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New Targeted Treatments for Fragile X Syndrome

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Abstract: Fragile X Syndrome (FXS) is the most common cause of inherited intellectual disability with prevalence rates estimated to be 1:5,000 in males and 1:8,000 in females. The increase of >200 Cytosine Guanine Guanine (CGG) repeats in the 5' untranslated region of the Fragile X Mental Retardation 1 (*FMR1*) gene results in transcriptional silencing on the *FMR1* gene with a subsequent reduction or absence of fragile X mental retardation protein (FMRP), an RNA binding protein involved in the maturation and elimination of synapses. In addition to intellectual disability, common features of FXS are behavioral problems, autism, language deficits and atypical physical features. There are still no currently approved curative therapies for FXS, and clinical management continues to focus on symptomatic treatment of comorbid behaviors and psychiatric problems. Here we discuss several treatments that target the neurobiological pathway abnormal in FXS. These medications are clinically available at present and the data suggest that these medications can be helpful for those with FXS.

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1. INTRODUCTION

Fragile X Syndrome (FXS) is the most common cause of inherited intellectual disability with prevalence rates estimated to be 1:5,000 in males and 1:8,000 in females [1]. Individuals with 55 and 200 CGG repeats carry the premutation, which results in excessive transcription of the *FMR1* gene, but are usually not intellectually impaired. An increase of >200 CGG repeats gives rise to the full mutation and FXS. The full mutation results in transcriptional silencing of the *FMR1* gene with a subsequent reduction or absence of fragile X mental retardation protein (FMRP), an RNA binding protein involved in the maturation and elimination of synapses. FMRP is important to dendritic maturity and synaptic plasticity, and its reduced levels, therefore, lead to intellectual impairment and FXS [2, 3].

Physical features have been described but are often non-specific, making diagnostic testing based on alterations found in the *FMR1* gene essential for the diagnosis of FXS. Common physical and medical features in FXS include

increased risk for chronic otitis media, esotropia, hyperextensible finger joints, long face, prominent ears, high arched palate, low muscle tone, seizures (occurring in 16% of patients with FXS) and macroorchidism with puberty [4]. As FXS is an X-linked disorder, the symptoms manifest markedly in males, who commonly present with moderate to severe cognitive impairment. Females have two X chromosomes with variable activation ratios and are thus generally less affected, presenting with a spectrum of impairments from mild learning difficulties to intellectual disabilities [5]. The behavioral phenotype involves poor eye contact, excessive shyness, anxiety, hand flapping, hand biting, aggression, tactile defensiveness, attention deficits, hyperactivity, impulsivity, hyperarousal to sensory stimuli, and autism spectrum disorder [6]. These symptoms are hypothesized to be caused by an altered balance in excitatory and inhibitory neurotransmission and by the absence of FMRP's effect on synaptic plasticity and activity-dependent protein translation [7].

Pharmacological approaches have comprised the focus of treatment due to the biological cause of FXS. However, no currently approved curative therapies exist, and clinical management continues to focus on symptomatic treatment of comorbid behaviors and psychiatric problems. There have been several clinical trials in FXS, as well as multiple recent reviews thereof [4, 8, 9]. Notably failed trials in FXS include

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the mGluR5 antagonists [10, 11]. These trials demonstrated a high rate of placebo response and did not involve quantitative outcome measures that could directly assess the brain's response to treatment to give unbiased results. Improved outcome measures are now in place for most newer clinical trials [4] to address these concerns. Lately, increasing emphasis on trials in young children with FXS reflects a recent effort to influence brain structure and development early on. For instance, the mGluR5 antagonist AFQ056 is now being studied in children 3 to 6 years old in a randomized controlled trial that also involves intensive Parent Implemented Language Intervention (PILI) with a speech and language therapist twice weekly *via* video call (NCT02920892).

