those in wild-type (wt) mice (*52*) suggesting MMP-9 is integral to the pathophysiology of FXS-associated defects at the neuronal level.

Minocycline is an antibiotic of the tetracycline class typically used to treat acne, but is known to decrease MMP-9 activity as well. Preclinical trials of minocycline in the *Fmr1* KO mouse have induced mature dendritic spine morphology and improved anxiety and cognitive measures within one month of use (*50,51*). However, while positive results in behavior have been seen in both young and adult *Fmr1* KO mice, research suggests younger mice have longer-lasting benefits, whereas improvements in adult mice disappear soon after cessation of treatment (*53*).

Initial open label studies of minocycline in both pediatric and adult populations showed positive results in areas of language, attention, anxiety, hyperactivity, and overall improvement (*54,55*). These data spurred a 6-month double-blind, placebo-controlled crossover trial in children with FXS (*56*). Sixty-six participants were randomized in the study with 55 children completing at least one arm and 48 finishing both arms of the study. Significant improvements were observed in one of the primary outcome measures, the Clinical Global Impression-Improvement scale (CGI-I), as well as in areas of mood and anxiety on the VAS. Overall, minocycline was safe and well-tolerated as most reported AEs were mild in nature. No significant differences in AEs were found between treatment and placebo groups, although one patient experienced a seizure while on placebo. Continued research should be conducted to confirm its safety profile because long-term treatment with minocycline may darken the skin, gums, and dentition of permanent teeth. Minocycline has also been associated with a rare lupus-like syndrome, and thus an antinuclear antibody (ANA) titer should be checked within the first 6 months of treatment and subsequently annually if stable or more frequently if elevated. Severe chronic headache, rash, or swollen joints should necessitate immediate cessation of treatment. Occasionally, loose stools can occur with minocycline treatment, so use of a probiotic daily while on minocycline will be beneficial to replenish normal flora in the intestine. Also, minocycline should not be taken at the same time as milk because they interfere with absorption when given together. One should wait at least 30 minutes before taking milk after the minocycline dose.

A subgroup of these study patients treated with minocycline (8 males and 4 females) also underwent an event related potential (ERP) study (*57*). Patients with FXS have exaggerated EEG amplitudes to auditory stimuli and lack a habituation response after repeated stimulation (*58-61*). In this trial, treatment with minocycline showed statistically significant reductions in temporal activation due to auditory stimuli, as well as improvements in habituation (*57*). There was also a significantly increased ERP response in the central P2 component, which correlated with improvements on the CGI-I. Despite the low sample size, this data suggests ERP measures may be a possible objective measure to detect treatment response in the FXS population. Additional studies are needed to evaluate ERP paradigms as an outcome measure that correlates with clinical improvement. In addition, MMP-9 levels in FXS are elevated in blood samples and minocycline lowered these levels in the minocycline trial (*50*). This measure also appears to be a good biomarker in FXS that can be used to monitor treatment response in future trials of minocycline and perhaps in the use of other targeted treatments for FXS.

5. Selective Serotonin Reuptake Inhibitors (SSRIs)

Serotonin dysfunction has been linked to some behaviors associated with FXS and ASD. A study in patients with FXS found that those with a 5-HT transporter polymorphism causing hyperactive serotonin reuptake receptors – and thus lower synaptic serotonin availability – had increased aggression and destructive behaviors (*62*). Metabolomic and PET imaging studies also demonstrate decreased serotonin production in patients with ASD as well (*63,64*). These deficits appear to be more pronounced in young children (< 5 years) (*63*), and are especially deficient in the frontal lobe in children with ASD, which has been associated with language impairment (*65*). Targeted

treatments toward serotonin not only provide a potential avenue to correct these behaviors in FXS, but serotonin also increases LTP, which could enhance learning and cognitive function (*66,67*). SSRIs, in particular, provide additional benefit as they stimulate Brain-Derived Neurotrophic Factor (BDNF) production (*68*), which could help ameliorate cellular abnormalities in the FXS brain.

An initial retrospective study of young children (12 to 50 months) with FXS who were treated with low-dose sertraline (2.5 to 5 mg/day) revealed significant improvement in the developmental trajectory of expressive and receptive language as measured by the Mullen Scales of Early Learning (MSEL) compared to those not treated with sertraline (*18*). These results spurred a 6-month randomized, placebo-controlled trial of low-dose sertraline (2.5 to 5.0 mg/day) in children ages 2-6 years with FXS (Hess *et al*, unpublished data). Fifty-seven participants were randomized in the study: 27 to sertraline and 30 to placebo. Fifty-two participants completed the study. Primary outcome measures including the CGI-I and the receptive and expressive language scales on the MSEL were not significantly improved compared to placebo; however, areas of fine motor, visual perception, and the Cognitive T score sum did show improvement. A subset of patients with ASD also demonstrated significant improvements in expressive language on the MSEL when treated with sertraline. The dosages used in this study were safe and well-tolerated, and all families opted to continue sertraline after the conclusion of the study.

