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REVIEW ARTICLE

Targeted drug development in fragile X syndrome: molecular medicine as a key tool in clinical pharmacology

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Summary

The full mutation and epigenetic silencing of the *FMR1* gene lead to a deficiency of its protein, FMRP, resulting in Fragile X Syndrome (FXS). Although significant advances have been made in understanding the molecular mechanisms underlying FXS, no cure or targeted pharmacological treatments have yet been approved for this neurodevelopmental disorder. Current clinical management primarily relies on symptomatic therapies, which often offer limited benefits and do not address the core molecular causes of the condition, especially given the multifaceted roles of the FMRP.

This review highlights the crucial role of molecular insights in guiding the development of drugs for FXS. It provides an overview of existing pharmacotherapies, discusses their benefits and limitations, and emphasizes the unmet need for interventions that target the specific pathways disrupted by *FMR1* dysfunction. Recent and ongoing clinical trials were examined, focusing on how a deeper understanding of FXS molecular biology can inform the design of more effective and precise therapeutic strategies. In summary, key molecular pathways relevant to FXS are presented, and the potential synergy between clinical pharmacology and molecular medicine is discussed as a means to promote the advancement of tailored therapeutic approaches.

Keywords: fragile X syndrome, pharmacotherapy, targeted treatment, *FMR1* gene

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INTRODUCTION

Fragile X syndrome (FXS; OMIM: #300624; ORPHA: 908), sometimes referred to as FRAXA syndrome, FraX syndrome, or Martin-Bell syndrome, is a neurodevelopmental condition recognized as the most studied single-gene cause of autism spectrum disorder (ASD), and associated with moderate to severe intellectual disability (ID) in the most affected males with FXS (reviewed in: (1, 2)). According to the Orphanet database, the estimated prevalence of FXS ranges from 1 in 2,400 to 1 in 6,000 in the general population (more information available at www.orpha.net). However, prevalence rates vary across different parts of the world and depend on availability and methods of genetic testing (3). FXS is most commonly caused by a full mutation (FM, >200 CGG repeats) in *the FMR1* gene, which is located at Xq27.3 on the X chromosome, leading to its hypermethylation and transcriptional silencing and the deficit of FMRP, its final product. (4-7). FMRP binds to ribosomes and regulates the translation of specific messenger RNAs (mRNAs) that are essential for synapse formation, which together play a critical role in neurological development and function (4). It is considered an "immediate early protein" at the synapse, coordinating synaptic development, plasticity, and elimination (8). In addition, FMRP is involved in subcellular transport and RNA stability (1, 3, 9). Furthermore, point mutations and deletions within the *FMR1* gene have also been described as causes of FXS (6, 10).

Clinically, FXS is characterized by a combination of neurobehavioral and physical features (reviewed in: (1, 11)). In addition to ID and ASD, individuals with FXS typically exhibit speech and language delays, significant behavioral and neurological challenges, and characteristic physical features. These include a long, narrow face, prominent ears, a high-arched palate, hyperextensible joints, flat feet, and, in males after puberty, macroorchidism (1, 11-14). Symptoms are generally more severe in males, whereas females, due to the presence of two X chromosomes, often present with a milder phenotype (15).

Although the body of knowledge on FXS has grown substantially over the past three decades, no cure or approved targeted treatment exists for FXS. Several clinical trials are ongoing; however, until pharmacotherapies that specifically target the molecular pathways associated with the *FMR1* gene and its product, FMRP, become available, symptomatic treatment remains the foundation of pharmacological management in this population. Building on current scientific understanding, this article aims to provide a comprehensive review of the critical interplay between molecular medicine and clinical pharmacology in advancing drug development for FXS. In other words, this review aims to highlight how a deeper understanding of molecular mechanisms directly supports and informs future drug development efforts, serving as the key tool in clinical pharmacology and targeted drug development.

METHODS

To prepare this review, a comprehensive literature search was conducted using the PubMed, MEDLINE, and Google Scholar databases, as well as the clinical trial registry at clinicaltrials.gov. The search included articles and clinical trials published over the past two decades, with an emphasis on studies published within the last five years. The following keywords and their combinations were used: Fragile X Syndrome (FXS), *FMR1* gene, *FMR1* Protein (FMRP), pharmacotherapy, targeted treatment, and clinical trials. Additional references were identified by manually screening the bibliographies of key articles. Studies were selected based on their relevance to the pathophysiology, molecular targets, and pharmacological treatment approaches for FXS. Specifically, this article reviewed peer-reviewed original studies, reviews, and case reports, without restrictions on inclusion/exclusion criteria, outcome measures, or sample size.