Here we discuss several medications that are currently available for off-label treatment of FXS, along with the data that support their therapeutic potential. While many other medications such as stimulants, alpha agonists, and atypical antipsychotics can also be used effectively to treat behavior problems in FXS, this discussion will be limited to modulators of the abnormal neurobiological pathways in FXS where there is evidence that the abnormalities are at least partially reversed. [6]. The term targeted treatment was originally introduced to describe molecular treatment in cancer; it has been adopted by other areas of research to elucidate developing treatment modalities targeting specific abnormal pathways, in our case in FXS.

2. CURRENTLY AVAILABLE TARGETED TREATMENTS FOR FRAGILE X SYNDROME

2.1. Sertraline

Sertraline, a selective serotonin reuptake inhibitor (SSRI), is widely used to treat anxiety in patients with FXS, often starting in the second or third year of life as symptoms emerge. There is a deficit in serotonin production in the brains of young children with autism [12, 13], and metabolomic studies of lymphoblastoid lines of all types of ASD, including those with FXS, demonstrate down-regulation of the enzymes leading to serotonin production from tryptophan [14]. Sertraline may therefore be considered a targeted treatment for FXS.

A retrospective study of language development using the Mullen Scales of Early Learning (MSEL) demonstrated that the trajectory of both receptive and expressive language development was significantly improved in young children with FXS between ages 16 to 60 months who received low-dose sertraline (2.5 to 5.0 mg per day) compared to those who did not receive sertraline [15]. Subsequently, a controlled trial of low-dose sertraline was carried out in 57 children ages 2 to 6 years with FXS, 60% of whom had comorbid ASD [16]. Although the primary outcome measures of expressive language MSEL age equivalent score and the Clinical Global Impression-Improvement (CGI-I) did not show efficacy, there was a significant improvement in the secondary measures, including the fine motor and visual perception scales and the composite T score sum on the MSEL, in those treated with sertraline versus placebo. In a post hoc analysis, among 60% of patients with comorbid ASD, there was a significant improvement in the expressive language MSEL scale [16]. Furthermore, of the 22 patients

able to carry out the Passive Viewing Eye Tracking (PVET) task at baseline and follow-up, there was a significant improvement in the measure of receptive language on this task when the study patient was on sertraline compared to placebo [17]. Notably, in these children, the receptive language MSEL scale did not improve, suggesting that the PVET task was able to detect receptive language benefits with higher sensitivity than other measures. Sertraline is now often used to both treat anxiety and to enhance early development in children with FXS.

2.2. Metformin

Metformin, a biguanide antihyperglycemic medication utilized most commonly for type 2 diabetes and weight loss, is derived from galegine, which is a natural product isolated from the plant *Galega officinalis*. This plant was used in herbal medicine in medieval Europe and by the ancient Egyptians [18]. In the 1920s, metformin was first synthesized and tested, but its clinical use as an antidiabetic drug began in the 1950s [19, 20]. Metformin became available in the United States in 1995. Throughout metformin's 60 years of clinical use, there have been thousands of studies regarding its varied mechanisms of action [18]. Before molecular studies with metformin were possible in the current era, this medication was established as a safe and effective therapy for diabetes mellitus type 2 through clinical studies alone [21, 22].

After oral dosing in humans, approximately 70 to 80% of metformin's dose is absorbed in the small intestine (duodenum and jejunum) [23]. The mean peak plasma concentration (C_{max}) after an oral dose occurs at about 2 hours. When metformin is used in clinical doses, steady-state plasma concentrations in the low micromolar range (< 1 µg/mL) are reached between 24 and 48 hours. Metformin does not bind to plasma proteins and crosses the blood-brain barrier (BBB), with higher concentrations found in plasma than in the brain after acute and chronic administration [24]. There are no metabolites of metformin, and unchanged metformin is excreted in urine (approximately 90% of the absorbed drug) within 24 hours of ingestion [23]. Creatine clearance is 3.5 times lower than the clearance of metformin, which indicates that tubular secretion is the major route of metformin elimination, and the renal uptake of metformin is mediated by organic cation transporter 2 (OCT2); it does not undergo hepatic metabolism nor biliary excretion [23]. The half-life (T_{1/2}) of metformin is approximately 4-5 hours [23]. Unabsorbed metformin accumulates in the gut mucosa of the distal small intestine at a concentration 30- to 300-fold greater than in the plasma and is ultimately eliminated in feces [21].

