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Molecular mechanisms for targeted treatments in fragile X syndrome

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Abstract

Fragile X syndrome (FXS) is caused by a full mutation (> 200 cytosine-guanine-guanine (CGG) repeats) in the 5′-untranslated region of the Fragile X Messenger Ribonucleoprotein 1 (*FMR1*) gene, which leads to methylation and silencing of expression, generating the total or partial absence of its product, FMR1 protein (FMRP). When the repetitions are between 55 and 200 CGG repeats, it is called a premutation and is related to a wide spectrum of conditions such as fragile X-associated tremor/ataxia syndrome, fragile X-associated primary ovarian insufficiency, and fragile X-associated neuropsychiatric disorders. High levels of *FMR1* messenger RNAs are implicated in premutation pathophysiology, which differs from the deficiency or absence of FMRP in FXS. In recent years, numerous attempts have been made to find treatments that can counteract the effects of the absence of FMRP and improve symptoms associated with the condition, such as intellectual disability, anxiety, autism, stereotypies, language delay, and aggressive behavior. Here, we review current treatments in addition to targeted treatments that can reverse some of the neurobiological abnormalities in those with FXS. We also review molecular interventions that will hopefully lead to a promising future for those affected by FXS and their families.

Keywords: Rare disease, FXS, treatment, metformin, sertraline, cannabidiol

INTRODUCTION

Much progress has been made in the diagnosis and management of fragile X syndrome (FXS) since it was

first described in 1943 and then known as Martin-Bell Syndrome. In a large meta-analysis, the frequency of the full mutation (> 200 cytosine-guanine-guanine (CGG) repeats) was determined to be 1.4 per 10,000 males and 0.9 per 10,000 females in the general population[1]. However, in different parts of the world, the prevalence can be much higher; for instance, in the village of Ricaurte (Colombia), the prevalence of FXS is approximately 1 per 100 because of a founder effect in this region[2].

FXS is the most frequent cause of intellectual disability (ID) and autism of hereditary origin, caused by an expansion of CGG trinucleotide repeats to ≥ 200 in the promoter region of Fragile X Messenger Ribonucleoprotein 1 (*FMR1*) gene, located on the long arm of the X chromosome at Xq27.3. This full mutation generates silencing through methylation of the gene, leading to the total or partial loss of its product, FMR1 protein (FMRP). This protein is mainly expressed throughout the brain and in the testes, but also in almost all other tissues and organs[3].

The premutation is 55 to 200 CGG repeats in the promoter region of *FMR1* and is usually not methylated. Carriers of the premutation usually produce normal FMRP levels and are not intellectually impaired; however, they do exhibit elevated levels of *FMR1* messenger RNAs (mRNAs)[4]. The elevated mRNA levels lead to toxicity to the neuron and other cells, which causes clinical manifestations such as fragile X-associated primary ovarian insufficiency (FXPOI) (resulting in menopause before age 40)[5], fragile X-associated tremor/ataxia syndrome (FXTAS) (a neurodegenerative disorder)[6], and fragile X-associated neuropsychiatric disorders (FXAND, associated with depression, anxiety, insomnia, chronic pain, and chronic fatigue and social deficits typically in adulthood, but sometimes also in childhood) affecting approximately 50% of carriers[7].

FMRP binds mRNAs, including brain cytoplasmic RNAs and microRNAs, to regulate their transport and translation, interacts with nuclear and cytoplasmic proteins, modulates ion channels, and is present in synaptic compartments, where it controls the translation of specific mRNAs[8]. Structurally, it has three main regions (N-terminal, central, and C-terminal), all of which bind to RNA molecules[9]. FMRP's role in mRNA transport, splicing, and metabolism is important for brain development and aging.

The symptoms and signs related to the total or partial loss of FMRP are variable, but usually compromise all areas of development, including cognitive, behavioral, sensory, verbal, and motor domains, which have lifelong consequences. In addition, characteristic physical manifestations of FXS include an elongated face, prominent ears, joint hypermobility, soft skin, and macroorchidism in puberty. The treatment of FXS manifestations requires complex management, which should be addressed by a multidisciplinary team. Here, we present a review of the literature pertaining to the current optimal therapeutic intervention strategies for children, adults, and their families affected by fragile X conditions. The literature was selected for controlled medication trials in FXS, the inclusion of systematic reviews in this field of study, key search engines, and follow-up studies. Single-case or abstract conference studies were excluded.