BENEFITS AND LIMITATIONS OF CURRENT SYMPTOMATIC PHARMACOTHERAPY FOR FXS

Available pharmacological therapies give symptomatic benefit and are effective in improving the quality of life in patients with FXS (16). According to previously published data, between 40% and 90% of individuals with FXS, depending on sex and age, have received a prescription for psychotropic medication (17). In 2012, an extensive national caregiver survey in the US included information about 1064 males and 299 females with FXS, where 61% of males and 38% of females were reported to be taking medication for at least one neurological or behavioral symptom (18). The most often treated symptoms were anxiety, with treatment persisting into adulthood. Attention difficulties and hyperactivity were also common symptoms, usually treated throughout adolescence (18). More recent analysis of data from 975 participants from the Fragile X Online Registry with Accessible Research Database (FORWARD) found that 63% of participants used psychotropic medications (17). Most commonly prescribed drugs were (i) selective serotonin reuptake inhibitors (SSRIs) (43%), (ii) stimulants (38%), and (iii) antipsychotics (33%), and these drugs were more frequently used in males, individuals with ASD as a comorbidity in FXS, and adolescents (17). The listed classes of medication are briefly described below.

Sertraline and other SSRIs are very effective in treating anxiety, which affects around 70% to 80% of individuals with FXS (19, 20). SSRIs inhibit the presynaptic reuptake of serotonin, leading to increased synaptic serotonin levels, which are an essential positive mood regulator in the central nervous system (21). Sertraline can be started for anxiety even in young children, and has been shown to have additional positive effects on language and

motor development (19). Although sertraline is usually well tolerated, children may occasionally exhibit signs of behavioral activation, such as restlessness and excitement, particularly with a rapid upward dose titration (22).

Stimulants are first-line therapy for the treatment of attention deficit hyperactivity disorder (ADHD) in children with FXS who are older than 5 years, characterized by a persistent pattern of impulsivity, hyperactivity, and/or inattention (23). Stimulants primarily exert their effects by increasing the levels of dopamine and norepinephrine in the prefrontal cortex, where they have a significant influence on motivation, attention, and impulsivity (24, 25). In individuals with FXS-associated ADHD, stimulants are effective in approximately 70% of cases, with effective doses similar to those in the general population (26). Stimulants are mostly well tolerated, with rare occurrences of serious side effects like palpitations and high blood pressure (27). In FXS, stimulant doses are kept relatively low because higher doses can suppress language, which is a significant side effect to prevent in nonverbal individuals or individuals with low language abilities (23).

Individuals with FXS often exhibit more intense behavioral issues, such as aggression, self-injury, and severe temper outbursts, especially during adolescence (28). These symptoms can be treated with atypical antipsychotics such as risperidone and aripiprazole, which affect serotonergic and dopaminergic receptors in the CNS (26). Antipsychotics are effective, generally safe, and well-tolerated, but must be used with caution due to potential side effects such as weight gain. Indeed, 30–60% of individuals with FXS may have issues with weight gain (29). Aripiprazole may be preferred over risperidone for its lower risk of weight gain (30).

In addition to the described classes of medications, alpha-2 adrenergic agonists, such as clonidine and guanfacine, are also helpful for the treatment of ADHD symptoms, particularly in individuals not responding or not tolerating stimulants, such as children younger than 5 years (2). These agents activate presynaptic alpha-2 adrenergic receptors, leading to increased norepinephrine levels in the prefrontal cortex, which improves attention modulation (31). Clonidine may be helpful for children with FXS who also have sleep issues (32). Guanfacine is recommended for treating problematic behavior during the day because it causes less drowsiness than clonidine (33). Drowsiness may occur as a side effect after initiating therapy with either drug, particularly with a rapid upward dose titration. In addition, to reduce the risk of rebound hypertension in clonidine use, abrupt withdrawal should be avoided (23).

Melatonin is the primary treatment used to manage sleep problems in FXS, which affects between 27% and 77% individuals and are usually mild-to-moderate (12). Clonidine and guanfacine are other possible options if melatonin is ineffective (2, 23).

Based on previous pharmacological studies, it is evident that more than two-thirds of individuals with FXS have required pharmacological treatment at some point in their lives (17). This highlights the necessity and importance of using pharmacotherapy in the treatment of FXS and its associated symptoms.

Nevertheless, currently available treatments are not specific and only partially alleviate symptoms. Therapeutic effects are variable, and many of the drugs have side effects that can complicate compliance and decrease therapeutic benefit (23). Moreover, complex underlying molecular mechanisms in FXS often require the administration of multiple drugs, leading to a high prevalence of polypharmacy (34).

Although symptomatic pharmacotherapy remains the mainstay of current clinical practice, it fails to treat the root cause of FXS (23). A significant limitation of symptomatic treatment is that it targets downstream behavioral manifestations without correcting the underlying molecular pathology caused by the absence of functional FMRP. As a result, this has led to growing interest in the development of targeted therapies aimed at reversing dysregulated pathways, such as metabotropic glutamate receptor 5 (mGluR5) signaling, gamma-aminobutyric acid (GABA) deficiencies, mammalian Target of Rapamycin (mTOR) activation, and endocannabinoid dysfunction, as discussed in the next section of this review.

