Review Article

Fragile X Syndrome and Targeted Treatments

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Abstract

Many targeted treatment studies have been carried out in individuals with Fragile X Syndrome (FXS) guided by animal studies from the Fragile X Mental Retardation 1 (FMR1) knock out (KO) mice and the fragile X Drosophila studies. Here we review the many medications that have been studied in patients with FXS and some of these medications are available for clinical use by wise clinicians. Other medications are not currently available by prescription because they are not approved by the FDA. No medication has received specific approval for treatment of FXS, although some have shown benefit from clinical studies. There is much to be done in the treatment of those with FXS and this report describes those pharmacological treatments that target the neurobiological mechanisms that are dysregulated by the lack of the FMR1 Protein (FMRP) in those with FXS.

Keywords: Fragile X syndrome; FMR1; targeted treatment

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INTRODUCTION

Fragile X syndrome (FXS) is the most common single gene disorder which causes intellectual disability and autism spectrum disorder (ASD). The estimated prevalence in the general population is 1: 3,600 to 4,000 in males and 1:4,000 to 6,000 in females. Expansion of the cytosine-guanine-guanine triplet with more than 200 repeats on the fragile X mental retardation 1 (FMR1) gene, called “full mutation” silences transcription leading to the inability to produce the FMR1 protein (FMRP).

FMRP is expressed in various tissues and most prominently in the CNS and it is a key promoter of synaptic plasticity. FMRP regulates mRNA translation of hundreds of genes usually with inhibition. Therefore, deficient FMRP expression usually leads to enhanced protein production which includes upregulation of the excitatory metabotropic glutamate receptor 5 (mGlur5) pathway causing long term depression (LTD) of synaptic connections, α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) receptor internalization, increasing eukaryotic translation initiation factor 4E (eIF4E) phosphorylation, enhanced activity of extracellular signal-regulated kinase (ERK), and elevated matrix metalloproteinase-9 (MMP-9) levels. In addition, the lack of FMRP leads to deficits in inhibitory gamma-aminobutyric acid (GABA) signaling as well as dopamine and cholinergic dysregulation. The endocannabinoid system, which promotes synaptic plasticity, is also dysregulated in FMRP deficient conditions as in FXS. Imbalance of these mechanisms causes increasing neuronal hyperexcitability, dysregulation of dendritic spine maturation, and disrupted synaptic connections leading to intellectual disability and the clinical phenotypes of FXS (i.e., over reactivity to stimuli, decrease ability in habituation, seizure, hyperactivity, anxiety, and cognitive deficit). Many research studies have been proposed to modify these dysfunctional pathways with the aim to find pharmacological treatments targeting the aberrant mechanisms in FXS. This review describes recent pharmacological studies of targeted treatments focused on reversing the neurobiological abnormalities of FXS.

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Metformin

Metformin is an antihyperglycemic agent which is approved by the Food and Drug Administration (FDA) for the treatment of non-insulin dependent diabetes mellitus. It has also been the treatment of choice in children and adults with obesity and insulin resistance. Metformin distributes across the blood-brain barrier and the main mechanism of action of metformin in FXS is the normalization of the mammalian target of rapamycin (mTOR) and mitogen-activated protein kinase/extracellular signal-regulated kinase (MAPK/ERK) pathways. Both pathways regulate cellular function particularly in the central nervous system (CNS) and both are abnormally hyperactivated by the FMRP deficient condition. Metformin lowers the level of blood glucose by enhancing peripheral glucose uptake and glycolysis while inhibiting hepatic gluconeogenesis and gastrointestinal glucose absorption. The reduction of blood glucose causes a decrease of insulin signaling and consequently inhibits the mechanistic target of rapamycin complex 1 (mTORC1) and MAPK/ERK pathways. Moreover, metformin also inhibits the mTORC1 and ERK pathways via activation of AMP-activated protein kinase complex (AMPK). MMP-9 which is upregulated in conditions of FMRP deficiency causes a negative impact on synaptic formation in FXS. Metformin also normalizes messenger RNA (mRNA) encoding of MMP-9 via reduction of eIF4E phosphorylation and mRNA translation. Furthermore, metformin indirectly acts on insulin, the risk of experiencing hypoglycemia is low in individuals with normal renal function and without taking other hypoglycemic agents.

The efficacy of metformin was proved in Fmr1-knocked out (KO) animal models by showing improvement in abnormal behaviors. Problematic behaviors like hyperphagia and stereotypy have also shown improvements in patients with FXS treated clinically with metformin. A randomized-controlled trial of metformin showed benefits in weight control in children and adolescents with ASD experiencing atypical antipsychotic-induced weight gain above normal body mass index. However, the effects of metformin on cognition in children with ASD needs more study.

Medical treatment with metformin in seven children aged 4.5-60 years old with FXS produced behavioral improvements following the intake of metformin clinically for 6-12 months. These improvements included decreasing in irritability, hyperactivity, and social avoidance as well as improvement in social responsiveness which were measured by using Aberrant Behavior Checklist (ABC). While individuals with FXS tend to have cognitive decline overtime especially in the verbal communication domain, improvements in language especially conversational skills is a welcome change. Two additional males with FXS (age 25 and 30 years old) treated clinically for over one year showed improvement in Stanford-Binet Intelligence Scale-5 which included visuospatial processing, working memory, numeracy skill, and quantitative reasoning after taking 1000 mg of metformin once or twice a day. Their parents also anecdotaly reported improvements in language, social communication, responsiveness as well as decreasing irritability and anxiety.

Metformin stimulates neurogenesis in animal models. Normalizing the excess protein production in the CNS in FXS with metformin leads one to predict that young children may demonstrate the greatest benefits from metformin treatment. A case series report of 9 children between 2-7 years old has recently illustrated benefits of metformin in both developmental and behavioral domains. Metformin was started at 25-50 mg at dinner and then titrated up gradually with age. The youngest children were 2 years old and the lowest stable dose of metformin was 200 mg twice daily (31.01 mg/kg/day). Aberrant Behavior Checklist-Community (ABC-C) scores were improved especially in lethargy and stereotypy. Their Mullen Scales of Early Learning (MSEL) score were increased in all domains. Some parents noted that their child’s communication, problem-solving abilities, and daily living skills were better in addition to decreased tantrums and aggressive behaviors. The only common side effect was loose stools which was self-limited. However, one child developed seizures during the metformin treatment, whereas another child demonstrated improvement in seizures that were already present before metformin treatment was started.

Besides cognitive and behavioral aspects which have been considerably improved in some cases, metformin was also reported to prevent the development of macroorchidism at puberty in an adolescent male with FXS who started taking metformin at age 12 before puberty. Metformin might alleviate over production of proteins promoting testicular growth. Moreover, metformin is specifically considered beneficial in FXS with the Prader-Willi-Phenotype, a subgroup of individuals with FXS who have severe obesity together with hyperphagia and a lack of satiation after meals.