Metformin is now the most widely prescribed antidiabetic agent in the management of diabetes mellitus type 2 worldwide. The drug employs several mechanisms of lowering glucose, with the primary effect of reduction of hepatic glucose production, or gluconeogenesis. The uptake of metformin into hepatocytes is primarily mediated by OCT1 [25, 26]. In addition, it is well-known that metformin also has pleiotropic effects, including regulation of lipid metabolism (reduction of plasma triglycerides by 15 to 20%), cardioprotection, anticancer activity, and decrease of body weight and food intake. The mechanism of action is dependent on the

dose and duration of treatment with clear differences between acute and chronic administration of the drug. In general, metformin's mechanism of action includes both AMP-activated protein kinase (AMPK)-dependent and AMPK-independent pathways [18, 27]. Several mechanisms are responsible for AMPK-dependent impairment of mammalian target of rapamycin complex 1 (mTORC1) pathway activity. Furthermore, metformin has an inhibitory effect on the mitogen-activated protein kinases (MAPK) pathway. Although this effect of metformin was investigated in different *in vitro* models (including bladder, pancreatic and ovarian cancer), the exact mechanism of MAPK inhibition remains unclear [20, 28].

After over 6 decades of clinical experience with metformin, its short- and long-term side effects are well characterized [21]. Gastrointestinal side effects (diarrhea, abdominal discomfort, nausea, metallic taste, and anorexia) occur in up to 20% of patients and can be minimized by taking the medication with or after meals and by starting at a low dose and slowly increasing to a maximum tolerated dose [25]. If necessary, the extended-release preparation of metformin could be administered, as this preparation has fewer gastrointestinal side effects [25]. Lactic acidosis is typical of some other biguanides such as phenformin and buformin, which were removed from the market in the 1970s in most countries due to unacceptable rates thereof; in contrast, lactic acidosis is an extremely rare side effect of metformin [26]. However, this condition is more likely to occur during severe illness or surgery, so temporary discontinuation of metformin treatment during such episodes is recommended [25, 26].

Preclinical studies have demonstrated that metformin ameliorates core deficits in animal models of FXS. Recently, Gantois and colleagues (2017) showed that metformin corrected many phenotypic deficits [social deficit, repetitive behavior, macroorchidism, aberrant dendritic spine morphology, and exaggerated long-term depression of synaptic transmission] in the adult FXS mouse model. Wild-type (WT) and *FMR1*^{-/y} mice aged between 8 and 12 weeks were used in this investigation. Metformin, at a dose of 200 mg per kg body weight per day, was administered intraperitoneally for 10 days. They hypothesized that chronic metformin treatment corrected the enhanced Raf–MEK–ERK signaling and matrix metalloproteinase 9 (MMP-9) overexpression in *FMR1*^{-/y} mice [29]. MMP-9 is responsible for the degradation of the components of the extracellular matrix, including proteins that are important for synaptic function and maturation, which have been implicated in FXS and ASD [30-32]. Hyperactivation of mTORC1 and extracellular-signal-regulated kinase (ERK) signaling pathways is present in the brains of humans and mice models with FXS due to the loss of FMRP [33]. Finally, according to results obtained from the mouse model of FXS, it seems that the observed phenotypic rescue by metformin is selectively mediated via ERK down-regulation and normalization of MMP-9 expression in the brain [29]. Similarly, data obtained on the *Drosophila melanogaster* FX model, which is based on loss of *dfmr1* function, demonstrated that metformin treatment corrected circadian and cognitive deficits [34]. In this study, Monyak and colleagues (2017) documented that insulin signaling (IS) is increased in the brains of the *Drosophila* mel-

anogaster FX model and that treatment with metformin reduced IS, leading to improvements in memory and circadian rhythm defects [34].