Molecular mechanisms of fragile X syndrome

FMRP is a multi-functional mRNA-binding protein that travels to and from the cell nucleus, with a role in the translation, stability, editing, and intracellular transport of hundreds of mRNAs. FMRP also directly interacts with proteins and regulates their function. Additionally, FMRP can regulate RNA synthesis by either controlling the expression of or modulating the activities of transcription factors and chromatin-modifying enzymes, many of which are involved in neurodevelopment and the maintenance of neural connections[10]. In the synaptic space, FMRP is associated with the activation of glutamate receptors, both metabotropic (group 1 metabotropic glutamate receptor (mGluR) 1 and 5) and ionotropic (α-amino-3-

hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) and *N*-methyl-D-aspartate receptor (NMDA)). Its deficit, therefore, translates into abnormal growth and synaptic plasticity[11]. The expression of these receptors is increased in the hippocampus of animals lacking FMRP[12]. Despite the successful use of glutamate receptor inhibitors to correct symptoms such as seizures, hyperreactivity, and neural structural changes in *Fmr1* knockout (KO) mice[12], studies in fully methylated FXS patients with an antagonist of mGluR5 were not successful in adults or children with FXS[13,14].

The endocannabinoid system is also regulated by FMRP, and endogenous cannabinoid ligands such as anandamide (N-arachidonoylethanolamine) and 2-arachidonoylglycerol (2-AG) are postulated to play a role in neuronal development and function[15]. These ligands bind to cannabinoid receptors 1 and 2 (CB1 and CB2) and modulate synaptic activity. CB1 is expressed in the brain and is present at lower concentrations in a variety of peripheral tissues and cells. CB2 receptors are expressed primarily in the immune and hematopoietic system, as well as in the brain, pancreas, and bone[16]. CB1 can be found at high levels in inhibitory terminals [GABAergic interneurons (GABA, γ-aminobutyric acid)] and at lower levels in excitatory terminals (glutamatergic) and both play a role in mood and behavior modulation. 2-AG is the most abundant endocannabinoid in the brain and is produced in dendritic spines by activating mGluR1 receptors. The absence of FMRP in FXS reduces the production of 2-AG, thereby decreasing the activation of CB1 receptors in the central nervous system (CNS)[17]. Administration of cannabidiol (CBD) appears to increase 2-AG availability, thereby increasing CB1 receptor activation and attenuating reduced endogenous cannabinoid signaling[18].

From a different perspective, FMRP modulates the activation of sodium-potassium channels and calcium channels[19,20], and its absence alters the transport and/or translation of specific mRNAs that can influence neuronal excitability.

FMRP is broadly expressed in GABAergic neuron populations, and the GABAergic system is also affected in FXS, leading to an excitatory-inhibitory imbalance. This is caused by reduced expression of GABA receptor subunits and a reduction in GABAergic interneuronal signaling[21], and plays a central role in the pathogenesis of autism spectrum disorder (ASD) and FXS[22]. The GABAergic system normally mediates the inhibitory neurotransmission in the CNS and the lack of FMRP disrupts this system. It was implicated in the pathogenesis of FXS based on studies of GABA A receptor expression in *Fmr1* KO mice[22]. Alterations in the GABAergic system increase neuronal excitability and further enhance the imbalance of excitation and inhibition. This imbalance leads to enhanced sensitivity to repeated sensory stimuli, a lack of habituation to repetitive stimuli, and subsequent sympathetic hyperactivation, causing anxiety in these patients[23,24].

The normal morphology of dendritic spines also derives from physiological processes modulated in part by FMRP activity. Its absence or decrease leads to a pathological hyperabundance of long, thin immature dendritic protrusions, which result in an abnormal post-synaptic maturation and a failure in the synapse elimination process[25].