Currently, a multi-site randomized controlled trial is being conducted which aims to assess efficacy of metformin in improving language, cognition and behavior in patients between 6 to 25 years old with FXS (NCT03479476). Two additional studies also seek to assess the safety and tolerability of metformin and its effect in behavior (NCT03722290, NCT04141163) (see table 1).

Minocycline

Minocycline is a semi-synthetic tetracycline derivative commonly used to treat acne. It has been of interest in the FXS treatment field since animal studies showed it to have an inhibitory effect on the activity of MMP-9. FMRP deficiency leads to elevated MMP-9 activity which has a negative impact in synaptic physiology and plasticity. Treatment of Fmr1-KO mice with minocycline normalized MMP-9 levels in brain tissues and led to improvement in multiple phenotypes of FXS, especially in behavior and cognition domains.

In an experimental study of chronic minocycline treatment in Fmr1-KO mice, Yau et al. showed an improvement in hippocampal synaptic structure and N-methyl-D-aspartate (NMDA) receptor function. These findings may explain part of the beneficial cognitive effects of minocycline in FXS. Toledo et al (2019). studied the effect of minocycline treatment in Fmr1-KO
mice on ultrasonic vocalization deficits during mating, which is an indicator of abnormal social communication. They found that minocycline reversed ultrasonic vocalization deficits through attenuation of MMP-9 levels, even when treated past the early developmental period. Minocycline has also been shown to be beneficial in studies involving patients with FXS. Leigh and colleagues (2013) published a randomized double-blind, placebo-controlled crossover trial of minocycline in children and adolescents with FXS. Study participants were given minocycline for 3 months and placebo for 3 months. Forty-eight of their subjects completed the full trial. They found improvements in the Clinical Global Impression-Improvement (CGI-I) scale and anxiety and mood-related behaviors in the Visual Analog Scale.

Although minocycline is available clinically, its use in patients with FXS requires safety monitoring. In a survey of clinical response to minocycline in 50 patients with FXS performed by Utari et al. (2010), the most common side effect reported was gastrointestinal issues such as loss of appetite, gastrointestinal discomfort and diarrhea. Fewer frequent side effects reported were worsening hyperactivity and moodiness, and one patient had darkening of nails. Furthermore, an open-label study by Paribello et al. involving 19 patients with FXS documented dizziness, diarrhea and seroconversion to a positive antinuclear antibody (ANA). Therefore, minocycline is well tolerated but requires clinical monitoring and testing for ANA and liver function.

Sertraline
Sertraline is a selective serotonin reuptake inhibitor (SSRI) approved for the treatment of anxiety and mood disorders in children age 6-17 years old. Compared to other SSRIs, sertraline has less activation side effects and drug-drug interactions and has been prescribed for treatment of anxiety, irritability, and social deficit in FXS.

Serotonin in the cortex usually peaks during the first five years of life when synaptogenesis is also highest. However, this serotonin trajectory and brain morphology in children with ASD was found to be disrupted. Serotonin dysregulation is also an abnormal neurochemical pathway in FXS. Serotonin and the brain-derived neurotrophic factor (BDNF) interact reciprocally to regulate neuroplasticity. Treatment with an SSRI not only normalizes serotonin levels, but also stimulates BDNF which is also dysregulated in FXS. In the Fmr1-KO mouse model, serotonin also restored glutamate A1-dependent long-term potentiation (LTP) and attenuated mGluR5-mediated synaptic LTD. Moreover, dopamine is found impaired in Fmr1-KO mice. Sertraline is an SSRI which inhibits dopamine reuptake, therefore, leading to an increased dopamine concentration at the nucleus accumbent and the striatum in rats. Normalization of dopamine may help maintain normal LTP and dendritic morphology in FXS. These mechanisms ultimately stabilize neural functions and synapse formation. Importantly, treatment with an SSRI in young children with FXS could maximize benefits since this is the golden period of neuroplasticity.

Benefits of sertraline have been confirmed in individuals with FXS. A retrospective chart review compared 11 children with FXS who took sertraline with 34 children with FXS who did not take sertraline. They were between 12-50 months old and the earliest age when sertraline was prescribed was 18 months old. The indication for sertraline prescription was to treat anxiety and social deficits and the average dose was 5.8±2.5 mg/day. Children who took sertraline had higher language scores measured by using the Mullen Scales for Early Learning (MSEL) compared to the control group at follow-up. Decreased anxiety, irritability, and social deficits were also observed.

A randomized-controlled trial was conducted in 52 young children with FXS who took low dose of sertraline (2.5 mg/day for children age 2-3 years old and 5 mg/day for children age 4-6 years old) for 6 months. The results showed significant improvements in visual reception, fine motor skills, and MSEL summary age-equivalent scores in addition to a measure of social perception. In children with concomitant FXS and ASD, their expressive language development on the MSEL was significantly improved on post hoc analysis. Some minor side effects commonly seen were upper respiratory tract infections and gastrointestinal issues, but these did not differ from the placebo group. Although the study was completed, parents preferred to continue sertraline because of desired developmental outcomes. Long-term monitoring of adverse events is necessary and measuring efficacy of combined sertraline with language intervention should be studied.

Cannabidiol
The term ‘cannabinoïd’ is used to refer to metabolites derived from Cannabis sativa and synthetic compounds that act on cannabinoid receptors. Cannabidiol (CBD) is a phytocannabinoid that has been studied due to its pharmacological potential. In contrast to delta-9-tetrahydrocannabinol (THC), CBD is not associated with psychomimetic properties. CBD has several mechanisms of action and therefore multiple potential therapeutic effects. Several studies have found possible beneficial pharmacological effects of CBD in different disorders such as epilepsy, anxiety, neurodegenerative diseases such as Alzheimer’s disease, and autoimmune diseases such as rheumatoid arthritis, among others.

In the mouse model of FXS, it has been shown that the endocannabinoid system is linked to the biological actions of MFRP and is therefore dysregulated when there is absence or deficiency of this protein. Preclinical models of FXS have shown loss of endocannabinoid signaling and many of the abnormalities described in FXS such as social and cognitive impairment, seem to be associated to the dysregulation of the endocannabinoid pathways in the CNS. The loss of endocannabinoid signaling in the FXS model is in part due to reduced production of 2-arachidonylethanolamine (AEA) and increased catabolic hydroxylation of N-arachidonylethanolamine (AEA). CBD has shown to increase both AEA and 2-AG availability improving one of the biological mechanisms of FXS.

There are other mechanisms that have been involved in the potential benefits of CBD in FXS. Altered synaptic function and structure has been established as one of the major mechanisms of FXS. It has been proposed that
CBD may increase synaptic plasticity in FXS which may be associated with an improvement in learning and cognition domains. Additionally, alterations in the GABAergic system have also been implicated in FXS pathogenesis and pharmacological treatment with agonists of the GABA receptor have been shown to improve several behavioral deficits in the FXS mouse model. CBD may also improve the GABAergic dysfunction since it acts as a positive allosteric modulator of GABAA receptors.

