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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.[2] In addition, the lack of FMRP leads to deficits in inhibitory gamma-aminobutyric acid (GABA) signaling as well as dopamine and cholinergic dysregulation.[1,2] The endocannabinoid system, which promotes synaptic plasticity, is also dysregulated in FMRP deficient conditions as in FXS.[2]

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).[2] 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.[3] 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)[3]. MMP-9 which is upregulated in conditions of FMRP deficiency causes a negative impact on synaptic formation in FXS.[4] 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.[5,6]

The efficacy of metformin was proved in *Fmr1*-knocked out (KO) animal models by showing improvement in abnormal behaviors.[7,8] Problematic behaviors like hyperphagia and stereotypy have also shown improvements in patients with FXS treated clinically with metformin[9,10]. 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[11,12]. However, the effects of metformin on cognition in children with ASD needs more study[13].

Medical treatment with metformin in seven individuals age 4.5-60 years old with FXS produced behavioral improvements following the intake of metformin clinically for 6-12 months.[9] 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[14], improvements in language especially conversational skills is a welcome change.[9] 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.[15] Their parents also anecdotally reported improvements in language, social communication, responsiveness as well as decreasing irritability and anxiety.

Metformin stimulates neurogenesis in animal models.[3] 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.[10] 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[16]. 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.[2,9,15,16]

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[17]. FMRP deficiency leads to elevated MMP-9 activity which has a negative impact in synaptic physiology and plasticity.[4] 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.[17,18] 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.[19] 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.[20] 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.[21]

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. Less frequent side effects reported were worsening hyperactivity and moodiness, and one patient had darkening of nails.[22] 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).[23] Therefore, minocycline is well tolerated but requires clinical monitoring and testing for ANA and liver function.[24]

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.[25]

Serotonin in the cortex usually peaks during the first five years of life when synaptogenesis is also highest[26]. However, this serotonin trajectory and brain morphology in children with ASD was found to be dysregulated.[1,26,27] Serotonin dysregulation is also an abnormal neurochemical pathway in FXS.[1] Serotonin and the brain-derived neurotrophic factor (BDNF) interact reciprocally to regulate neuroplasticity[26].Treatment with an SSRI not only normalizes serotonin levels, but also stimulates BDNF which is also dysregulated in FXS.[1] In the *Fmr1*-KO mouse model, serotonin also restored glutamate A1-dependent long-term potentiation (LTP)[28] and attenuated mGluR5-mediated synaptic LTD.[29] Moreover, dopamine is found impaired in *Fmr1*-KO mice.[30] Sertraline is an SSRI which inhibits dopamine reuptake[1], therefore, leading to an increased dopamine concentration at the nucleus accumbent and the striatum in rats.[31] Normalization of dopamine may help maintain normal LTP and dendritic morphology in FXS.[1] 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.[25]

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[25]. 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.[32] 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 ‘cannabinoid’ 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.[33] 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,[34,35] anxiety,[36–38] neurodegenerative diseases such as Alzheimer’s disease,[39,40] and autoimmune diseases such as rheumatoid arthritis,[41] among others.

In the mouse model of FXS, it has been shown that the endocannabinoid system is linked to the biological actions of FMRP and is therefore dysregulated when there is absence or deficiency of this protein.[42,43] 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[44]. The loss of endocannabinoid signaling in the FXS model is in part due to reduced production of 2-arachidonylglycerol (2-AG) and increased catabolic hydroxylation of N-arachidonoylethanolamine (AEA) .[43] CBD has shown to increase both AEA and 2-AG availability improving one of the biological mechanisms of FXS.[45]

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.[46] It has been proposed that

CBD may increase synaptic plasticity in FXS which may be associated with an improvement in learning and cognition domains.[47,48,49] 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[50]. CBD may also improve the GABAergic dysfunction since it acts as a positive allosteric modulator of $GABA_A$ receptors.[51]