MOLECULAR PATHWAYS RELEVANT TO DRUG DEVELOPMENT IN FXS

Numerous studies have demonstrated that low levels or loss of FMRP disrupts brain protein synthesis by changing neural mRNAs, resulting in reduced quantity and integrity of neuronal dendrites and dendritic spines (8, 9, 35, 36). FMRP inactivation may also lead to an imbalance between neuronal excitation and inhibition. The activation of mGluR5 in the absence of FMRP in brain cells promotes the expression of glutamate receptors (37, 38). Enhanced glutamatergic signaling via mGluR5 leads to increased protein synthesis and defects in synaptic plasticity, contributing to cognitive dysfunction and behavioral abnormalities (38-41). In addition to altered glutamatergic signaling, the lack of FMRP has been demonstrated to reduce the synthesis of both GABA and its receptor (40, 42). As a result, an imbalance in these neurotransmitters may lead to disturbances in neuronal plasticity (43). Moreover, dysfunction of the endocannabinoid system has been implicated in FXS pathology (44, 45).

FMRP is also associated with ion channel control and is essential for the functions necessary for effective synaptic transmission (46). FMRP regulates action potentials through the large conductance Ca^{2+}-activated potassium BK channel by interacting with the sodium-activated potassium Slack channels (47-49). In addition, FMRP

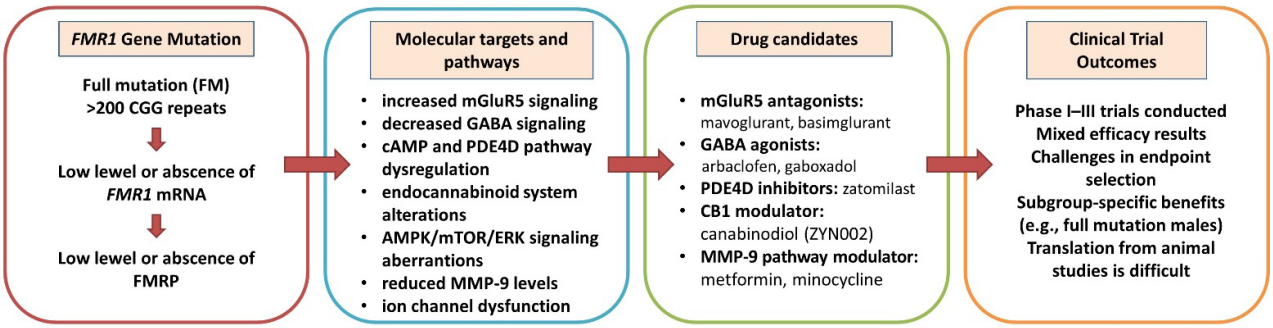

Figure 1. From gene mutation to clinical trials: molecular pathways and targeted therapies in fragile X syndrome

Abbreviations: *FMR1* gene - fragile X messenger ribonucleoprotein 1 gene; FMRP - fragile X messenger ribonucleoprotein; mGluR5 - metabotropic glutamate receptor 5; GABA - gamma aminobutyric acid; cAMP - cyclic adenosine monophosphate; PDE4D - phosphodiesterase 4D; CB1 - cannabinoid receptor type 1; AMPK - AMP-activated protein kinase; mTOR - mechanistic target of rapamycin; ERK - extracellular signal-regulated kinase; MMP-9 - matrix metalloproteinase 9.

regulates the voltage-gated potassium channels Kv3.1b and Kv4.2 (49). In the absence of FMRP, weak synaptic connections cannot undergo sufficient brain plasticity, thereby hindering normal intellectual growth (46).

FMRP also interacts with a large number of mRNA targets that encode proteins involved in synaptic structure and function, such as matrix metalloproteinase 9 (MMP-9) (50-52) and postsynaptic density protein 95 (PSD-95) (53). Finally, modest levels of FMRP were identified in the cell nucleus, indicating that FMRP may have many other previously undiscovered roles, such as DNA expression through DNA stabilization and epigenetic regulation, and DNA damage responses (54, 55).

The increasing understanding of these molecular mechanisms has not only improved our knowledge of the pathophysiology of FXS but also created a compelling framework for the development of targeted therapies in FXS (11). This involves identifying therapeutic targets such as matrix metalloproteinase 9 (MMP-9), mGluR5, GABA receptors, the PI3K-AKT-mTOR and ERK-MAPK signaling cascades, and components of the cAMP pathway (2, 23, 56, 57). Preclinical studies using *Fmr1* knockout mice, zebrafish, and *Drosophila* models have been crucial for identifying and validating therapeutic targets and testing pharmacological agents (58). By targeting the fundamental synaptic and molecular abnormalities of FXS, several promising drugs, including metformin, minocycline, cannabidiol (CBD), and other mGluR5 antagonists, have shown therapeutic promise (23, 56, 57).

While it has been difficult to successfully translate these findings into human trials, due to the variable clinical presentation of FXS, difficulties in selecting appropriate outcome measures, and the need for molecularly stratified trial populations (59, 60), these challenges have prompted the development of precision medicine approaches. Namely, medical interventions are tailored to individual genetic and molecular characteristics, including methylation status and FMRP expression levels (61, 62). Incorporating molecular biomarkers into clinical trial designs has begun to improve therapeutic endpoints and participant selection, ultimately increasing the likelihood of treatment success (61, 63).

As a result, FXS has evolved from a clinically defined disorder to a molecularly characterized condition that exemplifies the promise of mechanism-based therapeutics. As such, it offers a powerful model for translational research and the application of molecular medicine in clinical pharmacology (37).