Finally, CBD exerts its anxiolytic effects by its interaction with the serotonin system. Studies have identified serotonin-1A receptor (5-HT1A) as one of the targets through which CBD aids in the reduction of social anxiety experienced by patients with FXS.

Side effects of CBD appear to be minimal and it is generally well tolerated. In 2011, Bergamaschi et al. performed a systematic review on the safety and side effects of CBD and found that chronic use and high doses of CBD were reportedly well tolerated in humans. Some patients may experience transitory, dose-dependent mild to moderate effects including somnolence, decreased appetite and gastrointestinal disturbances. Studies in patients with treatment-resistant epilepsy being treated with CBD reported that the most common adverse effects were somnolence, diarrhea and decreased appetite. Significant elevation of liver enzymes was reported as a serious adverse effect, especially among patients treated with high dose CBD and concomitant valproic acid. In general, CBD has shown a favorable safety profile and tolerability which has allowed for several clinical trials to be approved.

CBD is a promising targeted treatment for FXS since it has effects in most of the pathways associated with FXS pathogenesis. A case series was published in 2019 in which a child and two adults with FXS were treated with oral botanical CBD+ solutions. In this case series there were parent-reported improvements in domains such as social avoidance, anxiety, sleep pattern, appetite, motor coordination, language and sensory processing while taking the CBD. Two of the patients re-experienced some of the FXS symptoms upon cessation of CBD and improved after the reintroduction of the treatment. A phase 2 open label trial of a CBD transdermal gel in 20 children with FXS was carried out in Australia. The results showed a significant reduction in anxiety and improvement in behavioral measures such as social avoidance and irritability. A phase 3 randomized, double-blinded, placebo-controlled, multicenter study assessing the efficacy and safety of a pharmaceutically manufactured CBD, formulated as a transdermal gel, for the treatment of patients with FXS is currently taking place in the United States and Australia (NCT03614663). The results are yet to be reported. CBD seems to be a promising intervention for individuals with FXS.

Ganaxolone
Ganaxolone is a 3β-methylated synthetic analog of allopregnanolone and is classified as a neurosteroid. Ganaxolone is a positive allosteric modulator of GABAA receptor in the CNS and it does not have inadvertent hormonal effects. Ganaxolone has been studied for treatment of epilepsy, anxiety, and depression. Preclinical studies found that treatment with ganaxolone can reduce repetitive and perseverative behaviors in the Fmr1-KO mice in a dose-dependent association in addition to modulating sensory response. A randomized controlled trial in 59 youth aged 6-17 years old with FXS found promising benefits of ganaxolone in hyperactivity, attention, and anxiety domains but only in participants who had a high level of anxiety and lower cognitive function (i.e., full-scale IQ ≤ 45). Although more adverse events were reported in the ganaxolone group (i.e., fatigue, drowsiness), no serious adverse events were observed, and most events were recovered. Further studies should specifically include younger children with low cognitive function and a high level of anxiety.

Gaboxadol (OV101)
Gaboxadol augments a δ-subunit-containing extrasympathetic GABAA receptor. A preclinical study observed that hyperactivity, anxiety, aggression, and repetitive behaviors in the animal model of FXS can be returned to typical behaviors after treatment with intraperitoneal gaboxadol. There is an ongoing randomized open label study in males aged 13 to 22 years old which aims to assess safety and improvement in the ABC-C after 12 weeks on gaboxadol (see table 1).

Arbaclofen
As previously mentioned, GABAergic system dysfunction has been implicated in FXS pathogenesis and therefore GABA agonists have been studied as potential targeted treatments. The administration of GABA agonist, alphaxalone, in Fmr1-KO mice resulted in a clear anxiolytic effect and improvement in elevated plus maze performance. Furthermore, studies in Fmr1-KO mice and GABAA agonist, baclofen, showed protection from audiogenic seizures. In other studies using baclofen in Fmr1-KO mice, improvement and correction of abnormalities involved in FXS pathophysiology were observed such as correction of excessive basal protein synthesis, which affects functional plasticity, and abnormal spine density. These promising results lead to clinical trials in patients with FXS.

Arbaclofen is the R-enantiomer of baclofen, a GABAA agonist. Two phase 3 placebo-controlled trials with arbaclofen involving patients with FXS showed that arbaclofen did not improve social avoidance in FXS. The pediatric study which recruited subjects aged 5-11, showed that the highest dose group had a beneficial effect over placebo on the ABC-C, FXS-specific (ABC-CFX) irritability sub scale and Parenting Stress Index, but the primary outcome measure of the study was not met.

Selective mGluR5 antagonist: Mavoglurant
FMRI loss in FXS leads to up regulation of mGluR and aberrant glutamate signaling. In Fmr1-KO mice, up regulation of the group I mGluR was associated with enhancement of synaptic LTD which is a mechanism involved in learning and memory. This provided the basis for the development of specific drugs targeting and antagonizing these receptors. Since mGluR5 is expressed in areas of the brain involved in emotion and motivation, it was proposed as a therapeutic target in FXS. Mavoglurant (AFQ056) was developed as a selective non-competitive mGluR5 antagonists capable of
blocking the excessive downstream signaling through mGluR5, which in FXS occurs due to loss of the negative regulatory function of FMRP.

In preclinical studies mavoglurant demonstrated positive neuronal and behavioral effects.71,72 Despite these promising results, mavoglurant failed to show beneficial behavioral effects in two 12-week randomized, placebo-controlled, double-blind, phase 2 studies in adult and adolescent patients with FXS73. The primary outcome in these studies was improvement on behavioral symptoms measured by the ABC-CFX after 12 weeks of treatment. None of the two studies showed efficacy in reducing the ABC-CFX total score after 12 weeks of treatment with any of the doses of mavoglurant studied vs placebo. In 2018 Hagerman et al. reported the results of two open-label extension trials. Long term safety was the primary endpoint and efficacy the secondary one. Although mavoglurant was well tolerated and there were no safety concerns, the trial was discontinued earlier than planned due to lack of proven efficacy in the core-controlled studies.74 It has been proposed that mavoglurant may be effective in specific experimental settings such as younger age groups and longer trial periods. Currently, there is a phase 2 clinical trial recruiting patients between 32 months to 6 years to evaluate if mavoglurant (AFQ056) can have a positive impact in language measured by the Weighted Child Intentional Communication Score (see table 1) (NCT02920892). It is estimated to be completed by July 2020. There is a need for future clinical trials to demonstrate if there are clinical behavioral and cognitive benefits of mavoglurant in patients with FXS.