The first clinical data of metformin's efficacy in FXS was obtained in 2017 [35]. Seven patients with FXS were treated clinically with metformin for at least 6 months. Almost all of these patients were prescribed metformin for obesity or treatment of type 2 diabetes and were additionally observed for behavior and metabolic improvements. The patients with FXS not only had improved weight and eating behavior but also experienced positive behavioral changes in areas such as irritability, social avoidance and aggression. Anecdotally, the parents and family members commented on language improvements. Metformin was well tolerated in all patients, even in a 4-year old child [35]. Subsequently, the first randomized, placebo-controlled trial of metformin to further assess safety and benefits in the areas of language/cognition, eating, and overall behavior was initiated. The study includes subjects from 6 to 25 years with FXS randomized to placebo or metformin over a 4-month period and is taking place at the MIND Institute at University of California, Davis Health System (NCT03479476). Identical trials are commencing at the University of Alberta in Edmonton and at St Justine Hospital in Montreal, to maximize analytical power to determine efficacy. The study results are expected by 2022. All in all, from a molecular perspective of looking at the metabolic effects and signaling pathways, as well as from a clinical vantage considering the data regarding metformin's benefit in FXS, this medication appears to be a strong candidate for a new targeted treatment for FXS.

2.3. Cannabidiol (CBD)

The clinical use of CBD has decades of research supporting its effects for a variety of clinical problems. It has been proven beneficial in the treatment of inflammation [36-38], ameliorating pain in autoimmune diseases [39], controlling the urge to smoke in nicotine addiction [40], and even providing neuroprotection that may be useful to prevent glaucoma and retinal neurodegenerative diseases [41]. The neuroprotective potential of CBD, based on the combination of its anti-inflammatory and antioxidant properties, showed certain benefits on the progression of striatal deterioration and proved highly effective in attenuating clasping behavior in the animal model of Huntington disease [42]; however, CBD did not show clinical improvement in humans with Huntington's disease [43, 44]. On the other hand, CBD may be able to improve general Parkinsonism in Parkinson's disease patients with no psychiatric comorbidities [45]. The therapeutic role of CBD in neuropsychiatric disorders has also been broadly studied. Crippa and colleagues have described the anxiolytic effects of CBD in generalized social anxiety disorder and its use in facilitating habituation of anticipatory anxiety [46-48]; other authors have reported that individuals taking CBD experience a reduction in anxiety, cognitive impairment, and discomfort during public speaking and performance [49, 50]. Furthermore, CBD is considered to have antipsychotic effects and may be useful to treat schizophrenia [51-54].

The anticonvulsant effects of CBD are the most studied to date; based on findings of recent controlled clinical trials,

the use of CBD has now been FDA-approved for the treatment of resistant epilepsy in Dravet and Lennox-Gastaut syndromes in children and adolescents [55-58].

Seizures and anxiety disorders are reported in approximately 20% and more than 80% of males with FXS respectively [59, 60], making CBD a promising treatment to address both comorbidities in FXS. Furthermore, the mGluR theory of fragile X [61] is based on the knowledge that FMRP is synthesized in response to mGluR activation, but FMRP inhibits this mGluR pathway [62]. Thus, it is hypothesized that most of the features of FXS, including epilepsy and cognitive impairment, are consequences of upregulation of Gp1 mGluR-dependent protein synthesis in the absence of the inhibition of FMRP in FXS [61]. Gp1 mGluR activation can mobilize endocannabinoids (eCBs), increasing excitability. In 2010, Zhang and colleagues reported consistent results on *Fmr1* KO mice models, revealing increased neuronal excitability mediated by eCBs compared to WT mice; this alteration is believed to be a factor contributing to the cognitive dysfunctions associated with FXS [63]. As such, the endocannabinoid system thence became a promising target for intervention in FXS. Further controlled trials in children and adults with FXS are recommended to better understand the efficacy and tolerability of CBD in the treatment of anxiety, seizures, and cognition in FXS.