Finally, CBD exerts its anxiolytic effects by its interaction with the serotonin system. Studies have identified serotonin-1A receptor ($5\text{-}HT_{1A}$) as one of the targets through which CBD aids in the reduction of social anxiety experienced by patients with FXS.[52,53]

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.[54] Some patients may experience transitory, dose-dependent mild to moderate effects including somnolence, decreased appetite and gastrointestinal disturbances.[55] 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.[56,57] 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[44] 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.[58] A phase 3 randomized, double-blinded, placebo-controlled, multi-center 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 $GABA_A$ 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.[59] 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[60]. 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 extrasy naptic $GABA_A$ 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[61]. 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 $GABA_A$ agonist, alphaxalone, in *Fmr1*-KO mice resulted in a clear anxiolytic effect and improvement in elevated plus maze performance[62]. Furthermore, studies in *Fmr1*-KO mice and $GABA_B$ agonist, baclofen, showed protection from audiogenic seizures.[63] 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.[64,65] These promising results lead to clinical trials in patients with FXS.

Arbaclofen is the R-enantiomer of baclofen, a $GABA_B$ 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 age 5-11, showed that the highest dose group had a beneficial effect over placebo on the ABC-C, FXS-specific ($ABC\text{-}C_{FX}$) irritability sub scale and Parenting Stress Index, but the primary outcome measure of the study was not met[66].

Selective mGluR5 antagonist: Mavoglurant

FMRP loss in FXS leads to up regulation of mGluR and aberrant glutamate signaling.[67] 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.[68] 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.[69] Mavoglurant (AFQ056) was developed as a selective non-competitive mGluR5 antagonists capable of

blocking the excessive downstream signaling through mGluR5,[70] 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 FXS[73]. The primary outcome in these studies was improvement on behavioral symptoms measured by the ABC-C_{FX} after 12 weeks of treatment. None of the two studies showed efficacy in reducing the ABC-C_{FX} 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.[75,76] 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.[77] 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 of lovastatin 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 weeks[81]. 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 astrocytes[87]. 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	NCT03722290/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	NCT01474746/published (Reference 32)	2	24-68 m	Changes in Mullen scales of early learning- expressive language raw Score and clinical global impression-improvement
Cannabidiol	Regulation of abnormal endocannabinoid signaling	NCT03614663/active, not recruiting	2,3	3-17 y	Improvement in aberrant behavior checklist-community fragile X factor structure
		NCT03802799/recruiting	2,3	3-18 y	Safety and tolerability
Ganaxolone	Neurosteroid Modulate $GABA_A$	NCT01725152/published (Reference 60)	2	6-17 y	Clinician's global impression-improvement
Gaboxadol (OV101)		NCT03697161/active, not recruiting	2	13-22 y, males	Safety
		NCT03109756/completed	1	13-17 y	Pharmacokinetic
Mavoglurant	mGluR5 receptor antagonists. Blocks excess mGluRI signaling.	NCT02920892/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[a]/completed	4	10-17 y	Expressive language sample composite score in the home
Acamprosate	Activate $GABA_A$ and $GABA_B$ receptor	NCT01300923/published (Reference 81)	2	5-17 y	Clinical global impression- severity scale
		NCT01911455/active, not recruiting	2, 3	5-23 y	Aberrant Behavior Checklist-Social Withdrawal subscale
		NCT02998151[b]/enrolling	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/ males	Safety
Donepezil	Enhances acetylcholine function in the brain	NCT01120626/published (Reference 91)	2	12-29 y	Contingency naming test performance score

[a]Combining lovastatin and a parent-implemented language intervention in a multimodal treatment for FXS
[b]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 dementia. A study found lower choline/creatinine ratio in the right dorsolateral prefrontal cortex of 9 males with FXS compared with typical developing males.[90] 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.[90] However, a subsequent 12 week-randomized controlled trial of donepezil in 42 individuals with FXS did not prove the effects[91]. 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[92].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.[93] 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[94]. There is ongoing research for the development of more sensitive and reproducible measurement tools such as white matter changes,[95] auditory evoked potentials measures[96], eye-tracking,[97] 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[24]. 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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