Lovastatin

Lovastatin is a 3-hydroxy-3-methylglutaryl-coenzyme A reductase inhibitor which is indicated for treatment of hypercholesterolemia. Ras-ERK1/2 signaling, mGluR-LTD, and excess proteins are downregulated by lovastatin which results in epileptogenic inhibition at the hippocampal area and blocks cortical hyperexcitability in the Fmr1-KO mouse model. In patients with FXS, an open-label phase I trial was conducted to assess safety and efficacy of lovastatin in 16 individuals with FXS aged between 10-40 years old. Subjects were initially treated with 20 mg of lovastatin for the first 4 weeks and titrated up to 40 mg for the next 8 weeks. Participants showed behavioral and adaptive skills improvement over time. Short-term safety of lovastatin was also reported by the trial. Ongoing randomized controlled trials to investigate efficacy ofLovastatin in combination with a language intervention and other medications in the pediatric population await results (see table 1).

Acamprosate

Acamprosate is a medication for the maintenance of abstinence from alcohol dependence. It is a gamma-aminobutyric acid agonist while it modulates NMDA receptor and decreases neuronal hyperexcitability in preclinical studies.78,79 Acamprosate might normalize the plasma amyloid-β precursor protein which is elevated in youth with FXS and ASD.80 A prospective open-label study in 12 youth with FXS age 6-17 years old found that 75% of participants had improvement in hyperactivity, irritability, social interaction, and communication after taking acamprosate for 10 weeks81. In this study, acamprosate was started at 333 mg a day for the first week and titrated up to an average of 1054±422 mg a day in the subsequent 5 weeks. Improvement in the BDNF level was also observed but a correlation between the BDNF level and behavioral improvement was not established. Currently, an active phase 2/phase 3 randomized-controlled trial aims to analyze the effect of treatment with acamprosate in a 10-weeks period in the problematic behaviors seen in patients with FXS (see table 1). Acamprosate has also been proposed as a beneficial treatment for patients with FXS syndrome diagnosed with alcohol dependency because it is helpful for both problems.82

Riluzole

Riluzole is a glutamate-modulating agent known for its use in amyotrophic lateral sclerosis. It acts by inhibiting glutamate release and enhancing its presynaptic reuptake.83 Since as stated earlier, glutamatergic dysregulation is part of the pathogenesis of FXS, riluzole was proposed as a potential targeted treatment. In the only published clinical trial studying riluzole in patients with FXS, 6 adults received open-label treatment with riluzole for six weeks. Investigators did not find significant clinical improvement and the primary outcome, which was improvement in repetitive and compulsive behaviors, was not met.84

Trofinetide (NNZ-2566)

Trofinetide (NNZ-2566) is a synthetic analogue of glypromate (glycine-proline glutamate; GPE). Glypromate is a derivate of insulin-like growth factor-1 (IGF-1) with neuroprotective properties.85 There are several neuroprotective mechanisms proposed for GPE including inhibition of caspase-3-dependent apoptosis by preventing amyloid beta-peptide mediated activation.86 Studies of GPE given as intravenous injection in hypoxic-ischemic adult rat models showed it was able to reduce caspase-3-dependent and caspase-independent apoptosis in the hippocampus, inhibit microglial proliferation and prevent injury-induced loss of astrocytes87. Due to its neuroprotective effects it was proposed as a treatment for neurodevelopmental disorders such as Rett syndrome. In clinical trials, it has been proven to have potential for treating core symptoms in patients with Rett syndrome, such as anxiety-like behaviors, disruptive behavior and mood dysregulation.88 Deacon et al. studied the effects of trofinetide in Fmr1-KO mice and found it normalized dendritic spine density and overactive ERK and protein kinase B (Akt) signaling, suggesting a unique disease modifying mechanism for trofinetide in FXS.89 In their study, normalization of activation of the Ras-MAPK ad PI3K-Akt-mTOR activation was accompanied by improvement in behavioral function such as restoration of social recognition. Furthermore, trofinetide was shown to be safe in a phase 2 clinical trial (NCT01894958), but there are to date no published results on further trials studying it in patients with FXS.
| Medication       | Mechanism                                                                 | Code/status                                | Phase | Age/sex | Primary outcome                                                                 |
|------------------|----------------------------------------------------------------------------|--------------------------------------------|-------|---------|--------------------------------------------------------------------------------|
| Metformin        | Normalizing ERK signaling, EIF4E phosphorylation, mTOR and PI3K activities in CNS, and lowering expression of MMP9 to normal | NCT01472290/recruiting                    | 2     | 10-40 y | Safety Changes in the total score of the fragile X-normed aberrant behavior checklist-community |
|                  |                                                                            | NCT04141163/recruiting                   | 1, 2  | 18-50 y/male | Safety and tolerability                                                        |
|                  |                                                                            | NCT03862950/recruiting                   | 2     | 6-25 y  | Changes in the expressive language sampling mean number of different words score |
|                  |                                                                            | NCT03479476/recruiting                   | 2, 3  | 6-25 y  | Changes in the expressive language sampling mean number of different words score |
| Low dose sertraline | Stimulation of BDNF                                                          | NCT03614463/active, not recruiting        | 2, 3  | 3-17 y  | Improvement in aberrant behavior checklist-community fragile X factor structure |
| Cannabidiol      | Regulation of abnormal endocannabinoid signaling                           | NCT03802799/recruiting                   | 2, 3  | 3-18 y  | Safety and tolerability                                                        |
| Ganalexolone     | Neurosteroid                                                               | NCT02902892/recruiting                   | 2     | 6-17 y  | Clinician's global impression-improvement                                      |
| Gaboxadol (OV101) | Stimulation of BDNF                                                         | NCT03697161/active, not recruiting        | 2     | 13-22 y/male | Safety                                                                        |
|                  |                                                                            | NCT03109756/completed                    | 1     | 13-17 y | Pharmacokinetic                                                                |
| Mavoglurant      | mGluR5 receptor antagonists. Blocks excess mGluRI signaling.                | NCT02902892/recruiting                   | 2     | 32 months−6 years | Greater improvement in language-weighted child intentional communication score |
| Lovastatin       | RAS signaling inhibitor                                                     | NCT02680379/completed                    | 2     | 8-45 y  | Change from baseline aberrant behavior checklist-community                     |
|                  |                                                                            | NCT02642653/completed                    | 4     | 10-17 y | Expressive language sample composite score in the home                        |
| Acamprosate      | Activate GABA<sub>A</sub> and GABA<sub>B</sub> receptor                    | NCT01300923/published (Reference 81)      | 2     | 5-17 y  | Clinical global impression- severity scale                                    |
|                  |                                                                            | NCT01911455/active, not recruiting       | 2     | 5-23 y  | Aberrant Behavior Checklist-Social Withdrawal subscale                        |
|                  |                                                                            | NCT02998151/active, not recruiting       | 2     | 15-55 y | Change in EEG aspects of auditory processing and clinical global impressions improvement |
| Trofinetide (NNZ-2566) | Block excess mGluRI signalling by normalizing activation of the Ras-MAPK and PI3K-Akt-mTOR pathway | NCT01894958/completed                    | 2     | 12-45 y/male | Safety                                                                      |
| Donepezil        | Enhances acetylcholine function in the brain                                | NCT01120626/published (Reference 91)     | 2     | 12-29 y | Contingency naming test performance score                                     |