In their review, Bergamaschi and colleagues, concluded that the controlled use of CBD may be safe in humans and animals regardless of the route of administration, even with chronic use and high doses up to 1,500 mg/day [64]. Recent studies have also found CBD to lack abuse potential [65] and have confirmed its safety and tolerability profiles via clinical trials [43, 45, 66]. Sultan and colleagues additionally concluded that acute and chronic administration of CBD had no effect on hemodynamics in vivo under controlled conditions; however, its use reduced blood pressure and heart rate under stressful conditions [67]. A Phase 2 open-label trial of a CBD transdermal gel in children with FXS was carried out in Australia with 20 children and demonstrated efficacy in reducing anxiety and improving other behavioral measures [68]. Currently, a phase 3 randomized double-blind controlled trial is being conducted at multiple sites in Australia and the United States to assess the efficacy of this CBD transdermal gel used twice a day on the skin (CONNECT-FX; NCT03614663). In many countries, CBD is legally sold at marijuana stores or through the internet and is thus available for clinical use.

2.4. Acamprosate

Acamprosate, a drug approved for the maintenance of abstinence from alcohol, has recently been the focus of research due to its potential pleiotropic effects impacting glutamate and GABA neurotransmission. Its exact mechanism of action remains incompletely understood but may include effects on multiple receptors with the implication of potential mGluR5 antagonism [69, 70]. In a case study of 3 participants, acamprosate was associated with the increased use of social communicative language, with content more appropriate to a given discussion; however, this benefit was not accompanied by significant improvements in nonverbal aspects of communication, such as eye gaze, based on par-

ent/caregiver report or physician interview [71]. A prospective open-label trial for 10 weeks in children ages 6 to 12 years with FXS showed treatment response in 9 of the 12 subjects (75%) in social behavior and inattention/hyperactivity using multiple standard behavior outcome measures. Moreover, the drug was well tolerated with no severe or serious adverse effects reported [72]. Acamprosate is suggested to be the preferred targeted treatment for those with FXS and comorbid alcohol dependence [73]. This medication is also currently available clinically.

2.5. Lovastatin

Research has also shown that lovastatin, 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) reductase inhibitor used in the treatment of hyperlipidemia and hypercholesterolemia, inhibits the RAS-MAPK-ERK1/2 activation pathway [74]. This drug has also been shown to normalize the excess protein synthesis and can prevent epileptogenesis in the *FMR1* KO mouse model, which is a functional consequence of increased protein synthesis in FXS [75]. An open-label study in 15 participants with FXS over 12 weeks showed significant improvement in aberrant behavior as measured by the global score of the Aberrant Behavior Checklist-Community (ABC-C) and adaptive skills across all three domains [communication, living skills and socialization] of the Vineland Adaptive Behavior Scales, Second Edition (VABS-II). Lovastatin was well tolerated with transient and mild adverse events [76]. Further clinical trials are being conducted with lovastatin with PILI (NCT02642653) and combined treatment of lovastatin with minocycline (LovaMix, NCT02680379). Lovastatin is currently available for clinical use, but the results of controlled trials are not yet available.

2.6. Minocycline

Minocycline is an antibiotic of the tetracycline class that is often used to treat acne. This drug has also been investigated as a treatment for FXS animal models due to its inhibition of the activity of MMP-9 [77]. FMRP negatively regulates the translation of MMP-9, such that FMRP deficiency leads to elevated MMP-9 activity, which alters synaptic plasticity and accounts for some of the cognitive and behavioral impairments in FXS. Animal studies in the *FMR1* KO mouse and in *Drosophila* have demonstrated that minocycline improved synaptic connections, brain structure, vocalizations, and behavior/cognition [77, 78]. These promising preclinical data led to clinical studies in patients with FXS.