Another association studied is the relationship between FMRP and metalloproteinase 9 (MMP-9) levels. MMP-9 is an enzyme that encodes an endopeptidase important for the maturation of dendritic spines and synaptic formation. MMP-9 levels are elevated in FXS because FMRP, which inhibits the translation of the MMP-9 mRNA, is missing[26]. However, MMP-9 levels can be lowered to normal with minocycline treatment[27,28]. In fact, in the *Fmr1* KO mouse, treatment with minocycline improved the maturation of dendritic spines, synapse formation, anxiety levels, and cognitive performance, as well as ultrasonic vocalizations[29,30]. In addition, metformin may decrease MMP-9 levels in the *Fmr1* KO mouse[31]. Such

studies led to the successful trial of minocycline in the treatment of FXS[28].

This knowledge has made it possible to generate therapeutic interventions with multiple medications that are already being applied in clinical practice or are in clinical trials.

Coping with FXS children and their family inheritance and social environment

The complexity of the children affected by FXS and the impact on their family and social environment requires that this condition be addressed by a multidisciplinary team guided by a developmental specialist (pediatrician, neurologist, or child psychiatrist), ideally with experience in FXS.

Treatment will then require pharmacological and non-pharmacological therapy such as speech and language therapy, occupational therapy, physical therapy, counseling, or behavioral interventions such as applied behavior analysis (ABA) and a healthy lifestyle[32]. The inheritance of the fragile X mutation through the pedigree needs to be explained to the family. If a child has the full mutation, the mother is always the carrier because the expansion to ≥ 200 CGG repeats occurs only when inherited from the mother. The mother usually has a premutation, but on occasion, she may have a full mutation, in which case she needs to undergo the fragile X DNA test herself. She has approximately a 50% probability of passing on the full mutation to her children because rarely do both X chromosomes carry the mutation. If her premutation is > 90 CGG repeats, then it will expand to a full mutation in the next generation. For premutation carriers with between 55 and 90 repeats, there is an increasing risk of progressing to a full mutation as the repeat number increases. Usually, after every 10 CGG repeats, there is an AGG anchor, and the presence of one or two anchors in the mother will decrease the risk of expansion to the full mutation up until 90 to 100 repeats, when the risk of expansion during transmission to the next generation reaches 100%[33,34].

If the family has a male premutation carrier, often the father of the mother, then this male will pass on the premutation to all his daughters. Therefore, it is helpful to request fragile X DNA testing for the grandparents because if the grandfather is the carrier, then all of the mother's sisters will be carriers too, and they will be at high risk of having children with FXS. In addition, premutation carriers need to know the risk of premutation disorders. FXPOI occurs in about 20% of female carriers[35]; FXTAS occurs in about 40% of aging male carriers and 16% of aging female carriers[6], and FXAND occurs in up to 50% of carriers[7,36,37].

Cognitive-behavioral challenges and neurological symptoms

Patients with FXS may present with significant behavioral problems, which usually manifest with greater intensity as they advance in their development, including tactile defensiveness, poor eye contact, hand flapping, hand biting, clothes chewing, hyperactivity, impulsivity, anxiety, aggressive behavior, and sensory over-reactivity from the second year of life onward. The manifestations of these problems include overfilling or stuffing their mouths with food, and this often translates into obesity. Autism (50%-60% of men, 20% of women)[38] or social deficits and attention deficits with or without hyperactivity (80% of boys and 30% of girls) are also more frequent than in the general population[9]. Often, in early childhood, sleep difficulties can be seen as well[39]. Physical therapy, occupational therapy, speech therapy, and ABA[40] for ASD are essential within non-pharmacological interventions[41].

ABA is a behavioral intervention commonly used as a therapeutic treatment for ASD. Its premise is to reinforce the positive socially involved actions of the child. The Early Start Denver Model (ESDM), another model of behavioral intervention, has ABA principles, but focuses on verbal and social skills through interactions with young children aged 1 to 3 in the family environment[40,42,43].