<sup>a</sup>Combining lovastatin and a parent-implemented language intervention in a multimodal treatment for FXS
<sup>b</sup>Evaluating the neurophysiologic and clinical effects of single-dose acamprosate, lovastatin, minocycline, and placebo in FXS
Donepezil

Donepezil is a cholinesterase inhibitor which has been used for the treatment of dementia. A study found lower choline/creatinine ratio in the right dorsolateral prefrontal cortex of 9 males with FXS compared with typical developing males. Eight participants aged 14-44 years old who took donepezil 5 mg for 3 weeks followed by 10 mg for 3 weeks showed improvement in cognitive and behavioral status. However, a subsequent 12 week-randomized controlled trial of donepezil in 42 individuals with FXS did not prove the effects. Perhaps improvement in cognitive-behavioral function needs a combination of behavioral interventions with medication treatment. Nevertheless, functional brain magnetic resonance imaging of participants in the donepezil group showed that the left superior frontal gyrus was less activated to stimuli tasks compared to the placebo group. This finding might reflect an effect of donepezil in restoring the abnormal neuroimaging phenotype in FXS. Future studies to assess the change in brain functioning may early capture the efficacy of treatment with donepezil.

Challenges in the search for a targeted treatment in FXS

Thanks to the advancement and the identification of multiple pathways involved in FXS pathogenesis, there are numerous studies targeting the altered pathways in the seek for therapeutic interventions that are specific for FXS. Several animal models have proven promising results in behavioral and cognitive endpoints and have led to clinical trials in humans with varying results. What are the main challenges for translation into beneficial therapy for humans? Zeidler and colleagues reviewed the limitations in the search for a targeted treatment for FXS in 2019. The main limitations identified where the extrapolation of results from animal models to humans, the outcome measures used in different studies, the trial design and the need to target more than one pathway.

In 2017 Budimirovic and colleagues evaluated the available outcome measures for trials involving FXS or other neurodevelopment disorders. They concluded that most of the outcome measures were of moderate quality level with limited information on reliability, validity, and sensitivity to treatment. There is ongoing research for the development of more sensitive and reproducible measurement tools such as white matter changes, auditory evoked potentials measures, eye-tracking, among others.

Additionally, since there are multiple pathways involved in FXS pathogenesis, a combination of medications or medications targeting multiple pathways could be more beneficial. Trials involving medication and non-pharmacological interventions such as Parent Implemented Language Intervention (PILI) could also be more sustainable and beneficial. Clinical trials focusing on multimodal interventions are promising and despite the limitations in the search for targeted treatment, there has been a significant advancement in implementing clinical trials in FXS.

CONCLUSION

Although there are currently no disease-modifying treatments for FXS with regulatory approval, there are several potential medications targeting different pathways involved in FXS pathophysiology. The current approach when treating a patient with FXS focuses mostly on symptomatic off-label treatment. Thanks to the increasing understanding of FXS pathogenesis, clinical trials have been performed and several agents show promising results.

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REFERENCES

1. Hanson AC, Hagerman RJ. Serotonin dysregulation in fragile X syndrome: implications for treatment. Intractable Rare Dis Res 2014;3:110–7.
2. Hagerman RJ, Berry-Kravis E, Hazlett HC, Bailey DB, Moine H, Kooy RF, et al. Fragile X syndrome. Nat Rev Dis Primers 2017;3:17065.
3. Gantois I, Popic J, Khoutorsky A, Sonenberg N. Metformin for treatment of fragile X syndrome and other neurological disorders. Annu Rev Med 2019;70:167–81.
4. Michaluk P, Wawrzyniak M, Alot P, Szcztot M, Wyrembek P, Mercik K, et al. Influence of matrix metalloproteinase MMP-9 on dendritic spine morphology. J Cell Sci 2011;124:3369–80.
5. Bodmer M, Meier C, Krähenbühl S, Jick SS, Meier CR. Metformin, sulfonylureas, or other antibiotics drugs and the risk of lactic acidosis or hypoglycemia: a nested case-control analysis. Diabetes Care 2008;31:2086–91.
6. van Dalem J, Brouwers MCGJ, Stehouwer CDA, Krings A, Leufkens HGM, Driessen JHM, et al. Risk of hypoglycaemia in users of sulphonylureas compared with metformin in relation to renal function and sulphonylurea metabolite group: population based cohort study. BMJ 2016;354:i3625.
7. Monyak RE, Emerson D, Schoenfeld BP, Zheng X, Chambers DB, Rosenfelt C, et al. Insulin signaling misregulation underlies circadian and cognitive deficits in a Drosophila fragile X model. Mol Psychiatry 2017;22:1140–8.
8. Gantois I, Khoutorsky A, Popic J, Aguilar–Valles A, Freemantle E, Cao R, et al. Metformin ameliorates core deficits in a mouse model of fragile X syndrome. Nat Med 2017;23:674–7.
9. Dy ABC, Tassone F, Eldeeb M, Salcedo-Arellano MJ, Tartaglia N, Hagerman R. Metformin as targeted treatment in fragile X syndrome. Clin Genet 2018;93:216–22.


10. Biag HMB, Potter LA, Wilkins V, Afzal S, Rosvall A, Salcedo-Arellano MJ, et al. Metformin treatment in young children with fragile X syndrome. Mol Genet Genomic Med 2019;7:e956.

11. Anagnostou E, Aman MG, Handen BL, Sanders KB, Shuai A, Hollway JA, et al. Metformin for treatment of overweight induced by atypical antipsychotic medication in young people with autism spectrum disorder: a randomized clinical trial. JAMA Psychiatry 2016;73:928–37.

12. Handen BL, Anagnostou E, Aman MG, Sanders KB, Chan J, Hollway JA, et al. A randomized, placebo-controlled trial of metformin for the treatment of overweight induced by atypical antipsychotic medication in young People with autism spectrum disorder: open-label extension. J Am Acad Child Adolesc Psychiatry 2017;56:849-856.e6.

13. Aman MG, Hollway JA, Veenstra-VanderWeele J, Handen BL, Sanders KB, Chan J, et al. Effects of metformin on spatial and verbal memory in children with ASD and overweight associated with atypical antipsychotic use. J Child Adolesc Psychopharmacol 2018;28:266–73.