Open-label studies with minocycline involving 19 patients with FXS ages 13 to 32 years showed significant improvements in behavioral outcomes in 4 out of 5 sub-scale scores of the ABC-C (irritability, stereotypy, hyperactivity and inappropriate speech). Adverse effects documented in this study were dizziness, diarrhea and seroconversion to a positive antinuclear antibody (ANA) in 2 trial subjects [79]. A study to document side effects and potential outcome measures for minocycline use for a minimum of 2 weeks was also undertaken on 50 patients. In this study, 21 patients (39.6%) reported side effects, with gastrointestinal problems being the most common (loss of appetite in 16%, gastrointestinal upset in 12%, and diarrhea in 8%); 1 patient had darkening of nails, and a small subset of patients experienced

worsening of hyperactivity and moodiness. There were no reports of photosensitivity, tooth discoloration, or skin rash. Parents reported improvements in language, attention, social communication, and anxiety [80]. These two studies prompted a randomized double-blind, placebo-controlled crossover trial of minocycline in 66 children and adolescents ages 3.5 to 16 years with FXS; 55 participants completed 1 arm while 48 finished both arms of the study. Study participants received 3 months of minocycline treatment and 3 months of treatment with placebo with no washout period in between treatments. There were significant but modest improvements in the Clinical Global Impression-Improvement (CGI-I) scale as well as anxiety and mood-related behaviors in the Visual Analog Scale (VAS). The majority of adverse events were mild except for one patient who had a seizure while on the placebo arm [31]. Minocycline is available clinically but its use with FXS requires testing of ANA and liver function studies at least once per year for safety monitoring, and more frequent testing is warranted if a rash develops.

CONCLUSION

Knowledge about FXS and other fragile X-associated disorders has grown significantly over the past decade. Taking into account the progress in the field of FXS, this article emphasizes that we are now entering a new era of the pharmacotherapeutic approach to treatment for patients with FXS. Nowadays, there is an increasing number of clinically available medications that fit the definition of a targeted treatment that can reverse the neurobiological abnormalities in genetic disorders as documented by animal models of specific genetic disorders. Here we reviewed several identified targeted treatments that are available clinically and can be prescribed for patients with FXS. Many of these medications are currently undergoing randomized controlled trials so that their safety and efficacy can be documented, but their off-label clinical use is ongoing and has already generated the preliminary positive results described here. Many of these medications can also be utilized with other psychotropic medications that are not described here because they are not considered targeted treatments for FXS but that have instead been helpful in treating the comorbid symptoms of FXS [6]. Since many pathways and proteins are disrupted in the absence of FMRP [3], it is likely that a combination of medications will be beneficial in those with FXS depending on the individual constellation of comorbid symptoms. Physicians should be encouraged to utilize targeted treatments as they become available clinically because they have the potential for improving not only behavior but perhaps cognition long term, as well. Additionally, there are many new targeted treatments under development that are not yet available clinically, such as trofinetide, AFQ056, arbaclofen and gaboxadol, and these new medications may show efficacy in current or future controlled trials. Therefore, clinicians should be alert to when these drugs would become available for clinical use [4, 8]. At some point in the future, it is possible that stem cell therapy and gene editing techniques may also become available for treatment of FXS.

These targeted treatments, with their promising results in FXS animal models and clinical settings, emerge as the cornerstone in the pharmacological therapy of FXS. The patho-

physiological mechanisms in FXS can be partially normalized by targeted treatment. Consequently, these new medications will not only improve the symptoms in individuals with FXS but hopefully also increase the level of their independent functioning in everyday life. For those individuals starting such treatments early in life, long-term follow-up studies will hopefully show a reversal of many of the symptoms of FXS.

CONSENT FOR PUBLICATION

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CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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