Anxiety is a cardinal symptom in FXS, especially after 2 years of age, and can increase with age, and it can also correlate with ASD[24]. Low doses of sertraline, a selective serotonin reuptake inhibitor (SSRI) that prevents the reuptake of serotonin from the synaptic space, has shown significant improvement in anxiety, visual perception, and fine motor coordination, and better T score on the Mullen scale for development in a controlled trial of young children (2 to 6 years of age) with FXS ages[44]. Those who do not respond to SSRIs, or who exhibit aggressive behaviors (potentially harming others or themselves), or who exhibit violent reactions, may respond well to the use of an atypical antipsychotic such as aripiprazole or risperidone, both of which are approved for aggression and irritability in ASD[45]. These atypical antipsychotics can block dopamine but also stimulate the serotonin receptors, leading to improvement in aggression and irritability, decreased hyperactivity, and less anxiety in FXS[46]. Cognitive behavioral therapy has proven helpful for emotional dysregulation in FXS[47,48].

Other neuropsychiatric manifestations of FXS include social or specific phobias, ritualistic and compulsive behaviors, restricted interests, aggressiveness, stereotypies, and self-harming behaviors. Early and intensive psychological and environmental interventions can play a positive role in the management of anxiety, attention-deficit hyperactivity disorder (ADHD), social challenges, and depression[46].

Most men with FXS present ID; however, up to 15% (predominantly those with mosaicism) and 70% of women may have an intelligence quotient (IQ) that is normal or close to normal but still have learning difficulties and emotional control problems. Cognitive abilities in FXS are known to decline with age in childhood and adolescence[49]. Most adult men with FXS have cognitive abilities around an IQ of 40 unless they are mosaic with a partial lack of methylation in the full mutation or a significant percentage of cells with the premutation. The size or methylation mosaicism is associated with a higher IQ than in those with a full mutation that is fully methylated because these mosaic individuals produce a higher level of FMRP[50,51]. This is the case of females with a full mutation, because their normal X chromosome produces FMRP and the amount of FMRP depends on the activation ratio (AR), the percentage of cells where the normal X chromosome is the active one, usually measured in blood. For example, an AR of 0.75 means that in 75% of the white blood cells, the normal X chromosome is the active chromosome, and usually the IQ is relatively high. Approximately one-third of girls with the full mutation have an IQ <70, one-third have an IQ in the borderline range (70 to 85), and one-third have an IQ in the normal range (above 85)[52].

By late adulthood, up to 17% of men with FXS may develop parkinsonian symptoms, often combined with some additional cognitive impairment[53].

Seizures and ASD

Seizures can also be associated with FXS, usually complex partial, although they can evolve into generalized tonic-clonic or absence seizures[54]. Seizures are common in FXS and up to 14% of males and 6% of females have them. Seizures often begin between the ages of 4 and 10 and data showed that autism was significantly associated with seizures as a co-occurring condition[55]. For treatment, levetiracetam and oxcarbazepine are used as the first line of treatment. Valproate is another choice and has a mood-stabilizing effect, allowing the improvement of symptoms of aggression or lack of control in some cases[56].

Growth, connective tissue, and involvement of other systems

People affected by FXS may have different associated medical conditions from the spectrum of connective tissue disorders. These include flat feet, scoliosis, joint subluxations, mitral valve prolapse, dilated ureters, and vesicoureteral reflux[57]. It is important to rule out aortic root dilation (25%) or mitral valve prolapse (3%-20%) if a murmur is heard on auscultation. Another possible finding of the cardiovascular system is the decrease in parasympathetic vagal tone, especially in children who are hyperaroused. Hypertension is

common in obese patients and in adulthood, and this may be related to enhanced anxiety. If hypertension or a murmur is heard, then referral to cardiology is required, where an evaluation, including an ultrasound, is carried out that can document mitral valve prolapse or other cardiac problems. It is worth mentioning that carriers of the premutation may also present with cardiovascular symptoms such as arrhythmias and dysautonomia[58].

Obesity occurs in about 35% of those with FXS before they reach adolescence, especially in those with hyperphagia and a lack of satiation after meals[59]. In about 10%, these behavioral features can lead to a Prader-Willi-like phenotype with severe obesity; low cytoplasmic FMRP-interacting protein (CYFIP) levels have been reported as a biomarker in these cases by Nowicki *et al.*[60]. These children present with severe hyperphagia and early obesity (6-9 years) and typically have small genitalia and delayed puberty. Knowing the increased risk of obesity, it is important to encourage regular physical exercise, ideally daily, and to promote a healthy diet by avoiding foods rich in saturated fats and sugar. The therapy is complex and requires multidisciplinary support from experts in the field for behavioral interventions and medications.