The following paragraph of this review article critically examines the most important clinical trials and drug development in FXS, based on its molecular landscape and the implications for targeted therapies, incorporating both established knowledge and recent advances. **Figure 1** provides a schematic illustration of the relationship between molecular medicine and clinical pharmacology in the field of FXS.

TARGETED DRUG DEVELOPMENT IN FXS

Metabotropic glutamate receptor 5 (mGluR5) pathway in FXS and related drug development

As mentioned above, one of the most studied molecular pathways in FXS is the mGluR5 signaling pathway. The absence of FMRP in FXS is believed to cause excessive activation of group 1 metabotropic glutamate receptors (mGluR1 and mGluR5), as proposed by the mGluR theory (64). This results in exaggerated long-term depression (LTD) and impaired neural signaling in parts of the brain like the hippocampus, which leads to the cognitive and behavioral symptoms seen in FXS (64).

Promising results from preclinical studies led to human clinical trials with mGluR5 antagonists, aimed at investigating their efficacy, safety, and tolerability. Mavoglurant (AFQ056) was identified as a non-competitive mGluR5 antagonist (65) and was further developed and entered human trials. A small, randomized, double-blind, crossover study of 30 adult males with FXS showed promising results: AFQ056 significantly improved behavior, as

measured by the Aberrant Behavior Checklist-Community for FXS (ABC-C_{FX}), in participants with complete *FMR1* promoter methylation and no detectable *FMR1* mRNA (66). However, the two Phase 2b, double-blind, placebo-controlled trials with mavoglurant failed to reach the primary efficacy endpoint of behavioral improvement, as measured by the ABC-C_{FX}. Studies included 139 adolescents (aged 12 to 17 years) and 175 adults (aged 18 to 45 years) with FXS, who were randomized to receive either a placebo or mavoglurant (25, 50, or 100 mg twice daily) for 12 weeks, after being stratified based on their methylation status (67). Similar disappointing results were obtained with basimglurant, a mGluR5 negative allosteric modulator (NAM). Two Phase II clinical trials, in children aged 5–13 years and in adults and adolescents aged 14–50 years, examined the efficacy of basimglurant based on changes from baseline scores in behavioral symptoms, using the Anxiety Depression and Mood Scale (ADAMS) total score as the primary efficacy endpoint (68). Two dosages of basimglurant (0.5 mg and 1.5 mg) were tested in participants over a 12-week treatment period in these randomized, double-blind, placebo-controlled, parallel-design trials (11, 68). Previous studies with mGluR5 antagonists have pointed to the need for future FXS trials to optimize endpoints, target younger age groups, extend study duration, reduce patient variability, and better control for placebo effects using biomarkers (2).

More recently, FXLEARN, a double-blind, placebo-controlled, parallel-group, flexible-dose, forced-titration design study with AFQ056 (mavoglurant), was conducted. A large multisite trial involving children aged 3-6 years with FXS found that the mGluR5 inhibitor AFQ056 did not show a significant improvement in language abilities compared to the placebo (69). Different publications analyzed blood biomarkers obtained from samples from FXLEARN study participants, including *FMR1* genotyping, methylation, mRNA, MMP-9, and Akt/mTOR pathway markers (70). Also, it found no treatment-related changes, supporting the lack of clinical benefit observed (69, 70).

GABA signaling pathway in FXS and related drug development

Dysregulation of the inhibitory GABAergic system is also involved in the pathophysiology of FXS. An imbalance in neuronal inhibition can contribute to anxiety, sensory hypersensitivity, and seizures, which are frequently present in FXS (2). The absence of FMRP leads to decreased expression of $GABA_A$ receptor subunits, as well as enzymes associated with GABA synthesis and metabolism, including Abat, Gad1, and Gad2 (42, 64).

Arbaclofen, a selective $GABA_B$ agonist, was tested in two Phase 3, placebo-controlled, flexible-dose trials; one in adolescents and adults (aged 12–50 years) and one in children (aged 5–11 years). The primary efficacy endpoint was assessed by using the social avoidance subscale of the ABC-C_{FX}. Arbaclofen failed to meet primary endpoints in both phase 3 trials. Still, in the child study, the group receiving the highest dose (10 mg twice daily) showed significant improvement compared to placebo on the ABC-C_{FX} Irritability subscale and Parenting Stress Index (71). In both studies, side effects were primarily mild, and overall, arbaclofen was well tolerated (71).

Another drug, ganaxolone, a positive allosteric modulator (PAM) of $GABA_A$ receptors, was also tested in a Phase 2 randomized, double-blind, placebo-controlled, crossover trial involving 59 children with FXS, aged 6-17 years. Ganaloxone demonstrated a favorable safety profile; however, there was no statistically significant difference in the primary endpoint, CGI-I, between the ganaloxone and placebo groups. Nevertheless, post hoc analyses showed ganaloxone efficacy in subgroups of participants with higher baseline anxiety and in those with low cognitive abilities (72).