This trial of low-dose sertraline in young children with FXS revealed significant benefits in areas of cognition and behavior, and was especially notable due to sertraline's positive effect on language. Additional trials should be conducted to replicate these results, and there is also a trial of sertraline in children with idiopathic ASD being conducted at the University of California, Davis MIND Institute (NCT02385799).

6. Lovastatin

Lovastatin is an HMG-CoA reductase inhibitor used in the treatment of hyperlipidemia and hypercholesterolemia. It has a well-known safety profile, and has been approved by the United States Food and Drug Administration to treat familial hypercholesterolemia in children as young as 10 years (*69*). Preclinical studies have also shown lovastatin inhibits RAS-MAPK-ERK1/2 activation (*70*), and treatment with lovastatin significantly improved cognitive deficits in the neurofibromatosis type 1 (NF1) mouse model through inhibition of these pathways (*71*).

Many of the proteins upregulated in FXS are believed to be downstream consequences of increased ERK1/2 activity (*72*), and trials of lovastatin in the *Fmr1* KO mouse have shown numerous benefits such as decreasing extracellular receptor kinase-mediated protein synthesis, correcting exaggerated mGluR-mediated LTD, blocking mGluR5-mediated epileptiform bursting hippocampal neurons, dampening hyperexcitability in the visual cortex, and reducing the incidence and severity of seizures (*73,74*). Recently, a 12-week open label trial of lovastatin in patients with FXS was completed (*75*); sixteen participants were initially enrolled (mean age = 18 ± 5 years), and 15 subjects finished the study. Results showed significant benefit on the ABC-C after 4 weeks of treatment, and further improvements were observed with continued use throughout the study specifically in areas of hyperactivity, lethargy, social avoidance, and stereotypy as determined by the ABC-C_{FX} (*41*). Participants also experienced significant improvements in communication, daily living skills, and coping skills on the VABS. Lovastatin was well-tolerated in these participants, and all AEs were transient and mild in intensity.

Lovastatin appears to be a promising therapy for patients with FXS, and future studies should continue to assess its effects. There are two clinical trials currently underway: one is a phase 4 trial looking at combined lovastatin and parent-implemented language intervention (PILI; NCT02642653) and another is a phase II trial evaluating combined minocycline and lovastatin (NCT02680379).

7. Additional trials

An open label trial of lithium in 15 patients with FXS demonstrated significant benefits in several behavioral measures and perhaps most importantly lithium treatment lead to normalization of ERK phosphorylation rates which is a quantitative measure of biological changes in the targeted pathway with lithium (*76*). Further controlled trials are warranted for lithium, although kidney toxicity after long term use is worrisome for pediatric patients with FXS, which may require prolonged treatment with lithium.

A small randomized, double-blind, placebo-controlled, single-dose trial of intranasal oxytocin was recently completed in adolescent and adult males with FXS (*77*). Oxytocin acts as both a hormone and neuropeptide, and has been shown to have anxiolytic and pro-social qualities. Eight subjects completed the study, and oxytocin was found to ameliorate behaviors such as anxiety and hyperarousal as suggested through improvements in eye gaze frequency and decreases in salivary cortisol levels. Additional studies are being conducted, although predominantly in ASD populations.

Another promising medication in FXS is trofinetide, a synthetic analogue of the terminal tripeptide tail of Insulin Growth Factor-1 (IGF-1) made by Neuren. Trofinetide demonstrated promising results in the KO mouse with improvement in behavior and also normalization of ERK and Akt levels (*78*). This led to a multicenter controlled trial in adolescents and adults with