14. Schneider A, Ligsay A, Hagerman RJ. Fragile X syndrome: an aging perspective. Dev Disabil Res Rev 2013;18:68–74.

15. Protic D, Aydin EY, Tassone F, Tan MM, Hagerman RJ, Schneider A. Cognitive and behavioral improvement in adults with fragile X syndrome treated with metformin-two cases. Mol Genet Genomic Med 2019;7:e00745.

16. Protic D, Kaluzhny P, Tassone F, Hagerman RJ. Prepupertal metformin treatment in fragile X syndrome alleviated macroorchidism: a case study. Adv Clin Transl Res 2019;3:100021.

17. Bilousova T V, Dansle L, Ngo M, Aye J, Charles JR, Ethell DW, et al. Minocycline promotes dendritic spine maturation and improves behavioural performance in the fragile X mouse model. J Med Genet 2009;46:94–102.

18. Siller SS, Broadie K. Neuronal circuit architecture defects in a Drosophila model of fragile X syndrome are alleviated by minocycline treatment and genetic removal of matrix metalloproteinase. Dis Model Mech 2011;4:673–85.

19. Yau SY, Bettio L, Vettrici M, Truesdell A, Chiu C, Chiu J, et al. Chronic minocycline treatment improves hippocampal neuronal structure, NMDA receptor function, and memory processing in Fmr1 knockout mice. Neurobiol Dis 2018;113:11–22.

20. Toledo MA, Wen TH, Binder DK, Ethell IM, Razak KA. Reversal of ultrasonic vocalization deficits in a mouse model of fragile X Syndrome with minocycline treatment or genetic reduction of MMP-9. Behav Brain Res 2019;372:112068.

21. Leigh MJS, Nguyen D V, Mu Y, Winarni TI, Schneider A, Chechi T, et al. A randomized double-blind, placebo-controlled trial of minocycline in children and adolescents with fragile X syndrome. J Dev Behav Pediatr 2013;34:147–55.

22. Utari A, Chonchaiya W, Rivera SM, Schneider A, Hagerman RJ, Faradz SMH, et al. Side effects of minocycline treatment in patients with fragile X syndrome and exploration of outcome measures. Am J Intellect Dev Disabil 2010;115:433–43.

23. Paribello C, Tao L, Folino A, Berry-Kravis E, Tranflaglia M, Ethell IM, et al. Open-label add-on treatment trial of minocycline in fragile X syndrome. BMC Neurol 2010;10:91.

24. Protic D, Salcedo-Arellano MJ, Dy JB, Potter LA, Hagerman RJ. New targeted treatments for fragile X syndrome. Curr Pediatr Rev 2019;15:251–8.

25. Indah Winarni T, Chonchaiya W, Adams E, Au J, Mu Y, Rivera SM, et al. Sertraline may improve language developmental trajectory in young children with fragile X syndrome: a retrospective chart review. Autism Res Treat 2012;2012:104317.

26. Sudhi MSK, Sanders-Bush E. Serotonin and brain development. Int Rev Neurobiol 2004;59:111–74.

27. Chandara SR, Behen ME, Juhász C, Muzik O, Rothermel RD, Mangner TJ, et al. Significance of abnormalities in developmental trajectory and asymmetry of cortical serotonin synthesis in autism. Int J Dev Neurosci 2005;23:171–82.

28. Lim C-S, Hoang ET, Viar KE, Stornetta RL, Scott MM, Zhu J. Pharmacological rescue of Ras signaling, GluA1-dependent synaptic plasticity, and learning deficits in a fragile X model. Genes Dev 2014;28:273–89.

29. Costa L, Spatuzza M, D’Antoni S, Bonaccorso CM, Trovato C, Musumeci SA, et al. Activation of 5-HT7 serotonin receptors reverses metabolitic glutamate receptor-mediated synaptic plasticity in wild-type and Fmr1 knockout mice, a model of Fragile X syndrome. Biol Psychiatry 2012;72:924–33.

30. Wang H, Wu LJ, Kim SS, Lee FJS, Gong B, Toyoda H, et al. FMRP acts as a key messenger for dopamine modulation in the forebrain. Neuropl 2008;59:634–47.

31. Kitaichi Y, Inoue T, Nakagawa S, Boku S, Kakuta A, Izumi T, et al. Sertraline increases extracellular levels not only of serotonin, but also of dopamine in the nucleus accumbens and striatum of rats. Eur J Pharmacol 2010;647:90–6.

32. Hess LG, Fitzpatrick SE, Nguyen DV, Chen Y, Gaul MM, Schneider A, et al. A randomized, double-Blind, placebo-controlled trial of low-dose sertraline in young children with fragile X syndrome. J Dev Behav Pediatr 2016;37:619–28.

33. Pisanti S, Malfitano AM, Ciaglia E, Lamberti A, Ranieri R, Cuomo G, et al. Cannabidiol: state of the art and new challenges for therapeutic applications. Pharmacol Ther 2017;175:133–50.

34. Devinsky O, Marsh E, Friedman D, Thiele E, Laux L, Sullivan J, et al. Cannabidiol in patients with treatment-resistant epilepsy: an open-label intervention trial. Lancet Neurol 2016;15:270–8.

35. Lattanzi S, Brigo F, Trinka E, Zaccara G, Cagnetti C, Del Giovane C, et al. Efficacy and safety of cannabidiol in epilepsy: a systematic review and meta-analysis. Drugs 2018;78:1791–1804.

36. Marinho ALZ, Vila-Verde C, Fogaça M V, Guimarães FS. Effects of intra-infralimbic prefrontal cortex injections of cannabidiol in the modulation of emotional behaviors in rats: contribution of 5HT1A receptors and stressful experiences. Behav Brain Res 2015;286:49–56.
37. Almeida V, Levin R, Peres FF, Niigaki ST, Calzavara MB, Zuardi AW, et al. Cannabidiol exhibits anxiolytic but not antipsychotic property evaluated in the social interaction test. Prog Neuropsychopharmacol Biol Psychiatry 2013;41:30–52.

38. Bergamaschi MM, Queiroz RHC, Chagas MHN, de Oliveira DCG, De Martinis BS, Kapczinski F, et al. Cannabidiol reduces the anxiety induced by simulated public speaking in treatment-naive social phobia patients. Neuropsychopharmacology 2011;36:1219–26.

39. Scuderi C, Steardo L, Esposito G. Cannabidiol promotes amyloid precursor protein ubiquitination and reduction of beta amyloid expression in SHSY5Y APP+ cells through PPARy involvement. Phytother Res 2014;28:1007–13.

40. Cheng D, Spiro AS, Jenner AM, Garner B, Karl T. Long-term cannabidiol treatment prevents the development of social recognition memory deficits in Alzheimer’s disease transgenic mice. J Alzheimers Dis 2014;42:1383–96.