Gaboxadol (OV101), a δ-subunit-selective, extra-synaptic $GABA_A$ receptor agonist, was evaluated for safety, tolerability, efficacy, and optimal dosage regimen in a phase 2a randomized, double-blind, parallel-group clinical study, known as the ROCKET study. The results showed that gaboxadol was generally well tolerated, with no serious adverse events reported. Based on CGI-I scores, approximately 60% of 23 participants were identified as treatment responders (73). These findings support the continued investigation of gaboxadol in larger, placebo-controlled trials, and one such trial is currently ongoing.

Phosphodiesterase-4D pathways in FXS and related drug development

A deficiency of FMRP also leads to dysregulation of cyclic adenosine monophosphate (cAMP) signaling, characterized by reduced levels of cAMP in FXS. cAMP is a crucial molecule regulating synaptic function, and its degradation is mediated by phosphodiesterases (PDEs) (74). Inhibiting PDEs represents a therapeutic strategy aimed at restoring normal cAMP signaling, and PDE inhibitors have shown positive effects on behaviors in animal models of FXS (11).

BPN14770 (zatolmilast), a selective PDE4D inhibitor, was evaluated for safety, tolerability, and cognitive efficacy in a phase 2 randomized, placebo-controlled, two-way crossover trial in 30 adult males aged 18-41 years with FXS (75). The study met its primary endpoint, demonstrating that BPN14770 (zatolmilast) was well-tolerated with no significant differences in adverse events between the treatment and placebo arms (75). Significant improvements were observed in secondary efficacy outcomes, including cognition and daily functioning. Notably, participants demonstrated cognitive benefits on the National Institutes of Health (NIH) Toolbox assess-

ments, specifically in Oral Reading Recognition, Picture Vocabulary, and the Crystallized Cognition Composite scores. Caregiver-reported visual analog scales also indicated clinically meaningful improvements in language and daily functioning (75). This study opened an exciting avenue for future research on this compound, as it is the first clinical trial to demonstrate cognitive improvements in individuals with FXS (2). The EXPERIENCE (Evaluation of Fragile X Experience in Cognition Expression) Clinical Trials, a Phase 2b/3 clinical trial, is currently underway. It consists of two randomized, double-blind, placebo-controlled trials: in adolescent males aged 9-17 years and in adult males aged 18-45 years. The main objective of these studies is to assess the cognitive effects of zatolmilast, using the Crystallized Cognition Composite Score from the NIH Toolbox Cognitive Battery (NIH-TCB) as the primary measure. Secondary endpoints include evaluations of daily functioning, language, emotional/behavioral, and other cognitive domains assessed by the NIH-TCB. Additionally, studies evaluating the safety and tolerability of the drug are conducted.

Cannabinoid pathway in FXS and related drug development

The absence of FMRP leads to alterations in the endocannabinoid system, particularly to reduced levels of endocannabinoids 2-arachidonoylglycerol (2-AG) and anandamide (AEA), as well as desensitization of cannabinoid receptor 1 (CB1). Cannabidiol (CBD), a non-psychoactive compound from cannabis, has emerged as a potential therapy and is commonly used by families to manage anxiety, sleep problems, and tantrums in children with FXS. CBD is a negative allosteric modulator of CB1, which helps restore synaptic balance.

Additionally, CBD can bind to multiple receptors, including those involved in serotonin, dopamine, and GABA signaling, which can repair the imbalance between excitatory and inhibitory signaling observed in FXS (76). ZYN002, a transdermal CBD gel, is the most studied CBD-based treatment in clinical trials for FXS, offering systemic delivery without psychoactive effects of THC. A phase 1/2, open-label, multi-site trial of transdermal CBD (ZYN002) assessed its safety, tolerability, and efficacy in 20 children and adolescents, aged 6-17 years with FXS (77). Treatment with ZYN002 was associated with good tolerability, with only minor adverse effects observed. The study met the primary efficacy endpoint, as measured by a statistically significant reduction in the ADAMS total score. Additionally, the trial achieved its secondary endpoints, demonstrating significant improvements in behavioral symptoms, anxiety, and quality of life (77). These findings led to the CONNECT-FX study, a Phase 3 randomized, double-blind, placebo-controlled trial that included 212 children and adolescents aged 6-17 years with FXS (76). In this 12-week trial, participants received 250 mg or 500 mg of ZYN002 twice daily or a placebo. The primary endpoint, improved social avoidance on ABC-C_{FX}, was not met by the whole cohort. However, a post hoc analysis indicated that participants with *FMR1* gene promoter methylation of 90% or higher experienced significant benefits. In this subgroup, ZYN002 also led to improvement in caregiver-rated social avoidance, social interaction, and irritable behaviors. Consistent with observations from the earlier open-label trial, ZYN002 was found to be safe and well-tolerated (76). Based on learnings from the CONNECT-FX trial, another trial, RECONNECT, is ongoing and is expected to provide further data on the effectiveness of ZYN002. RECONNECT is a phase 3, multi-center, randomized, double-blind trial in children, adolescents, and young adults, aged 3-30 years, with FXS. The RECONNECT trial improves upon CONNECT-FX by focusing on patients with fully methylated *FMR1* genes, expanding to three weight-based dosing levels (250, 500, and 750 mg/day), and extending treatment to 18 weeks. It also utilizes an FXS-anchored Clinical Global Impression of Severity/Change (CGI-S/I) scale, which is anchored on three behavioral symptoms: social avoidance, social interactions, and irritability. The CANAX study is an upcoming randomized, double-blind, placebo-controlled, single-center, crossover trial investigating the effects of an oral CBD solution on anxiety and GABAergic function in individuals with FXS. The study will evaluate the effects of oral CBD on anxiety, disruptive behavior, and behavioral inhibition.