Annual pediatric health checkups should include otoscopy and appropriate treatment of otitis media, which is often recurrent in the first years of life. It is recommended to have early and frequent hearing screens, especially if there is a history of recurrent ear infections, to avoid a greater impact on language acquisition[61]. Children with FXS may have difficulty communicating discomfort or pain, so in the face of otitis, they may hit their head, and this may be the only outward symptom of infection. Recurrent otitis media infections often require insertion of pressure equalizer tubes and sometimes adenoidectomy and/or tonsillectomy.

An evaluation by an ophthalmologist is recommended between 3 and 4 years of age to evaluate and correct visual acuity problems (astigmatism, hypermetropia, strabismus). If strabismus is noted (it occurs in about 20% of FXS patients), it should be evaluated as early as possible to avoid the development of amblyopia. Nystagmus, convergence insufficiency, and eyelid ptosis have also been reported but are less common[54,56].

In the gastrointestinal system, loose stools are usually the norm in FXS, although in some rare cases, constipation may occur. Toilet training is typically delayed because of sensory problems and cognitive deficits; however, the biggest problem is training the patients to wipe themselves successfully after a bowel movement. Occupational therapy is often helpful in addressing this problem. Gastroesophageal reflux is more common in infants with FXS (leading to frequent emesis) than in the general population. These symptoms should be screened carefully, diagnostic studies performed, and treatment implemented if appropriate[62].

Therapies

Almost all young children with FXS need early intervention beginning by 2 years of age or earlier, and they benefit from speech and language therapy, occupational therapy, and physical therapy[41]. In general, motor involvement is one of the first findings noticed in early development. Most children affected by FXS are hypotonic, which will translate into delayed onset of motor milestones such as the ability to sit without support and the onset of walking. In the neonatal and infant period, they may have difficulties in sucking and frequent regurgitation. Physical therapy and occupational therapy are important for supporting motor development.

They usually have poor language development, and some children, especially boys and those with autism as a comorbidity, may be completely nonverbal for several years. Speech problems of males with FXS include

variability in rate and stuttering-like repetition of sounds. Also notable among the features displayed by males is perseveration on a word, phrase, or topic in conversation, which is probably related to the hyperarousal and frontal-lobe-executive function deficits[63]. Speech therapy is incorporated as a central element of intervention in the first years of life. Prompts for restructuring oral muscular phonetic targets (PROMPT) therapy can be helpful for the nonverbal child at 2 years of age, and this therapy involves tactile stimulation to the mouth to facilitate the expression of language[64]. For those who continue to be nonverbal, sign language can be a bridge to oral communication, in addition to the use of augmentative and assistive communication devices, which are usually initiated by the speech and language therapist[41].

School integration programs with special education are necessary for these children when accessing the school system. Resources or plans for these children can be found on online portals such as www.fragileX.org, www.fragilex.org.uk, or www.fragilex.org.au.

PSYCHOPHARMACOLOGY

Attention-deficit hyperactivity disorder and treatment

As previously noted, ADHD can be diagnosed in approximately 80% of boys and about 30% of girls with FXS. However, the usual treatment of ADHD with stimulants can cause irritability when given to children less than 5 years old with FXS[46]. For those who are 5 and older, stimulants are usually tolerated well, especially the long-acting preparations, when once-a-day dosing in the morning leads to a stable level in the blood during the day. These first-line medications improve norepinephrine and dopamine levels at the synapses in the prefrontal cortex, which play a role in improving motivation, attention, and impulse control. For children under 5 years of age with excessive hyperactivity, guanfacine or clonidine may be considered. Clonidine is especially helpful for sleep problems when given at bedtime because of its sedative effect. Guanfacine has an overall calming effect, which helps reduce hyperactivity and hyperarousal when given in the morning and in the afternoon after school. In some countries, such as Italy, stimulants are not authorized; therefore, alternatives for attention-deficit symptoms are L-acetylcarnitine or valproic acid[65,66].