41. Malfait AM, Gallily R, Sumariwalla PF, Malik AS, Andreakos E, Mechoulam R, et al. The nonpsychoactive cannabinoid constituent cannabidiol is an oral anti-arthritic therapeutic in murine collagen-induced arthritis. Proc Natl Acad Sci U S A 2000;97:9561–6.

42. Maccarrone M, Rossi S, Bari M, De Chiara V, Rapino C, Musella A, et al. Abnormal mGlu5 receptor/endocannabinoid coupling in mice lacking FMRP and BC1 RNA. Neuropsychopharmacology 2010;35:1500–9.

43. Jung KM, Sepers M, Henstridge CM, Lassalle O, Neuhofer D, Martin H, et al. Uncoupling of the endocannabinoid signalling complex in a mouse model of fragile X syndrome. Nat Commun 2012;3:1080.

44. Tartaglia N, Bonn-Miller M, Hagerman R. Treatment of fragile X syndrome with cannabidiol: a case series study and brief review of the literature. Cannabis Cannabinoid Res 2019;4:3–9.

45. Maione S, Piscitelli F, Gatta L, Vita D, De Petrocellis L, Palazzo E, et al. Non-psychoactive cannabinoids modulate the descending pathway of antinoceptive in anaesthetized rats through several mechanisms of action. Br J Pharmacol 2011;162:584–96.

46. Bagni C, Zukin RS. A synaptic perspective of fragile X syndrome and autism spectrum disorders. Neuron 2019;101:1070–88.

47. Huber KM, Gallagher SM, Warren ST, Bear MF. Altered synaptic plasticity in a mouse model of fragile X mental retardation. Proc Natl Acad Sci U S A 2002;99:7746–50.

48. Zhang L, Alger BE. Enhanced endocannabinoid signaling elevates neuronal excitability in fragile X syndrome. J Neurosci 2010;30:5724–9.

49. Melis M, Greco B, Tonini R. Interplay between synaptic endocannabinoid signaling and metaplasitc性 in neuronal circuit function and dysfunction. Eur J Neurosci 2014;39:1189–201.

50. Van der Aa N, Kooy RF. GABAAergic abnormalities in the fragile X syndrome. Eur J Paediatr Neurol 2020;24:100–104.

51. Bakas T, van Nieuwenhuijzen PS, Devenish SO, McGregor IS, Arnold JC, Chebib M. The direct actions of cannabidiol and 2-arachidonoyl glycerol at GABA,A receptors. Pharmacol Res 2017;119:358–70.

52. Campos AC, Guimarães FS. Involvement of 5HT1A receptors in the anxiolytic-like effects of cannabidiol injected into the dorsolateral periaqueductal gray of rats. Psychopharmacology (Berl) 2008;199:223–30.

53. Norris C, Loureiro M, Kramar C, Zunder J, Renard J, Rushlow W, et al. Cannabidiol modulates fear memory formation through interactions with serotonergic transmission in the mesolimbic system. Neuropsychopharmacology 2016;41:2839–50.

54. Bergamaschi MM, Queiroz RHC, Zuardi AW, Crippa JAS. Safety and side effects of cannabidiol, a Cannabis sativa constituent. Curr Drug Saf 2011;6(4):237–49.

55. Anciones C, Gil-Nagel A. Adverse effects of cannabinoids. Epileptic Disord 2020;22:29–32.

56. McCoy B, Wang L, Zak M, Al-Mehmadi S, Kabir N, Alhadid K, et al. A prospective open-label trial of a CBD/THC cannabis oil in Dravet syndrome. Ann Clin Transl Neurol 2018;5:1077–88.

57. Devinsky O, Nabbout R, Miller I, Laux L, Zolnowska M, Wright S, et al. Long-term cannabidiol treatment in patients with Dravet syndrome: an open-label extension trial. Epilepsia 2019;60(2):294–302.

58. Heussler H, Cohen J, Silove N, Tich N, Bonn-Miller MO, Du W, et al. A phase 1/2, open-label assessment of the safety, tolerability, and efficacy of transdermal cannabidiol (ZYN002) for the treatment of pediatric fragile X syndrome. J Neurodev Disord 2019;11:16.

59. Braat S, D’Hulst C, Heulens I, De Rubeis S, Mienjttes E, Nelson DL, et al. The GABAA receptor is a FMRP target with therapeutic potential in fragile X syndrome. Cell Cycle 2015;14:2985–95.

60. Ligsay A, Van Dijck A, Nguyen DV, Lozano R, Chen Y, Bickel ES, et al. A randomized double-blind, placebo-controlled trial of ganaxolone in children and adolescents with fragile X syndrome. J Neurodev Disord 2017;9:26.

61. Cograp P, Deacon RMI, Warner-Schmidt JL, von Schimmelmann MJ, Abrahams BS, During MJ. Gaboxadol normalizes behavioral abnormalities in a mouse model of fragile X syndrome. Front Behav Neurosci 2019;13:141.

62. Heulens I, D’Hulst C, Van Dam D, De Deyn PP, Kooy RF. Pharmacological treatment of fragile X syndrome with GABAergic drugs in a knockout mouse model. Behav Brain Res 2012;229:244–9.

63. Pacey LKK, Heximer SP, Hampson DR. Increased GABA(B) receptor-mediated signaling reduces the susceptibility of fragile X knockout mice to audiogenic seizures. Mol Pharmacol 2009;76:18–24.