Matrix metalloproteinase-9 (MMP9) molecules as a target in drug development in FXS

Matrix metalloproteinase-9 (MMP-9) is a zinc-dependent enzyme involved in remodeling the extracellular matrix, and it is essential for brain development and synaptic plasticity (78). Normally, FMRP binds to MMP-9 mRNA in dendrites and inhibits its translation (79). The absence of FMRP in FXS leads to excessive local synthesis and activity of MMP-9 at synapses, which causes aberrant synaptic architecture and cognitive impairments (79). Measurement of MMP-9 levels has shown significantly higher active MMP-9 plasma levels in FXS individuals compared to healthy controls (50). In individuals with FXS, a high active MMP-9 level is correlated with aberrant behavior (measured using the ABC-C_{FX}) and an ADAMS score, suggesting its role as a biomarker and therapeutic target (50).

Minocycline, a tetracycline antibiotic known to inhibit MMP-9 activity, has been tested in clinical trials for FXS. An open-label add-on treatment trial has shown significant improvement in ABC-C Irritability Subscale and CGI-I scores in 20 FXS participants treated with minocycline (80). A randomized, double-blind, placebo-controlled, crossover trial further supported these findings.

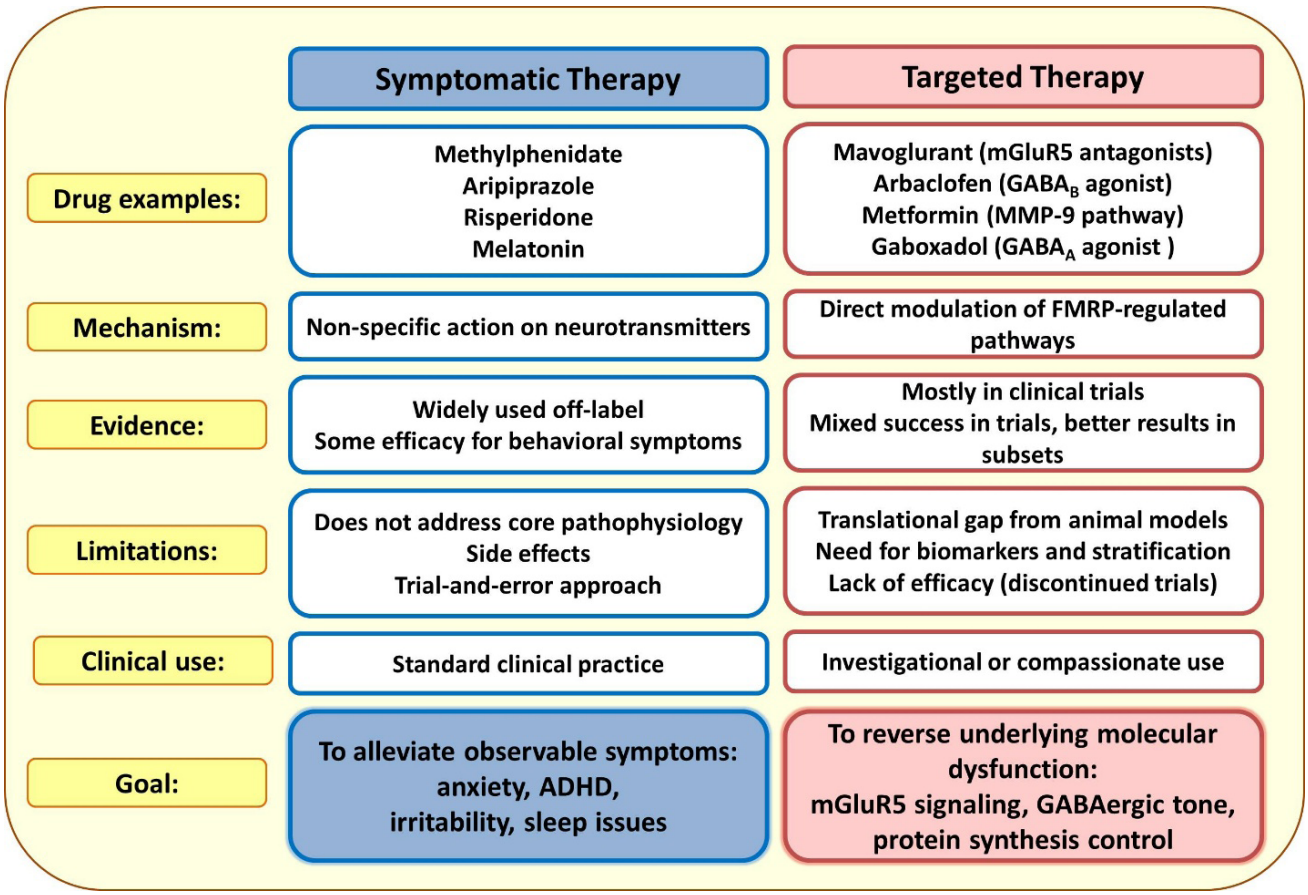

Figure 2. An overview of symptomatic and targeted treatment in fragile X syndrome