In general, the use of stimulants such as methylphenidate or mixed amphetamine salts should start at a low dose, and then follow-up visits should follow weight and vital signs, particularly blood pressure, since both can be affected in children with FXS. If weight loss occurs, then the dose should be lowered or even discontinued on weekends, and increased food intake can occur before bed when the medication has worn off. In general, the stimulants are well tolerated; however, it is important to note that at higher doses, they can have a quieting effect on language. Therefore, it is advisable to avoid administering high doses[46].

Sleep

At each medical visit, it is suggested to check sleep quality and time because these parameters influence the development, learning, and functioning of any child, including those affected by FXS. Children under 3 years of age with FXS often have wakefulness at night and may get up and search for their parents. Seizures can also occur at night in about 15% of young children with FXS[67]. Sleep apnea can be present in young children as well, especially if there is a history of snoring and obstruction heard at night by the parents. Referral to an ear, nose and throat specialist and a sleep study can clarify if sleep apnea is occurring, and an adenoidectomy/tonsillectomy typically eliminates sleep apnea in a young child with FXS.

Melatonin is the most effective medication as a primary treatment of sleep awakening in young children with FXS[68]. Not only does melatonin play a role as a sleep inducer, but it also has antioxidant properties, and it facilitates neuronal plasticity[69]. Melatonin should not be given in the daytime because it can cause drowsiness.

Targeted treatments for fragile X syndrome

Most of the targeted treatments have been promoted in the last decade and are supported by research conducted in *Fmr1* KO animal models of FXS and the neurobiological effects that this entails. The current objectives are to be able to, on the one hand, reactivate the damaged gene and, on the other hand, replace the effects of the absence of the FMRP protein.

Early attempts used negative modulators of mGluR5 receptors (mGluR antagonists), since this pathway is upregulated in FXS. Favorable results have been shown in animal studies using these antagonists, but in humans, the results have been negative[70-72]. Even the use of the mGluR5 antagonist AFQ056 in young children with FXS combined with parent-implemented language intervention (PILI) has not demonstrated efficacy over a prolonged period of time in a controlled trial[73].

GABA A and GABA B receptor agonists can reverse some of the symptoms in the animal model of FXS and would be useful under the theory that GABA pathways are also affected in individuals with FXS and that problems such as seizures and sleep disturbances are associated with this pathway. FMRP acts as a modulator of these GABA receptors. Clinical studies with arbaclofen (a selective GABA B agonist) showed improvement in the symptoms of social isolation and behavioral problems reported by parents of patients with FXS, compared to a placebo[74]; however, in phase 3 studies with adolescents and adults, arbaclofen did not show improvement[75]. In the group aged 5 to 11 years, an observable improvement was maintained in the application of the Aberrant Behavior Checklist-Community Edition, factored for FXS (ABC-C FX) test[76]; therefore, arbaclofen will be studied again in FXS by the Allos company. Acamprosate (a positive allosteric modulator of the GABA A receptor)[77] and metadoxine (an indirect GABA activator) have been tested in small placebo-controlled groups of FXS individuals. Metadoxine has shown improvement when evaluated with the Vineland Daily Living Skills subscale and in the computerized cognitive Test of Attentional Performance for Children (KiTAP) test (executive function tasks) compared with individuals who received placebo[78]. Ganaxolone has shown improvement in anxious symptoms in a specific subgroup of participants with greater severity of anxiety; however, it was not efficacious for the overall group of FXS[79]. A placebo-free study of three doses of gaboxadol, a GABA A receptor agonist, was carried out in 28 adolescent and adult males with FXS, and there was a 60% response rate, suggesting that further studies are warranted in a controlled trial with placebo[80].

Minocycline, an antibiotic of the tetracycline family, acts by lowering MMP-9 expression and activity levels, which, in turn, can improve the maturity and strength of synaptic connections in the *Fmr1* KO mouse[29]. One controlled study demonstrated significant improvement in behavior in children with FXS measured with the Clinical Global Impression Scale and the Visual Analogue Scale[28]. Thus, this medication is used in clinics to help with behavioral problems, anxiety, and attention in children with FXS at times. Other benefits of minocycline are its antioxidant power and anti-apoptotic effects. Unfortunately, it has certain side effects, such as the permanent darkening of the teeth when used in children under 8 years, and sometimes an increase in the levels of antinuclear antibodies that can trigger a lupus-like syndrome with rash or inflammation of joints that reverts when treatment is stopped. Therefore, if minocycline is used, the antinuclear antibodies should be tested every 6 months or once a year if the patient is stable without side effects.