64. Henderson C, Wijetunge L, Kinoshita MN, Shumway M, Hammond RS, Postma FR, et al. Reversal of disease-related pathologies in the fragile X mouse model by selective activation of GABAB receptors. J Neurodev Disord 2020;12:19.
receptors with arbaclofen. Sci Transl Med 2012;4:152ra128.
65. Qin M, Huang T, Kader M, Krych L, Xia Z, Burlin T, et al. R-Baclofen reverses a social behavior deficit and elevated protein synthesis in a mouse model of fragile X syndrome. Int J Neuropsychopharmacol 2015;18:pii:pyv034.
66. Berry-Kravis E, Hagerman R, Visootsak J, Budimirovic D, Kaufmann WE, Cherubini M, et al. Arbaclofen in fragile X syndrome: results of phase 3 trials. J Neurodev Disord 2017;9:3.
67. Telias M. Molecular mechanisms of synaptic dysregulation in fragile X syndrome and autism spectrum disorders. Front Mol Neurosci 2019;12:51.
68. Bear MF, Huber KM, Warren ST. The mGluR theory of fragile X mental retardation. Trends Neurosci 2004;27:370–7.
69. Gomez-Mancilla C, Berry-Kravis E, Hagerman R, von Raison F, Apostol G, Ufer M, et al. Development of mavoglurant and its potential for the treatment of fragile X syndrome. Expert Opin Investig Drugs 2014;23:125–34.
70. Vranesic I, Ofner S, Flor PJ, Bilhe G, Bouhelal R, Enz A, et al. AFQ056/mavoglurant, a novel clinically effective mGluR5 antagonist: identification, SAR and pharmacological characterization. Bioorg Med Chem 2014;22:5790–803.
71. Gantois I, Pop AS, de Esch CEF, Buijsen RAM, Pooters T, Gomez-Mancilla B, et al. Chronic administration of AFQ056/Mavoglurant restores social behaviour in Fmr1 knockout mice. Behav Brain Res 2013;239:72–9.
72. Pop AS, Levenga J, de Esch CEF, Buijsen RAM, Nieuwenhuizen IM, Li T, et al. Rescue of dendritic spine phenotype in Fmr1 KO mice with the mGluR5 antagonist AFQ056/mavoglurant. Psychopharmacology (Berl) 2014;231:1227–35.
73. Berry-Kravis E, Des Portes V, Hagerman R, Jacquemont S, Charles P, Visootsak J, et al. Mavoglurant in fragile X syndrome: results of two randomized, double-blind, placebo-controlled trials. Sci Transl Med 2016;8:321ra5.
74. Hagerman R, Jacquemont S, Berry-Kravis E, Des Portes V, Stanfield A, Koumaras B, et al. Mavoglurant in fragile X syndrome: results of two open-label, extension trials in adults and adolescents. Sci Rep 2018;8:16970.
75. Osterweil EK, Chuang S-C, Chubykin AA, Sidorov M, Bianchi R, Wong RKS, et al. Lovastatin corrects excess protein synthesis and prevents epileptogenesis in a mouse model of fragile X syndrome. Neuron 2013;77:243–50.
76. Muscas M, Louros SR, Osterweil EK. Lovastatin, not simvastatin, corrects core phenotypes in the fragile X mouse model. eNeuro 2019;6:pii:ENEURO.0097-19.2019.
77. Çaku A, Pellerin D, Bouvier P, Riou E, Corbin F. Effect of lovastatin on behavior in children and adults with fragile X syndrome: an open-label study. Am J Med Genet A 2014;164A:2834–42.
78. Mason BJ, Heyser CJ. Acamprosate: A prototypic neuromodulator in the treatment of alcohol dependence. CNS Neurol Disord Drug Targets 2010;9:23–32.
79. Kalk NJ, Lingford-Hughes AR. The clinical pharmacology of acamprosate. Br J Clin Pharmacol 2014;77:315–23.
80. Erickson CA, Ray B, Maloney B, Wink LK, Bowers K, Schaefer TL, et al. Impact of acamprosate on plasma amyloid-β precursor protein in youth: a pilot analysis in fragile X syndrome-associated and idiopathic autism spectrum disorder suggests a pharmacodynamic protein marker. J Psychiatr Res 2014;59:220–8.
81. Erickson CA, Wink LK, Ray B, Early MC, Stiegemeyer E, Mathieu-Frasier L, et al. Impact of acamprosate on behavior and brain-derived neurotrophic factor: an open-label study in youth with fragile X syndrome. Psychopharmacology (Berl) 2013;228:75–84.
82. Salcedo-Arellano MJ, Lozano R, Tassone F, Hagerman RJ, Saldarriaga W. Alcohol use dependence in fragile X syndrome. Intractable Rare Dis Res 2016;5:207–13.
83. Sabus A, Feinstein J, Romani P, Goldsen E, Blackmer A. Management of self-injurious behaviors in children with neurodevelopmental disorders: a pharmacotherapy overview. Pharmacotherapy 2019;39:645–64.
84. Erickson CA, Weng N, Weiler IJ, Greenough WT, Stigler KA, Wink LK, et al. Open-labelriluzole in fragile X syndrome. Brain Res 2011;1380:264–70.
85. Lu XC, Chen RW, Yao C, Wei H, Yang X, Liao Z, et al. NNZ-2566, a glycyrhizic analog, improves functional recovery and attenuates apoptosis and inflammation in a rat model of penetrating ballistic-type brain injury. J Neurotrauma 2009;26:141–54.
86. Ioudina M, Uemura E. A three amino acid peptide, Gly-Pro-Arg, protects and rescues cell death induced by amyloid beta-peptide. Exp Neurol 2003;184:923–9.
87. Guan J, Thomas GB, Lin H, Mathai S, Bachelor DC, George S, et al. Neuroprotective effects of the N-terminal tripeptide of insulin-like growth factor-1, glycine-proline-glutamate (GPE) following intravenous infusion in hypoxic-ischemic adult rats. Neuropharmacology 2004;47:982–903.
88. Glaze DG, Neul JL, Kaufmann WE, Berry-Kravis E, Condon S, Stoms G, et al. Double-blind, randomized, placebo-controlled study of trofinetide in pediatric Rett syndrome. Neurology 2019;92:e1912–25.
89. Deacon RMI, Glass L, Snape M, Hurley MJ, Altimiras FJ, Biekofsky RR, et al. NNZ-2566, a novel analog of (1–3) IGF-1, as a potential therapeutic agent for fragile X syndrome. Neuromolecular Med 2015;17:71–82.
90. Kesler SR, Lighthoby AA, Reiss AL. Cholinergic dysfunction in fragile X syndrome and potential intervention: a preliminary 1H MRS study. Am J Med Genet A. 2009;149A:403–7.
91. Bruno JL, Hosseini SH, Lighthoby AA, Manchanda MK, Reiss AL. Brain circuitry, behavior, and cognition: a randomized placebo-controlled trial of donepezil in fragile X syndrome. J Psychopharmacol 2019;33:975–85.
92. Berry-Kravis EM, Lindemann J, Jønch AE, Apostol G, Bear MF, Carpenter RL, et al. Drug development for neurodevelopmental disorders: lessons learned from fragile X syndrome. Nat Rev Drug Discov 2018;17:280–99.

93. Zeidler S, Willemsen R. Fragile X syndrome, the search for a targeted treatment. Journal of Biomedicine and Translational Research [Online]. 2019 https://doi.org/10.14710/jbtr.v5i1.3925.

94. Budimirovic DB, Berry-Kravis E, Erickson CA, Hall SS, Hessl D, Reiss AL, et al. Updated report on tools to measure outcomes of clinical trials in fragile X syndrome. J Neurodev Disord 2017;9:14.

95. Swanson MR, Hazlett HC. White matter as a monitoring biomarker for neurodevelopmental disorder intervention studies. J Neurodev Disord 2019;11:33.

96. Ethridge LE, De Stefano LA, Schmitt LM, Woodruff NE, Brown KL, Tran M, et al. Auditory EEG biomarkers in fragile X syndrome: clinical relevance. Frontiers Integr Neurosci 2019;13:60.

97. Klusek J, Moser C, Schmidt J, Abbeduto L, Roberts JE. A novel eye-tracking paradigm for indexing social avoidance-related behavior in fragile X syndrome. Am J Med Genet Part B 2020;183B:5-16.