Table 1. Overview of Clinical Trials in fragile X syndrome

Clinical Trial Registration Number	Compound (Drug Class)	Clinical Trial Phase	Target Population	Principal Investigator	Status/Results
Metabotropic glutamate receptor 5 (mGluR5) antagonists					
NCT01357239/ NCT01253629	Mavoglurant (AFQ056)	Phase IIb/Phase IIb	Adolescents/Adults	Novartis Pharmaceuticals	Completed (*23*)
NCT00718341	Mavoglurant (AFQ056)	Phase IIa	Adults	Novartis Pharmaceuticals	Completed (*22*)
NCT01517698/ NCT01750957	Basimglurant (RO4917523)	Phase IIb (adult/ adolescents), Phase IIa (children)	Adults and adolescent/Children	Hoffmann-La Roche	Completed (*24,25*)
γ-aminobutyric acid (GABA) modulators					
NCT01282268/ NCT00788073	Arbaclofen ($GABA_B$ agonist)	Phase III	Adults and adolescents/Children	Seaside Therapeutics	Completed (*81*)
NCT01013480	Arbaclofen ($GABA_B$ agonist)	Phase II	Adults, adolescents and children	E. Berry-Kravis, MD, PhD	Completed (*40*)
NCT01911455	Acamprosate (GABA agonist)	Phase II	Adults, adolescents and children	E. Berry-Kravis, MD, PhD & C. Erickson, MD	Recruiting
NCT01725152	Ganaxolone ($GABA_A$ agonist)	Phase II	Adolescents and children	R. Hagerman, MD	Completed/Pending
NCT02126995	Metadoxine (ion-pair salt of pyridoxine, GABA activator)	Phase II	Adults and adolescents	E. Berry-Kravis, MD, PhD	Completed (80)
Minocycline					
NCT01053156	Minocycline (Tetracycline)	Phase II	Adolescents and children	R. Hagerman, MD	Completed (*56-57*)
Selective Serotonin Reuptake Inhibitors (SSRI)					
NCT01474746	Sertraline	Phase II	Children	R. Hagerman, MD	Completed (Hess *et al*, unpublished data)
Additional Trials					
NCT00054730	CX516 (Ampakine)	Phase II	Adults	E. Berry-Kravis, MD, PhD	Completed (*82*)
NCT01894958	Trofinetide (NNZ-2566; neurotrophic peptide)	Phase II	Adult and adolescent males	E. Berry-Kravis, MD, PhD	Completed/Pending
NCT01254045	Oxytocin (neuropeptide)	Phase II	Adults and adolescent	A. Reiss, MD	Completed (*77*)
NCT01329770	Ascorbic acid and α-tocopherol	Phase II	Adolescents and children	Y. de Diego-Otero, PhD & L. Pérez Costillas, MD, PhD	Completed (*83*)
NCT01120626	Donepezil (cholinergic drug)	Phase II	Adults and adolescents	A. Reiss, MD	Completed (*84*)
Combined Trials					
NCT02642653	Combined Lovastatin (HMG-CoA reductase inhibitor) and PILI	Phase IV	Children	R. Hagerman, MD	Open for recruitment
NCT02680379	Combined Minocycline (Tetracycline) and Lovastatin (HMG-CoA reductase inhibitor)	Phase II	Adults and adolescents	F. Corbin, MD, PhD	Not yet open for Recruitment

HMG-CoA = 3-hydroxy-3-methylglutaryl-coenzyme A, PILI = Parent-Implemented Language Intervention.

FXS. Although the initial study has not been published at this time, preliminary results look promising (*79*), and additional studies are planned for the future.

Metadoxine, a combination of vitamin B6 and a 2-pyrrolidone-5-carboxylate ring, is a medication developed for alcohol toxicity with GABA agonist effects. Metadoxine has been beneficial in the KO mouse by lowering ERK and Akt and has been studied in adolescents and adults with FXS in a multicenter controlled trial (*80*). The preliminary results were

promising so it is likely to be further assessed in children with FXS (Table 1).

8. Conclusions and future directions

Although significant improvements outside of subgroup and *post hoc* analyses have yet to be reproduced, these clinical trials highlight many salient points as we work toward future trials in FXS. While the outcomes measures utilized in the aforementioned studies have been validated in multiple populations, our toolbox of assessments is largely limited to questionnaires, which are often subjective. Moreover, patients with FXS may bottom out on rating scales depending on the severity of their phenotype. There has already been progress in developing questionnaires specifically graded to monitor changes in patients with FXS (*41*), and there is a movement toward objective measures to monitor treatment response such as studying ERPs as was carried out in the minocycline trial (*57*) or MMP-9 levels that were lowered in the minocycline trial (*50*). Upcoming trials are also studying combination therapy, as this may be another avenue toward unveiling beneficial effects of targeted treatments as well (NCT02642653, NCT02680379). Nevertheless, the positive results found in previous studies should not be diminished, but rather should serve as a sign of progress toward a better understanding of the pathology and clinical treatment of patients with FXS.

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