Another drug being investigated is trofinetide, a terminal analog of the tripeptide of the insulin-like growth factor, which can decrease abnormal extracellular signal-regulated kinase (ERK) and protein kinase B (Akt) activity, normalizing the phenotype in the *Fmr1* KO mouse. An exploratory, phase 2, multicenter, double-blind, placebo-controlled, parallel-group study of the safety and tolerability of orally administered

trofinetide in adolescent and adult males with FXS was performed. Trofinetide was well tolerated and showed a consistent signal of efficacy at the higher dose, as observed in caregiver and clinician assessments, despite the relatively short treatment duration[81].

Lovastatin, a statin, is another drug under study. In *in vitro* studies, it corrected excessive protein synthesis in the hippocampus of the *Fmr1* KO mouse and, when administered orally or injected, was able to inhibit the expression of audiogenic-induced seizures. Being approved by the U.S. Food and Drug Administration (FDA) and widely used in adults and children for the management of hypercholesterolemia, it was exciting to continue testing it as a specific therapeutic alternative for FXS. Unfortunately, a controlled trial of lovastatin combined with an open-label PILI in youth with FXS did not show greater benefit than the use of PILI alone[82].

Metformin

Another specific targeted treatment for FXS is metformin, a first-line medication for type 2 diabetes that acts by decreasing hepatic glucose production, decreasing intestinal glucose absorption, and increasing insulin sensitivity, leading to lower plasma glucose levels. In the *Fmr1*-KO mouse, metformin has been shown to reduce ERK signaling and decrease the levels of phosphorylated eukaryotic translation initiation factor 4 (EIF4E) and MMP-9[31]. Metformin can also alleviate features of FXS in the *Drosophila* model of FXS by lowering the upregulation of the mammalian target of rapamycin (mTOR) pathway observed in FXS[83]. Metformin is also known to reduce obesity, especially in children and adults with ASD who have gained weight because of antipsychotic use[84]. Initially, metformin was tried in patients with FXS and Prader-Willi-like phenotype with obesity, and it helped to reduce the obesity[85]. In addition, it was tried in FXS without obesity and the families saw an improvement in language and conversational abilities. In young children aged 3 to 7 years, an open-label trial of metformin improved both behavior and cognitive abilities on the Mullen Scales of Early Learning compared to the early development of FXS documented in the literature[86]. Subsequent cases of FXS have demonstrated improvement in the IQ in two adult males treated clinically with metformin[87], and another case reported that macroorchidism did not develop when metformin was started before puberty[88]. Currently, a controlled trial of metformin treatment over 4 months for individuals with FXS from ages 8 to 45 is taking place at three centers: the MIND Institute at the University of California, Davis (USA), the University of Edmonton in Alberta (Canada), and the St Justine Hospital in Montreal (Canada). Results should be available in early 2024. Because of clinical reports demonstrating its benefits, metformin is often used clinically and we all await the results of the controlled trial.

Cannabidiol

CBD has antioxidant, anti-inflammatory, and neuroprotective effects; therefore, it could be useful in patients with neurodevelopmental disorders where neuroinflammation and immune dysregulation play a pathogenic role, such as in ASD[89]. Moreover, its anxiolytic effect, via GABA enhancement and activation of the CB1 receptor, has been shown to improve anxious symptoms in patients with autism[90]. Its positive medicinal effects include the control of seizures in children with Lennox-Gastaut syndrome and Dravet syndrome[91]. Moreover, parents of children with refractory epilepsy reported improvement in attention and behavior when treated with CBD[92].