Abbreviations: ADHD - attention deficit hyperactivity disorder; mGluR5 - metabotropic glutamate receptor 5; GABA - gamma aminobutyric acid; GABA$_A$- gamma aminobutyric acid type A receptor; GABA$_B$- gamma aminobutyric acid type B receptor; MMP-9 - matrix metalloproteinase 9.

The trial included 66 participants with FXS, aged 3.5 years to 16 years, confirming statistically significant improvement in CGI-I scores, along with greater improvement in anxiety and mood-related behaviors, in the minocycline group compared to the placebo (81).

Metformin, an antidiabetic drug, reduces MMP-9 levels by activating both AMP-activated protein kinase (AMPK)-dependent and independent pathways, leading to suppression of the aberrant mTORC1 signaling observed in FXS (82, 83). The first published case series described improvement in behavior and language in seven individuals with FXS treated with metformin (84). No significant side effects were observed, and metformin treatment led to weight loss as seen in three cases with obesity (84). Another case series of nine children aged 2-7 years with FXS, reported that clinical treatment with metformin improved behavior and language, as measured by improvement in ABC-C$_{FX}$ and Mullen Scales of Early Learning (MSEL) before and after metformin treatment (85). The authors of the publication emphasized the need for a controlled trial of metformin in children younger than 7 years, since their brains are still developing and may benefit more from the medication (85).

Contrary to typical IQ decline seen in FXS, clinical treatment with metformin in two adult men with FXS for one year showed significant cognitive improvement, measured as increases in Full Scale IQ, Nonverbal IQ, and Verbal IQ on the Stanford–Binet Intelligence Scale, Fifth Edition (SB-5)(86). Additionally, significant improvements in communication, social engagement, and behavior, along with better eating habits, were observed in both individuals (86). Protic et al (2019) reported the first case of a 14-year-old pubertal male with FXS, who did not develop macroorchidism after two years of treatment with metformin (87).

An open-label, phase 2 study in 15 individuals with FXS (aged 17–44 years) who received metformin 500 mg twice daily for nine weeks showed a favorable safety profile of metformin in normoglycemic individuals (88). Using transcranial magnetic stimulation to assess excitatory and inhibitory neural mechanisms, the study revealed elevated corticospinal inhibition mediated by GABAergic pathways (88). Recently, a multi-site, double-blind, placebo-controlled trial has been conducted, but the results have not yet been published and are highly anticipated (58). This 4-month trial included individuals aged 6–25 years with FXS. It was designed to evaluate the effects of metformin on expressive language, as well as its potential benefits on challenging behaviors, cognition, eating behavior, adaptive functioning, mood and anxiety symptoms, sleep habits, ADHD symptoms, and overall quality of life. A longitudinal follow-up study, published

in 2024, included individuals with FXS who completed the previously described 4-month clinical trial and were followed up for a period of 1 to 3 years (89). Twenty-six participants with FXS, aged 6–25 years, were recruited from three different sites and treated with metformin at doses ranging from 500 to 1,000 mg twice daily. Baseline and follow-up assessments conducted after at least one year of metformin treatment revealed no significant changes over time in nonverbal IQ and adaptive behavior, as measured by the Leiter-III and Vineland-III, respectively. More importantly, the results indicated stability in cognition and adaptive behavior, suggesting that metformin may help prevent the typical decline seen in FXS. However, the small sample size and short follow-up limited the conclusions (89). Currently, two additional clinical trials are recruiting individuals with FXS: one in Canada for participants aged 6 to 35 years, and another in China for participants aged 2 to 16 years (58). Metformin shows excellent promise as a targeted treatment for FXS; however, controlled trial results are needed to confirm its effectiveness.

An overview of symptomatic and targeted treatment in FXS is presented in **Figure 2**.

CONCLUSION

FXS remains without a cure or an approved targeted pharmacological therapy despite substantial progress in understanding its molecular basis. Current treatments are primarily symptomatic and do not address the underlying mechanisms driven by *FMR1* dysfunction. Advances in molecular medicine are paving the way for more rational and precise drug development approaches in FXS. Integrating clinical pharmacology with molecular insights is essential for translating these discoveries into effective therapies. Continued research into key signaling pathways will be crucial for achieving personalized and disease-modifying treatments for individuals with FXS.