Zynerba has manufactured CBD that is free of tetrahydrocannabinol and has carried out a twelve-week controlled trial of a topical ointment in children with FXS at a dose of 250 mg to 500 mg (weight-dependent) rubbed on the shoulder skin twice a day. This topical preparation of CBD demonstrated significant benefit for the primary outcome measure of Social Avoidance, a subtest of the ABC-C FX test in

children with >90% methylation[93]. However, because it did not demonstrate efficacy for those with FXS who were mosaic, the FDA asked Zynerba to carry out a second treatment study where the molecular subgroups were further studied, and this trial, called RECONNECT, is currently ongoing at multiple international sites. CBD not only improved social avoidance but also benefited anxiety and outburst behavior, resulting in improved quality of life for the families, who found that they could go on outings with their children without severe behavioral manifestations. However, this medication is currently not available from *marijuana* stores, and the closest alternative is hemp-derived CBD, which has a very low level of tetrahydrocannabinol (< 1%) and is typically available in tinctures or gummies[94]. CBD may also be helpful for premutation carriers who often have symptoms of insomnia, anxiety, and chronic pain associated with FXAND[36].

Phosphodiesterase 4D inhibitors: BPN14770

Perhaps the most exciting treatment study in FXS was the use of BPN14770, an inhibitor of cyclic adenosine monophosphate (cAMP) breakdown, as cAMP levels are deficient in FXS. This inhibitor raised cAMP levels and improved cognition substantially in a controlled trial. Thirty adults with FXS were treated for 14 weeks with either BPN14770 at a dose of 25 mg twice a day or a placebo[95]. The cognitive outcome measure was the National Institutes of Health (NIH) Toolbox, which had been modified by Dr. Hessl ęĬ ĕ̊. to be useful in individuals with ID[96]. They found that multiple measures on the NIH Toolbox improved in those treated with BPN14770 compared to placebo, including Oral Reading Recognition, Picture Vocabulary, and the Crystallized Cognition Composite Score, in addition to the parents' documentation of improvements in language and daily functioning on the Visual Analogue Scale. Such significant improvements in cognition in adults with FXS have never been seen before; therefore, this medication has brought hope to the FXS field that cognitive deficits can be improved even in adulthood. Currently, phase 3 controlled trials of BPN14770 are underway in adolescents and adults with FXS at multiple centers, and by the end of 2023, such trials will likely take place in children with FXS.

Gene therapy

Advances in DNA technology have been used to correct CGG expansions in pluripotent cells. Eventually, in the future, these cells will be introduced into the CNS of patients affected with FXS to correct the expansion. Recently, an approach to correct the genetic defect in FXS has been described via recruitment of endogenous repair mechanisms that then drive excision of the long CGG repeat. FMRP restoration through *de novo* transcription can be induced by demethylation and resolution of the aberrant R-loops in the *FMR1* gene in cellular models, identifying a potential method for treating FXS in the future[97]. A study that sought to deliver antisense oligonucleotides into the brain of FXTAS-affected mouse models showed reduced inclusion formation, improved motor response, and correction of gene expression profiling[98]. These studies raised hope for the development of future curative treatments, and it is exciting to witness the development of therapeutic alternatives that until recently were unthinkable.

CONCLUSION

Animal models and advances in understanding the molecular dysregulation present in FXS have led to several targeted treatments described here that have improved the lives of many patients with this condition. Support and intervention for patients should extend to their family and environment. The quality of life of each of these individuals will be affected differently depending on the level of ID, behavioral challenges, the presence of aggressive or disruptive behaviors, and the comorbidities present in both the patient and their caregivers. Parents can be affected emotionally, having to assume the responsibility of ensuring the health of their child, and battling chronic stress or major depression, especially those caregivers who have the premutation with FXAND and/or FXPOI. Family support is a fundamental pillar in

therapeutic approaches. PILI is an alternative that strengthens communication channels between parents and children. It is recommended to provide counseling, parent group support, educational alternatives, and psychological and/or psychiatric intervention when necessary.

DECLARATIONS

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Authors' contributions

Wrote the first draft of this paper: Miranda I
Added additional information and edits: Hagerman R

Availability of data and materials

Not applicable.

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Conflicts of interest

Dr. Hagerman R has received research funding from Zynerba to conduct a CBD clinical trial in individuals with Fragile X syndrome and from the Azrieli Foundation to conduct a metformin trial. These grants are unrelated to the present study, and the sponsors had no role in the study design, data analysis, or manuscript preparation. The sponsors had no role in the study design, data analysis, or manuscript preparation. Dr. Miranda I declares no conflicts of interest.

Ethical approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

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