In addition, based on the previous results of failed and successful clinical trials in the field of fragile X, there is a need to reassess outcome measures and revise recommendations for FXS, as presented in the review article published by Budimirovic et al. in 2017 (90). Clinical trials in FXS often failed because appropriate outcome measures had not been fully developed. It appeared that the ultimate success of clinical trials in FXS largely depended on the choice of outcome measures; some trials were judged as unsuccessful not because the treatments were ineffective, but because the selected endpoints were not optimally aligned with the specific challenges of this field. Although progress had been made in creating cognitive and behavioral instruments, most tools remained only of moderate quality, with limited evidence of reliability, validity, and sensitivity to treatment effects. Biomarkers and other objective measures, which could have provided more quantitative endpoints, had advanced slowly, partly due to the industry's reluctance to invest in costly projects with uncertain regulatory approval. As a result, trials relied heavily on parent-reported outcomes in the behavioral domain, which lacked objectivity and consistency. Despite continuous efforts and some tangible progress, the limited availability of validated, clinically meaningful endpoints hindered the demonstration of treatment efficacy and contributed to the failure of past studies.

From a clinical perspective, certain drugs already available on the market (e.g., metformin, sertraline, etc.) and their combinations entered clinical use as off-label therapies for FXS even before results from clinical trials were available. This approach was supported by the clinical experience of experts in the field and the positive effects observed in individual cases. However, once the results of the ongoing clinical trials in FXS are published, we will have much more data to guide our decisions in selecting pharmacotherapy for individuals diagnosed with FXS.

Currently, there are no approved medications that target the root cause of FXS; available pharmacological strategies only address comorbid behavioral and psychiatric symptoms. As summarized in the paper published by Protic and Hagerman in 2023 (23). Gene-based approaches, including antisense oligonucleotide (ASO) therapy, adeno-associated virus (AAV) vectors, and CRISPR-based techniques, are being explored to reactivate or correct the *FMR1* gene. Still, none have yet advanced to clinical trials in FXS. Significant obstacles remain, particularly the delivery of therapeutic agents across the blood–brain barrier, the risk of uneven distribution within brain tissue, vector-related toxicity, and the need for sustained, regulated expression without off-target effects. Advances in nanotechnology, viral vector engineering, and delivery methods are being investigated to overcome these limitations. Importantly, the timing of FMRP restoration is critical, as earlier interventions—ideally before or during the onset of symptoms—may offer greater therapeutic benefit, although this raises complex ethical considerations. Long-term or even lifelong administration may also be necessary, underscoring the need for careful evaluation of safety, efficacy, and feasibility in future gene therapy trials for FXS.

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List of all abbreviations:

2-AG - 2-arachidonoylglycerol
ABC-C_{FX} - Aberrant Behavior Checklist-Community for FXS
ADAMS - Anxiety Depression and Mood Scale
ADHD - attention deficit hyperactivity disorder
AEA - anandamide
AMPK - AMP-activated protein kinase
ASD - autism spectrum disorder
cAMP - cyclic adenosine monophosphate
CB1 - cannabinoid receptor 1
CBD - cannabidiol
FM-full mutation
FMR1 gene - Fragile X Messenger Ribonucleoprotein 1 gene
FMRP - *FMR1* protein
FXS - fragile X syndrome
GABA - gamma-aminobutyric acid
ID - intellectual disability
mGluR5 - metabotropic glutamate receptor 5
MMP-9 - matrix metalloproteinase 9
mRNAs - messenger RNAs
MSEL - Mullen Scales of Early Learning
NAM - negative allosteric modulator
PAM - positive allosteric modulator
PDEs - phosphodiesterases
PSD-95 - postsynaptic density protein 95
SSRIs - selective serotonin reuptake inhibitors

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RAZVOJ CILJANE FARMAKOTERAPIJE ZA FRAGILNI X SINDROM: MOLEKULARNA MEDICINA KAO KLJUČNO SREDSTVO U KLINIČKOJ FARMAKOLOGIJI

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Sažetak

Puna mutacija i epigenetsko utišavanje gena *FMR1* dovode do nedostatka njegovog proteina, FMRP, što rezultira fragilnim X sindromom (FXS). Iako je ostvaren značajan napredak u razumevanju molekularnih mehanizama koje su u osnovi FXS-a, za ovaj neurorazvojni poremećaj još uvek ne postoji lek niti je odobrena ciljana farmakološka terapija. Trenutno lečenje se uglavnom oslanja na simptomatsku terapiju, koja često donosi ograničene koristi i ne utiče na osnovne molekularne uzroke poremećaja.

Ovaj pregledni rad ukazuje na značaj integracije molekularnih saznanja u procesu razvoja lekova za FXS. Prikazan je pregled postojeće farmakoterapije, sa analizom njihovih prednosti i ograničenja, uz naglašavanje potrebe za terapijama koje ciljaju specifične puteve poremećene disfunkcijom *FMR1* gena. Razmatrana su savremena i tekuća klinička ispitivanja, sa fokusom na to kako dublje razumevanje molekularne biologije FXS-a može doprineti razvoju efikasnijih i preciznijih terapijskih strategija. Na kraju, analizirani su ključni signalni putevi uključeni u patofiziologiju FXS-a i diskutovano je na koji način klinička farmakologija i molekularna medicina mogu zajednički doprineti razvoju personalizovanih terapija za osobe sa ovim složenim poremećajem.

Ključne reči: fragilni X sindrom, farmakoterapija, ciljana terapija, *FMR1* gen

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